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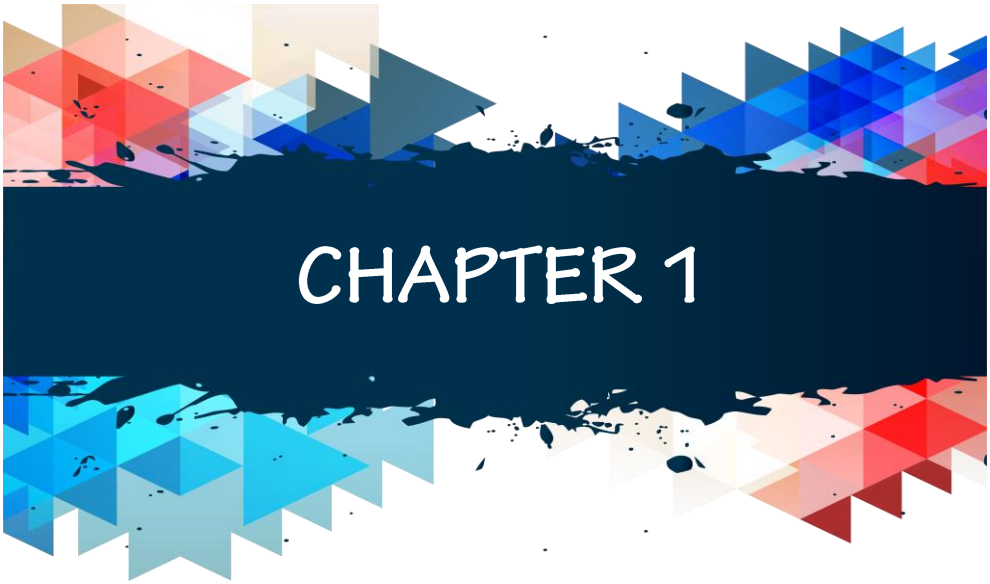

Metacognition and Psychiatric Disorders

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Introduction

Metacognition is recognizing, controlling and regulating individuals' thought processes. This ability plays a vital role in the management of psychological functions, especially psychiatric disorders. Metacognitive skills include the capacity of individuals to be aware of their thoughts, regulate them and change them when necessary. In recent years, research on the relationship between metacognition and psychiatric disorders has contributed to the strengthening of treatment approaches in this field. Psychiatric disorders are conditions that affect the emotions, thoughts and behaviors of individuals, and impaired metacognitive functions may exacerbate the symptoms of these disorders. This chapter will examine the effects of metacognition on psychiatric disorders and discuss the role of metacognitive therapies in treatment processes.

Definition and Components of Metacognition

Metacognition was first defined by Flavell (1979) and accepted as the awareness of cognitive processes. Metacognition is the ability of individuals to observe their mental processes, make sense of these processes and regulate them when necessary. Metacognition consists of two main components: metacognitive knowledge and metacognitive regulation. Metacognitive knowledge is the individual's understanding of their cognitive processes and when and how they are used. Metacognitive regulation is the ability to manage and change thoughts using this knowledge (Flavell, 1979). Metacognition is a function that can affect the psychological states of individuals and has the power to manage these states.

Metacognition and Psychiatric Disorders

The impact of metacognition on psychiatric disorders occurs when individuals' thought processes become uncontrolled. Many psychiatric disorders are associated with metacognitive disorders. In particular, conditions such as depression, anxiety disorders, obsessive-compulsive disorder and schizophrenia are characterized by weakened metacognitive functions. In these disorders, individuals often struggle with negative thoughts and have difficulty controlling these thoughts. Improving metacognitive functions has become an essential goal in treating these disorders (Wells, 2018).

Anxiety Disorders and Metacognition

Anxiety disorders are characterized by individuals feeling excessive worry, fear and anxiety. In individuals with anxiety disorders, metacognitive processes are often impaired. In particular, these individuals usually tend to overthink about adverse future events constantly, and these thoughts can reinforce anxiety-

producing rumination. Individuals with anxiety disorders may have difficulty making metacognitive adjustments, which may lead to increased levels of anxiety (Wells, 2018). Metacognitive therapy (MCT) helps these individuals manage their anxiety by making them aware of their thoughts. MCT is recognized as a practical treatment approach for managing anxiety.

Depression and Metacognition

Depression is characterized by low mood, loss of interest and negative thoughts about oneself. People with depression are often confronted with frequent negative thoughts and these.

Thoughts can darken an individual's view of the world. Depression is closely related to impaired metacognitive functions. Individuals with depression often have difficulty controlling their thoughts, which increases rumination, i.e., obsessive thoughts. Metacognitive impairments associated with depression can make it challenging to respond to treatment. Metacognitive therapy aims to address these disorders and change negative thinking patterns in individuals with depression (Koster et al., 2020). Metacognitive therapies can help individuals organize their thoughts more effectively and alleviate the symptoms of depression.

Obsessive-Compulsive Disorder (OCD) and Metacognition

Obsessive-compulsive disorder leads to a cycle between unwanted and repetitive thoughts (obsessions) and behaviors aimed at preventing these thoughts (compulsions). Individuals with OCD often believe that their thoughts are uncontrolled, and this belief leads them to over-evaluate and repeat their thoughts. Metacognitive therapies are used to question these beliefs and develop more functional thought organizations. In OCD, metacognitive disorders may affect the way individuals cope with their obsessions, and therapeutic intervention offers an opportunity to correct this process (Günay et al., 2022).

Schizophrenia and Metacognition

Schizophrenia is a severe psychiatric disorder characterized by disturbances in thought and perception. Patients with schizophrenia have marked impairments in metacognitive functions. These individuals may often lose touch with reality and develop delusions (unrealistic beliefs) or hallucinations (unrealistic perceptions). Metacognitive impairments can lead to a strengthening of such disorders. The role of metacognitive therapies in the treatment of schizophrenia is to help individuals evaluate their thoughts more realistically and reduce the impact of delusions. In addition, metacognitive treatment can accelerate the

recovery process by improving cognitive and emotional regulation in patients with schizophrenia (Morrison, 2019).

Use of Metacognitive Therapies in Psychiatric Disorders

Metacognitive therapy (MCT) is a psychotherapy approach that aims to improve metacognitive processes. MCT has been particularly effective in the treatment of psychiatric disorders such as depression, anxiety disorders, OCD and schizophrenia.

The main goal of therapy is to make individuals aware of their thought processes and teach them to regulate these processes functionally. MCT plays an essential role in the treatment process by facilitating the control of individuals' thoughts and the management of negative thoughts (Wells, 2016). In addition, MCT increases individuals' metacognitive flexibility, enabling them to gain a more effective and efficient intellectual regulation skill.

The Effect of Metacognitive Therapies on Psychological Disorders

The effect of metacognitive therapies on psychiatric disorders has been concretely demonstrated in clinical studies. In studies on conditions such as depression and anxiety, metacognitive therapy increased the response to treatment and reduced symptoms. In particular, as the metacognitive functions of individuals become more muscular, obsessive thoughts, rumination, and anxiety may decrease. Metacognitive therapies have also been successfully applied in more complex disorders such as schizophrenia and OCD and have helped improve the symptoms of these disorders (McAuliffe & Gregson, 2020). These findings emphasize the importance of metacognitive therapies in the field of psychiatry.

Conclusion

Metacognition plays a vital role in the treatment of psychiatric disorders. Psychiatric disorders such as depression, anxiety disorders, OCD and schizophrenia are associated with impaired metacognitive functions. Metacognitive therapies contribute to treatment by enabling individuals to recognize and regulate their thought processes when managing these disorders. Metacognitive therapies have been found to help individuals develop more flexible and effective coping strategies against negative thoughts. These therapies have a great potential to provide faster recovery in the treatment process and support alleviating symptoms.

Research on the effect of metacognitive therapies has shown positive results, especially in common psychiatric disorders such as depression and anxiety

disorders. It is also used as an effective treatment tool for more complex and severe disorders such as schizophrenia and OCD. Understanding the impact of this treatment approach on psychiatric disorders may increase the use of metacognitive strategies in a broader range of clinical practice.

Future research will be directed towards further clarifying the role of metacognitive therapies in psychiatry and increasing the effectiveness of these therapies. In particular, a better understanding of the biological and neurological mechanisms associated with metacognition will allow metacognitive therapies to be applied in a more targeted and effective manner. In addition, it may be possible to develop more comprehensive treatment methods by combining metacognitive therapy and other psychotherapy approaches.

Improving metacognitive functions will remain an essential goal in the treatment of psychiatric disorders. Metacognitive therapies can improve individuals' ability to cope with psychological disorders by increasing their cognitive flexibility. Therefore, metacognition's importance in psychiatry will be further strengthened by its contributions to treatment processes and future research.

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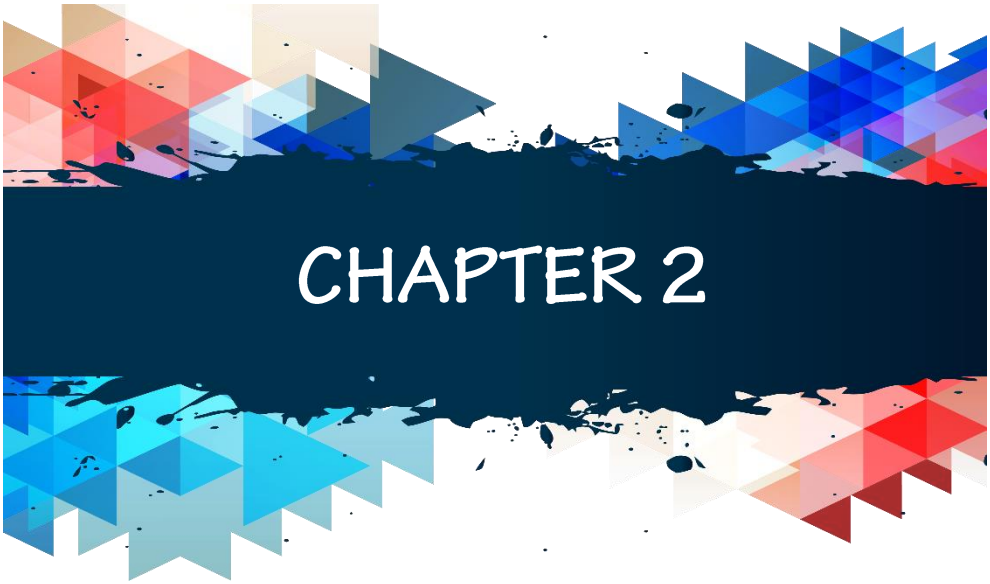

EMG-Biofeedback for Pelvic Floor Dysfunction

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Introduction

Pelvic floor dysfunction (PFD) is a general term that covers a variety of functional problems that occur in any of the compartments (anterior-middle-posterior) that make up the pelvic floor muscles. The presentations that may occur in PFD include lower urinary system symptoms (LUTS), bowel symptoms, pelvic organ prolapse (POP), sexual dysfunction and pelvic pain syndromes (Skaug et al., 2022). It affects all genders in all age groups. Treatment modalities for pelvic floor dysfunction include surgical, medical and physiotherapy and rehabilitation approaches. The aim of physiotherapy and rehabilitation in pelvic floor dysfunction is to reduce clinical symptoms, prevent progression, prevent or delay the need for surgery and improve quality of life. Physiotherapy and rehabilitation approaches applied for these purposes consist of pelvic floor muscle training. Pelvic floor muscle training consists of manual therapy applications, behavioral modification approaches and functional exercise training. Functional pelvic floor muscle exercises can be performed with vaginal cone, biofeedback, three-dimensional real-time ultrasound (US) and virtual reality applications (Quaghebeur et al., 2022). In this section, a current perspective on the effectiveness of EMG-Biofeedback in pelvic floor dysfunction will be emphasized.

Pelvic Floor Muscles

Pelvic Floor Muscles (PFM) consist of the levator ani, anal sphincter complex, pelvic sidewall muscles, and anterior perineal muscles. The PFM is responsible for voiding, defecation, sexual function and support of the organs in the pelvis (Jorge et al., 2022). In addition, PFM works in synergy with the anterolateral abdominal muscles and diaphragm muscle in changing intra-abdominal pressure and providing stability. It also has important contributions in providing trunk stabilization (Hodges et al., 2007).

The PFM has been compared to a trampoline due to its position in the pelvis. If the trampoline is too tense or too loose, jumping will be difficult. But a trampoline with normal tension provides a fast response with an effective upward thrust. The PFM is the only transversely positioned muscle group that provides structural support to the pelvic organs and pelvic openings such as the urethra, vagina and anus. With PFM contraction, the perineum moves in the ventral and cranial direction and the anus, vagina and urethral openings are closed. With these contractions, continence is achieved and involuntary leakage of urinary and rectal contents is prevented (Yakıt Y. et al., 2019).

EMG studies have shown that continuous motor activity of the PFM persists during rest. This physiologic spontaneous contractions at rest is termed 'tonic activity'. With tonic activity, pelvic floor organs are supported in resting state. PMF is the only muscle group that carries load in the horizontal position in the organism and it is reported to be active for 24 hours (van Reijn-Baggen et al., 2022).

The International Continence Society (ICS) standardized the pelvic floor muscles as follows.

-Normal pelvic floor muscles,

-Underactive pelvic floor muscles,

-Overactive pelvic floor muscles,

-Non-functional pelvic floor muscles (Frawley et al., 2021).

Pelvik Floor Dysfunctions (PFD)

The pelvic floor (PF) has a complex anatomical structure consisting of endopelvic fascia, ligaments, perineal membrane, levator ani muscles, urogenital diaphragm muscles and superficial perineal muscles. The PF supports pelvic organs such as the bladder, bowel and uterus. Structural and functional damage to PF components may cause complex compartmental dysfunction (Himmler et al.,2021).

Integral system theory (IT)

Integral theory explains the suspensory function of the pelvic floor on the bladder and bowel. The suspension of the pelvic organs is achieved by being held in suspension by the ligaments, which counteract the muscle contractions that perform the mechanism of opening and closing the anus, urethra and vaginal openings. Contraction of the pelvic floor muscles also supports the suspension role of the suspensory ligaments by providing form and strength to the perineal bed (Liedl et al., 2018). The ligamentous system in this region divides the pelvic region into three levels: anterior, middle and posterior. The first level consists of the uterosacral ligaments (USL), the arcus tendineus fascia pelvis (ATFP) and the pubocervical fascia (PCF). The second level consists of the pubourethral ligaments (PUL) and the rectovaginal fascia (RVF). The third level consists of the perineal body (PB), the perineal membrane, the post-anal plate and the external ligament of the urethra (EUL). Şekil 1'de pelvis içindeki organları askıya alan bağlar ve seviyeler gösterilmektedir. Connective tissue changes occurring in the ligaments or laxity of the vaginal wall or suspensory ligaments due to trauma

reduce PFM strength. As a result, complex pelvic floor dysfunctions such as pelvic organ prolapse, bladder and bowel symptoms may occur (Quaghebeur et al., 2022).

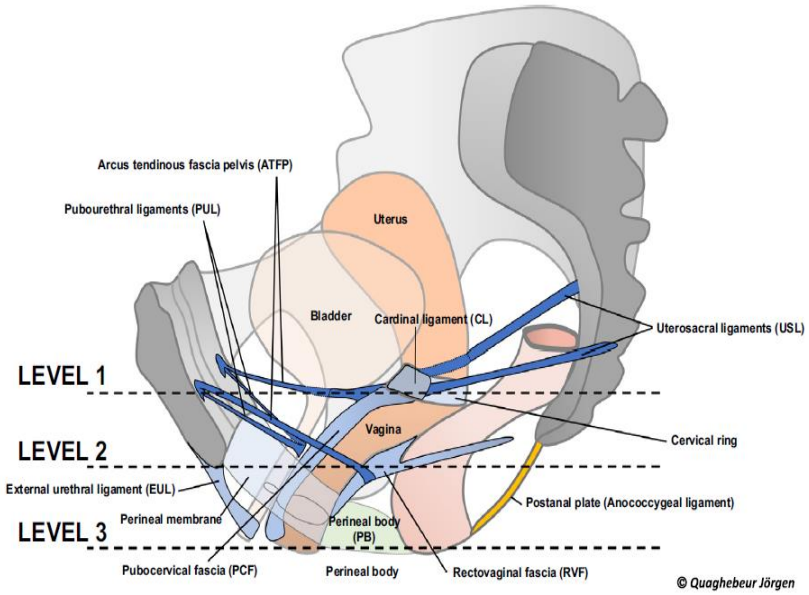

Figure 1. Ligaments that hold the pelvic organs suspended (Quaghebeur et al., 2022).

PFD is a concept characterized by complex symptoms. Clinical symptoms associated with PFD are analyzed under five subgroups (Yıldırım et al., 2017);

1. Lower urinary tract symptoms (LUTS): Urinary incontinence (UI), stress UI, urgency, frequency, slow flow, hesitancy, feeling of not being able to empty completely, intermittent urination.

2. Bowel symptoms: constipation, obstruction of defecation, rectal or anal prolapse, fecal incontinence.

3. Vaginal symptoms: Pelvic organ prolapse, vaginismus.

4. Sexual symptoms: Dyspareunia, erectile dysfunction, ejaculatory dysfunction, orgasmic dysfunction.

5. Pain symptoms: Interstitial cystitis, pelvic pain.

EMG-Biofeedback in PFD

Since the anatomical structure and functional disorders of the pelvic floor are complex, diagnosis and treatment are quite difficult (Grimes et al., 2020). Pelvic

floor muscle exercise (PFME) is recommended as a first-line conservative treatment approach in PTD. By strengthening the PFM, the closing pressure of the urethra increases, pelvic organs are supported and the feeling of urgency is reduced (Martinho et al., 2016). PFME can be performed using biofeedback (BF) modality (Hagen et al., 2020). BF has been used for more than half a century in treatment programs to improve normal movement patterns for muscle tissue-related injury and dysfunction. Electromyography (EMG)-biofeedback, which belongs to the neuromuscular BF category, is the most preferred type of BF in the clinic. EMG-biofeedback is also used in the evaluation and treatment of PFD (Giggins et al., 2013; Chiang et al., 2021). EMG-biofeedback, a neuromuscular training method, teaches PFM to contract and relax with external stimuli in a visual and auditory manner (Faubion et al., 2012). Individuals who will receive EMG-biofeedback treatment should have good cooperation and be motivationally ready. Therefore, it is reported that EMG-biofeedback therapy is not effective in children under 5 years of age. Adequate mental maturation is one of the most important issues for this treatment method. Success in EMG-biofeedback treatment depends on the patient's effort, motivation for the treatment program, appropriate mental maturation and a safe, practical and feasible treatment (Ladi-Seyedian et al., 2019). There are many studies in the literature showing the effect of EMG-biofeedback therapy in PFD. In a systematic review including seven studies investigating the efficacy of biofeedback in PFD, it was reported that treatment programs including any type of biofeedback increased the treatment success rate six times compared to other treatments (Koh et al., 2008). In a study with 41 participants diagnosed with overactive bladder, the participants were divided into two groups. One group received only PFME while the other group received biofeedback treatment in addition to PFME. As a result of the study, the superiority of PFME with biofeedback was emphasized (Wang et al., 2004). Wu et al. included 21 studies in their systematic review by searching PubMed, EMBASE, Cochrane Library, Web of Science, Wanfang and CNKI databases. In this systematic review, the efficacy of PFME alone and EMG-Biofeedback combined treatment in addition to PFME in the treatment of women with stress urinary incontinence was compared. In the study, 1967 women received combined biofeedback and PFME training while 1898 women received only PFME training. Pelvic floor muscle strength, urodynamic Qmax value, Sexual Quality of Life (FSFI) and urinary incontinence quality of life (I-QOL) were reported to increase more in the direction of improvement. This result suggests that PTKE is more effective when applied in combination with EMG-Biofeedback (Wu et al., 2021).

Aalaie et al. conducted a study examining the superiority of electrical stimulation and EMG-biofeedback applications in the improvement of sexual dysfunctions, which are important PTDs in women with stress incontinence. They reported that EMG-biofeedback was more effective in improving quality of life by increasing sexual function scores. In addition, they stated that both applications were effective in improving pain (Aalaie et al., 2021).

In a study conducted by Bertotto et al. in postmenopausal women with stress UI, they investigated the efficacy of EMG-Biofeedback in combination with PFME in improving muscle strength, myoelectric activity, precontraction reflex and quality of life. They reported at the conclision a significant improvement in maximum voluntary contraction strength, endurance contraction duration and ICIQ-SF quality of life. This study suggests that PFME performed with EMG-Biofeedback in postmenopausal women with stress UI is associated with increased muscle strength myoelectric activity, precontraction reflex of pelvic floor muscles and improved quality of life (Bertotto et al.,2017).

In another study investigating the effect of EMG-Biofeedback and electrical stimulation on UI in a three-month early rehabilitation program after radical prostectomy surgery, the patient group given PFME combined with EMG-Biofeedback and electrical stimulation was compared to the group given only pelvic floor home exercise. It was reported that there was a significant difference between the control group and the treatment group in one-hour pad test, 24-hour pad test and ICIQ-SF scores. The results of this study show that combined EMG-Biofeedback and electrical stimulation applied in the early period after radical prostectomy surgery is effective in reducing incontinence symptoms, helping continence and improving quality of life (Soto et al., 2020).

Conclusion

The International Continence Society (ICS) reports pelvic floor rehabilitation as the first-line treatment in PTD. EMG-Biofeedback, one of the applications of pelvic floor rehabilitation, is applied together with pelvic floor muscle exercises. Evidence shows that EMG-Biofeedback in combination with PFME increases the effectiveness of treatment in PTD such as voiding and defecation dysfunctions, sexual dysfunctions and pelvic pain.

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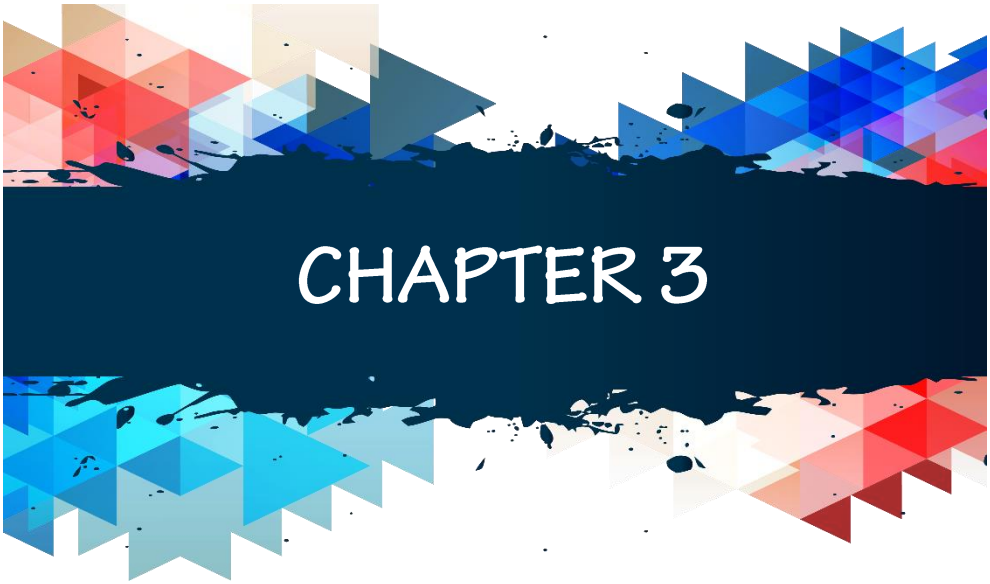

A Current Look at the Transvaginal Approach in the Surgical Treatment of Vesicovaginal Fistulas

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INTRODUCTION

Vesicovaginal fistula (VVF) is a pathological connection between the bladder and vagina and is a health problem that causes serious physical and psychological issues for women. While it mostly occurs after gynecological or pelvic surgeries in developed countries, it is usually seen in developing countries due to traumas during birth (Härkki-Sirén, 1998). Results such as constant urine leakage, bad odor, and irritation in the genital area significantly reduce the daily life quality of patients and create psychological effects such as social isolation and depression (Wall, 2006).

The most commonly used methods in the treatment of VVF are transvaginal and transabdominal surgical approaches. Although the popularity of minimally invasive methods (laparoscopic and robot-assisted surgeries) is increasing, the transvaginal approach is more advantageous, especially when the fistula is located in the inferior bladder wall. It is reported that this method offers many advantages such as shorter operation time, low blood loss, short hospital stay and fewer complications. The transvaginal approach is also more cost-effective, accelerates the recovery process of patients, and has better cosmetic results (Colenbrander, Heesakkers, & Martens, 2021). In addition, almost all vesicovaginal and urethro-vaginal fistulas that develop after synthetic mesh urinary incontinence and pelvic organ prolapse (POP) surgeries, which have become increasingly common in recent years, can be treated with the transvaginal approach. Therefore, urology specialists who deal with synthetic mesh surgery and its complications must be familiar with the transvaginal approach (Fikret Fatih Önol&ÜmitYıldırım, 2015).

The transabdominal approach is preferred in larger and more complex fistulas, in cases where the ureters are involved, or in cases where access to the pelvic structure is difficult. However, this method carries a higher risk of complications and generally requires a longer recovery period (Kumar et al., 2022).

In this section, the effectiveness of the transvaginal surgical approach used in the treatment of vesicovaginal fistula will be discussed, the positive effects of this method on patient outcomes will be discussed, and recommendations for achieving optimal surgical results will be presented.

CLASSIFICATION

Various classification systems exist to categorize vesicovaginal fistulas (VVF) depending on their causes and complexity. Generally, VVF can be classified into two main types: simple and complex. Simple VVFs are typically 2

cm or smaller, are usually isolated cases, and are not caused by radiation damage. In contrast, complex VVFs are characterized by a larger size (exceeding 2 cm), involvement of the ureter, recurrence after prior unsuccessful repair attempts, or they may result from radiation exposure (Önol & Yıldırım, 2015).

For obstetric fistulas, two widely used classification systems are the Goh and Waaldijk systems. A comparative analysis of these systems found that the Goh classification was significantly more effective than the Waaldijk system in predicting the likelihood of fistula closure. This advantage suggests that the Goh system may offer more reliable guidance in the context of obstetric VVFs(Capes, Stanford, Romanzi, Foma, & Moshier, 2012).

There has been interest in applying the Goh classification, which defines VVFs by types 1 through 4, to non-obstetric cases as well. A study by Beardmore-Gray and colleagues examined the Goh classification in a non-obstetric context through a retrospective analysis of 63 cases managed by a single surgeon. Their findings revealed that while the Goh classification types (1 to 4) were not predictive of closure success for these non-obstetric cases, continence outcomes did worsen as the Goh classification type increased. This trend indicates that as the Goh classification type progresses, patients may experience a decline in continence, regardless of the success in achieving fistula closure (Beardmore-Gray, Pakzad, Hamid, Ockrim, & Greenwell, 2017; Stamatakos, Sargedi, Stasinou, & Kontzoglou, 2014).

The study identified that two specific factors (patient age and fistula size) were the most significant predictors of successful outcomes. This insight underscores the importance of these characteristics, suggesting that classification systems might be most effective when they take into account the overall clinical picture, including fistula size and patient demographics, in order to predict outcomes more accurately (Beardmore-Gray et al., 2017; Capes et al., 2012).

In summary, while the Goh classification has shown some predictive value in obstetric fistulas, its application to non-obstetric cases appears limited in terms of closure success but may still inform expectations for continence outcomes. The findings emphasize that beyond classification systems, specific patient factors such as fistula size and age are crucial in determining the success of VVF treatment (Beardmore-Gray et al., 2017; Capes et al., 2012; Stamatakos et al., 2014). Table 1 provides information summarizing the VVF classification.

Table 1. Comparative summary of VVF classifications

Classification Category	Characteristics
General Classification	
Simple VVF	≤ 2 cm in size - Often solitary - Not induced by radiation
Complex VVF	- > 2 cm in size - Involves the ureter - Related to recurrence after previous failed repairs - Caused by radiation damage
Obstetric Fistula Classification Systems	
Goh Classification	- Based on fistula types (Types 1-4) - More effective at predicting fistula closure success compared to Waaldijk
Waaldijk Classification	- Alternative classification
Study Findings	
Goh Classification (for Non-Obstetric VVF)	- Not predictive of closure success based on Types 1-4 - Continence outcomes deteriorated with increasing Goh type
Most Significant Predictor of Success	- Age and size of the fistula

PREOPERATIVE EVALUATION

Vesicovaginal fistulas (VVF) require distinct approaches depending on whether they are non-irradiated or irradiated/malignant, as these categories affect surgical complexity, repair route, and tissue interposition needs based on surgeon expertise. Non-irradiated, post-hysterectomy VVFs are generally less challenging, while irradiated VVFs can present significant challenges due to compromised tissue quality, which may necessitate urinary diversions, such as ileal or colonic diversions (Lee & Zimmern, 2019).

Clinically, VVFs often present soon after gynecologic or obstetric procedures, with symptoms such as continuous urine leakage into the vagina. In small VVFs, symptoms may include minimal watery discharge with normal voiding. It's crucial to review the original surgery report for any bladder injury and note any history of cesarean operation or radiation, as these can impact tissue vascularity and complicate repair. Radiation-induced VVFs can also have delayed presentations years after treatment (Lee & Zimmern, 2019; Önol & Yıldırım, 2015).

A thorough pelvic examination is essential to determine fistula size, location, and surrounding tissue quality, assessing for signs of infection, necrosis, or

scarring. If diagnosis is uncertain, instilling diluted methylene blue or performing a tampon test with bladder filling may help confirm the presence of VVF. Imaging, like CT urograms or retrograde pyelograms, is essential to rule out ureterovaginal fistulas, which co-occur with VVFs in about 10% of cases. Cystoscopy is also valuable for confirming diagnosis, assessing fistula positioning relative to the ureter, and evaluating for malignancy (Dwarkasing, Hussain, Hop, & Krestin, 2004; Goodwin & Scardino, 1980; Lee & Zimmern, 2019).

Additional investigations, including voiding cystourethrograms for both preoperative and postoperative assessments, may be necessary for medicolegal purposes. Less commonly used modalities, such as MR fistulography or transvaginal ultrasound, can be useful depending on radiology expertise and availability (Dwarkasing et al., 2004; Goodwin & Scardino, 1980).

TRANSVAGINAL REPAIR OF VESICOVAGINAL FISTULAS

The best opportunity for successful VVF repair is the initial surgical operation (Thomas E. Elkins, 1994). The vast majority of VVFs seen in developed countries are suitable for transvaginal repair (Margolis & Mercer, 1994). Because of its benefits, such as a shorter surgical and hospital stay and reduced blood loss, the transvaginal method is preferred for the treatment of small fistulas (distally situated, primary, <4 cm, post-hysterectomy) (Goodwin & Scardino, 1980) (Table 2). However, previous unsuccessful attempts may cause scarring and anatomical deterioration in the surgical field, making transvaginal intervention difficult, and may also compromise the local tissue flaps that could be used. One major drawback of the transvaginal approach is that it can potentially shorten the vagina. Another issue is that most urologists don't have a good understanding of the vaginal vault's anatomy, which can make exposure difficult. This is particularly true for women with deep or narrow vaginas or fistulas that are high-located and retracted close to the vault (Table 2).

Although the transabdominal approach is considered more advantageous in the treatment of complex fistulas, the vast majority of complicated VVFs can currently be treated with the transvaginal approach, except for fistulas that require intraabdominal interventions such as simultaneous augmentation cystoplasty and ureteral reimplantation (Onol et al., 2014; Önol & Yıldırım, 2015; V. Singh et al., 2011). There are basically two methods for transvaginal repair of VVFs (11). The first is the partial colpoclesis method described by Sims and modified by Latzko (Latzko, 1942). The second method, which is used more frequently today and is

described below, is the “split-flap” method based on the dissection of tissues and layered repair (Kumar et al., 2022).

Table 2: Advantages and Disadvantages of Transvaginal Repair for Vesicovaginal Fistulas

Advantages	**Disadvantages**
Avoids morbidity associated with laparotomy and related complications	Possibility of vaginal shortening and narrowing
Shorter operation time	Difficulty in exposure for high-positioned fistulas
Shorter hospital stay	Lack of familiarity of urologists with vaginal cuff anatomy
Faster recovery and return to normal activities	Unable to perform simultaneous augmentation cystoplasty and ureteral reimplantation (if necessary)
Less postoperative pain	
Minimal blood loss	
No need for extensive bladder opening or division	
Unaffected by previous abdominal surgeries	
Allows concurrent anti-incontinence or prolapse surgery	
Proximity to local interpositional flaps	
Potential for multilayer closure (3-4 layers)	
Low risk for future abdominal interventions in case of failure	

Timing of VVF Repair

Vaginal fistula (VVF) repair time is affected by a number of variables. When there is no ongoing infection, necrosis, or inflammation, most surgeons choose to do the surgery. While some have found success by waiting to intervene after a VVF diagnosis, others have found comparable results by acting immediately. In most cases, the repair may be done no later than twelve weeks after the diagnosis (Blaivas, Heritz, & Romanzi, 1995). Key factors influencing the timing of repair include:

1. Nature of the injury causing the fistula
2. Patient’s nutritional status
3. Presence of infection and foreign bodies
4. Immunocompromised status

The timing of the repair is critical since it has the best chance of success if done initially. It is crucial for women with VVF to have emotional and psychological support from their treating physician and family members since they typically feel worry and sadness while waiting for surgery (O. Singh, Gupta, & Mathur, 2010).

Post-Obstetric Fistulas:

Obstetric fistulas may heal more slowly, letting dead tissue slough off and inflammation go down. An early repair could be possible if the fistula is simple and not infected. Repairing a wound when necrosis and infection have not yet healed presents unique challenges (Hillary, Osman, Hilton, & Chapple, 2016).

Postsurgical Vesicovaginal Fistulas:

After surgery, the 12-week recommendation is usually followed for VVF repair as well. This gives the body a chance to heal and identify any necrotic tissues (Altaweel, Rajih, & Alkhudair, 2013). Within this norm, there are exceptions:

1. VVF, which verified a few days after the main operation

2. Coexisting ureteral injury necessitating surgical intervention

3. The patient's wish for or need for prompt treatment

Radiation-Induced Vesicovaginal Fistulas:

It is recommended to postpone surgery for radiation-induced VVF until the immediate post-radiation tissue response has subsided. Radiation reactions typically heal within about a year, so repair is often delayed until after six months (Pushkar, Dyakov, & Kasyan, 2009).

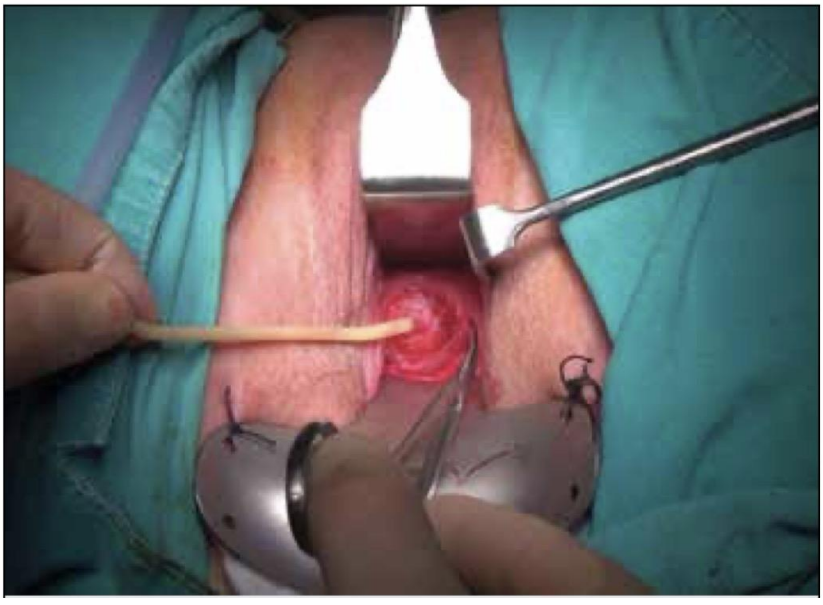
Transvaginal VVF Repair Technique

Positioning and Preparation: The patient is placed in the dorsal lithotomy position, and the perineal area is cleansed with standard surgical solutions. Positioning the patient’s hips to extend off the table, using the Trendelenburg position, and applying weighted speculums facilitate access to deeply located fistulas. After positioning and cleaning, hooks/ring retractors or silk sutures are used for retraction to ensure adequate exposure. A cystoscopy is then performed to assess the location of the VVF and its relation to the ureters. It is necessary to insert and carefully monitor a ureteral catheter during the repair process if the fistula is near or encompasses the ureteral orifice. Before fistula repair, any other

vaginal or anti-incontinence procedures scheduled for the same session should be canceled so as not to interfere with the finished reconstruction (Onol et al., 2014).

Vaginal Incision: A Foley catheter, with a balloon inflated to match the VVF's diameter, is inserted through the fistula tract into the bladder and pulled downward, drawing the VVF towards the introitus (Figure 1). If the Foley catheter cannot be passed through the tract or if the fistula is wide, bougie dilators inserted via the urethra allow for probing and circumferential dissection. The vaginal flaps are then marked, and a circumferential incision is made around the fistula tract. To increase exposure in dissection areas, an additional reverse "J" or "U" incision can be made from the circumferential incision around the fistula tract, extending toward the vaginal apex. When the ureteral orifice is close to the fistula tract, the circumferential incision should be made 0.5-1 cm away to protect the ureter during dissection(Onol et al., 2014; Önol & Yıldırım, 2015).

Figure 1. Retraction of the tract to the introitus with the help of a Foley catheter passed through the VVF tract. After the incision of the fistula tract all around, the dissection between the vaginal wall and the bladder is shown.



Creating Vaginal Flaps: Proximal, distal, and lateral dissections are used to produce flaps in the vaginal wall as they move away from the fistula tract. Dissection continues between the vaginal flaps and bladder tissue surrounding the fistula tract until strong bladder tissue is exposed to allow for tension-free closure of at least 2-3 layers. This dissection can be challenging due to scar tissue, making it essential to stay within the correct plane without disrupting blood flow.

To adequately cover the fistula tract, it may be required to extend the proximal flap distally, hence it is crucial to mobilize the vaginal wall flap distally to the VVF (Margolis & Mercer, 1994). Complete circumferential dissection of the fistula tract is usually unnecessary, as excising the tract can enlarge the defect, making tension-free closure difficult for the bladder and vaginal walls. Only the portions of the fistula tract at the suture line should be locally excised ("shaved") as needed (Onol et al., 2014; Önol & Yıldırım, 2015).

Closing the Fistula: After removing the Foley catheter from the tract, the initial layer of repair is carried out. Sutures (2-3/0 vicryl) are placed transversely or vertically along the fistula. Remaining fistula tract tissue supports the first layer by providing strong anchorage. For larger VVFs, a bougie dilator advanced from the urethra into the bladder retracts the fistula tract towards the introitus, providing better access for suturing challenging corners. The bladder's deep muscle layer and perivesical fascia are then advanced over the initial suture line to bury the previous layer. Although their routine use is controversial, a Martius flap or interpositional peritoneal flap might be utilized at this stage to strengthen the suture line. After completing the fistula closure, the bladder is filled with 200-300 cc of saline diluted with methylene blue to check for leaks at the vaginal incision site (Onol et al., 2014).

Advancing and Closing Vaginal Flaps: The flaps made from the vaginal wall are used for the third and last layer of closure. It is possible to remove the extra anterior (distal) vaginal flap in situations where there isn't a major abnormality. Suturing the posterior flap forward with absorbable sutures allows healthy tissue to cover the suture line and prevents the suture lines from overlapping.

A Foley catheter is left in place for at least 10-14 days postoperatively. A cystography is performed 14-21 days after surgery, and if leakage is observed, catheterization continues with follow-up imaging every 2-3 weeks to monitor healing (Önol & Yıldırım, 2015).

Should the Fistula Tract Be Excised:

The classical view in vesicovaginal fistula repair is to completely remove the fistula tract and the surrounding scar tissue. According to this approach, excision of the fistula tract provides well-perfused, viable tissue for the closure of the first layer of the repair (Wein, Malloy, Carpiniello, Greenberg, & Murphy, 1980).

It is not always essential to remove the fistula tract, and doing so may compromise the healing process (Margolis & Mercer, 1994). The removal of the fistula tract may have certain unintended consequences. The operation leaves a

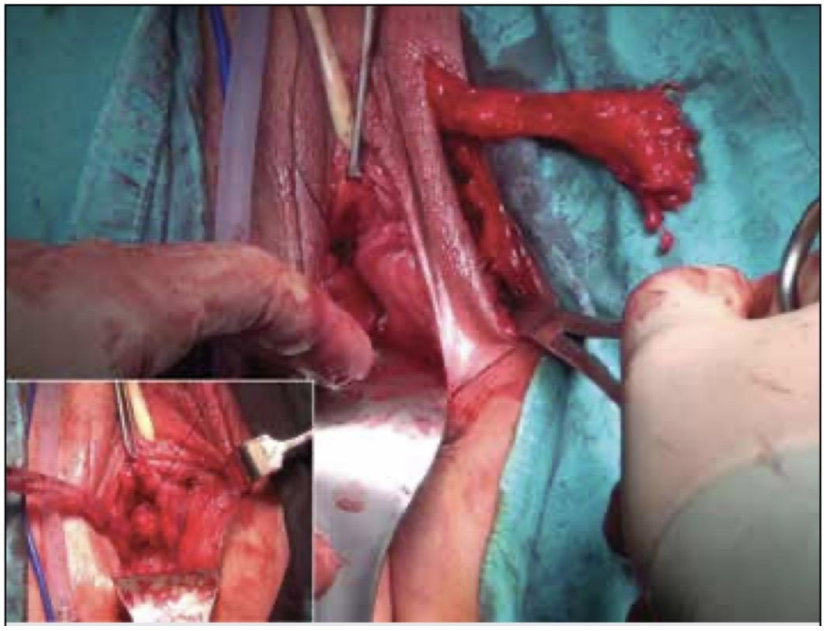
huge defect in the soft tissue. Using cautery to stop bleeding after removing the fibrous tract can slow healing since it causes necrosis (Eilber, Kavaler, Rodríguez, Rosenblum, & Raz, 2003). It may be necessary to reimplant the ureterovesical system after removing the fistula tract if the VVF is located near the ureter.

Tissue Interposition in Transvaginal VVF Repair

Fistula surgeons should know the grafts or flaps that provide healthy tissue interposition in vesicovaginal fistula repair. The indications for tissue interposition have not been clearly defined. However, it is widely used in cases such as tissues exposed to radiation, obstetric fistulas causing large soft tissue defects, previous failed repairs, large fistulas, and poor repair due to inadequate tissue quality (Önol & Yıldırım, 2015). In a study of 49 patients (25 primary, 24 recurrent VVF) who underwent transvaginal fistula repair after excluding patients with malignant etiology and those receiving radiotherapy, it was reported that there was no difference in fistula recurrence in the groups with and without tissue interposition (Pshak, Nikolavsky, Terlecki, & Flynn, 2013).

Martius flap: The preferred flap for fistulas involving the trigone, bladder neck, and urethra is obtained by making a vertical incision in the labium majus. It consists of adipose and connective tissue (Figure 2) (Rangnekar, Imdad Ali, Kaul, & Pathak, 2000; Wilson, Pillay, & Greenwell, 2017). It can also be used in proximal fistulas in experienced centers. Blood supplies the Martius flap from the posterior labial arteries inferiorly, the external pudendal artery posteriorly, and the obturator artery laterally.

Figure 2. Creation of a Martius flap from the left labium majus for tissue interposition after posthysterectomy vaginal cuff fistula repair. The small picture shows the flap transferred to the repair area.



Peritoneal flap: It is frequently used in the transvaginal repair of high-lying post-hysterectomy fistulas. It can also be used in the treatment of complex VVFs repaired transabdominally due to its ease of obtaining (Eilber et al., 2003).

Other Transvaginal Techniques

The Latzko technique is another option with a success rate of over 90% in transvaginal fistula repair (Käser, 1977). On the other hand, with huge obstetric fistulas, this procedure may not be effective as well as the vaginal flap technique (T E Elkins, Drescher, Martey, & Fort, 1988). Using this method, the vaginal epithelium is circularly peeled back up to 1-2 cm to expose the tissue around the VVF tract. The bladder and perivesical fascia are areas that should not be penetrated by deep peeling. After the peeling is complete, the fistula tract is re-attached using interrupted absorbable sutures. A second layer of closure can be applied to the vaginal wall borders after a partial colpoclesis procedure, which is an option for certain individuals.

Less blood loss, elimination of the necessity for ureteral reimplantation, and a speedy recovery are some of the benefits of the Latzko procedure. Possible

drawbacks include vaginal shortening and overlapping suture lines (Enzelsberger & Gitsch, 1991).

RESULTS OF TRANSVAGINAL REPAIR

The outcomes of transvaginal vesicovaginal fistula (VVF) repair, often noted in the literature, underscore the procedure's efficacy, especially when success criteria encompass not only fistula closure but also postoperative continence and sexual function.

Studies reveal that transvaginal VVF repair achieves a high success rate (over 85%) across various populations. In a review of patients in a high-volume hospital, the modified Latzko technique (a popular transvaginal approach for VVF repair) showed effective closure rates without significant complications, such as urinary incontinence (Cardenas-Trowers, Heusinkveld, & Hatch, 2018; Luo & Shen, 2019). Another study compared transvaginal and minimally invasive techniques, highlighting that the transvaginal method can deliver shorter operative times, reduced blood loss, and lower costs, with a success rate nearing 99% compared to 96.5% in minimally invasive approaches (Kumar et al., 2022).

A critical aspect of evaluating VVF repair success, as noted in the literature, is the "continence gap." This term describes cases where, despite fistula closure, patients continue to experience urinary leakage, underscoring the importance of holistic criteria in assessing VVF outcomes (Kumar et al., 2022).

For postoperative sexual function, transvaginal repair has generally been favorable. While it can theoretically lead to complications such as vaginal stenosis or shortening, findings suggest that it often enhances sexual function compared to preoperative levels, with no clear superiority between transvaginal and transabdominal approaches for sexual function outcomes (Cardenas-Trowers et al., 2018).

Complications

Appropriate dissection plans are important for limiting surgical bleeding. Excessive use of cautery to prevent bleeding may impair the nutrition of tissue flaps. Repair of a VVF that is near to the ureteral orifices carries the risk of ureteral damage. In such a case, intravenous indigo carmine should be administered, and cystoscopy should be performed. Vaginal stenosis and shortening are late consequences of transvaginal VVF surgery. Hence, it is important to refrain from removing too much of the vaginal wall before closing. Fistula recurrence is the most serious risk associated with VVF repair. In these cases, repeating the transvaginal approach may provide satisfactory success

(Margolis & Mercer, 1994). However, since the tissue quality is insufficient in recurrent VVF repair, care should be taken to use appropriate interposition flaps.

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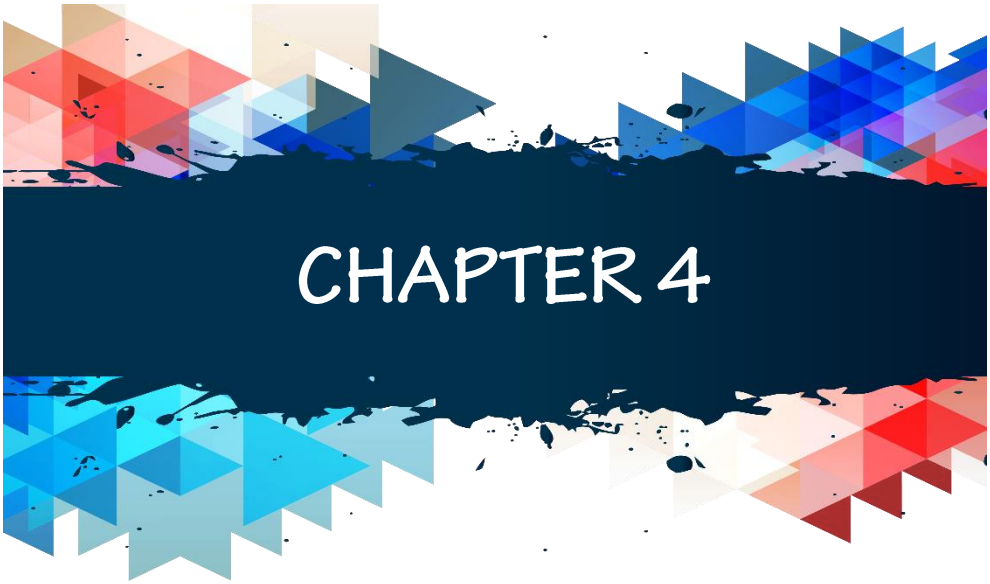

Finite Element Analysis of the Hip Joint

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FINITE ELEMENT ANALYSIS OF THE HIP JOINT

As a critical component of the human musculoskeletal system, the hip joint plays a vital role in mobility and stability. Its anatomy is characterized by a ball-and-socket structure consisting of the femoral head and the acetabulum of the pelvis, allowing a wide range of motion in multiple planes. This unique structure facilitates a variety of movements such as flexion, extension, abduction, adduction and rotation, which are essential for activities of daily living and athletic performance (Samaan et al., 2016;, Pollard et al., 2010). The biomechanics of the hip joint are influenced by various factors such as joint loading, muscle activation patterns and the integrity of the surrounding soft tissues, which collectively contribute to its functional capacity.

1. Structure and Biomechanical Function of the Hip Joint

Biomechanical studies in the field show that the hip joint is subjected to significant stresses during dynamic activities such as walking, running and jumping. For example, in individuals with conditions such as femoroacetabular impingement syndrome (FAI), loading during gait may change, leading to increased hip flexion moments and compensatory mechanisms in the distal joints of the lower extremity (Samaan et al., 2016). These different loading scenarios and compensatory mechanisms can result in joint degeneration and pain. This highlights the importance of understanding hip joint mechanics in both healthy individuals and those with pathologic conditions (Solomonow-Avnon et al., 2014).

The hip joint also has a role in maintaining stability during dynamic movements. The hip extensors, especially the gluteus maximus, are crucial for controlling deceleration and reducing reaction forces (Pollard et al., 2010). Research suggests that limited hip and knee flexion during descent can lead to increased frontal plane motion in the knee, which can predispose individuals to injury, especially in a sporting context (Pollard et al., 2010). However, the ability of the hip joint to absorb and dissipate forces is significantly influenced by the integrity of the surrounding soft tissues, including the joint capsule and ligaments, which provide passive stability (Karunaseelan et al.) Furthermore, the response of the hip joint to different physical activities is influenced by factors such as body weight and muscle coordination. Obesity has been shown to alter lower limb biomechanics and potentially contribute to the development of osteoarthritis by increasing loads on the hip joint (Runhaar et al., 2011). In addition, poor motor coordination may lead to altered biomechanics during activities such as jumping, increasing the risk of injury.

The effects of hip joint biomechanics play an important role in injury prevention as well as rehabilitation and recovery. Understanding the mechanical loading acting on the hip joint during various activities can help to improve rehabilitation protocols and guide clinicians in developing effective treatment strategies for patients after hip joint problems or surgery. Exercises that promote optimal loading patterns and joint stability can improve recovery outcomes and reduce the risk of re-injury.

In conclusion, the anatomy and biomechanics of the hip joint are complex and multifaceted, influenced by various factors such as joint loading, muscle activation and the integrity of surrounding structures. A comprehensive understanding of these elements is crucial for both clinicians and researchers, as it informs both the prevention and management of hip joint pathologies.

2. Use of Finite Element Method in Biomechanical Studies

Finite Element Analysis (FEA) is a powerful computational technique widely used in engineering and applied sciences to solve complex structural, thermal and fluid problems. The method involves breaking down a structure that is difficult to analyze into a finite number of smaller, simpler parts known as finite elements. This approach allows simplification of complex geometries and boundary conditions, making it particularly useful in fields as diverse as structural engineering, materials science and biomechanics.

The Finite Element Method (FEM) has emerged as a crucial tool in biomechanics, especially in the analysis and simulation of complex biological structures and their mechanical behavior. This computational technique allows researchers to create detailed models of anatomical structures, enabling the evaluation of stress, strain and overall biomechanical performance under various loading conditions. The utility of FEM in biomechanics is highlighted by its ability to simulate internal stress and strain, which are often difficult to measure experimentally. Cai et al. demonstrated the effectiveness of FEM in the anterior atlantoaxial joint and emphasized its capacity to accurately reflect cervical motion (Cai et al., 2013). Similarly, Hu and Wang demonstrated the method's role in providing biomechanical assessments for various orthopedic conditions, including tibia fractures, by reflecting interactions within bone structures (Hu & Wang, 2017). This capability is crucial for developing treatment strategies and predicting outcomes in clinical settings.

The FEM serves as a cornerstone in biomechanical research by enabling detailed simulations of complex biological systems. Its ability to model internal stresses and predict mechanical behavior under various conditions has made it an

important tool in the fields of orthopedics research. As computational techniques continue to advance, the potential applications of FEM in biomechanics are likely to expand and offer deeper insights into mechanical interactions within biological systems.

3. Obtaining the Finite Element Model of the Hip Joint

3.1 Creating Model Geometry

Obtaining a finite element model (FEM) of the hip joint requires a thorough understanding of the model geometry and the simplifications that can be made in the modeling process. An important issue here is the acquisition of accurate geometric data through advanced 3D imaging techniques. Several studies emphasize the importance of subject-specific geometries obtained from imaging modalities such as computed tomography (CT) and magnetic resonance imaging (MRI) in the creation of realistic FEMs. The feasibility of using μCT imaging to create a finite element model of a cadaveric hip joint has been demonstrated, highlighting the importance of subject-specific geometry and biphasic cartilage properties in simulating cartilage contact mechanics (Li et al., 2016). The study underlines the need to accurately represent the collagen fiber organization within the cartilage to improve the fidelity of the model. Furthermore, the inclusion of subject-specific geometries allows for a more precise characterization of the mechanical effects in the hip joint, as demonstrated in the study by Ng et al. who investigated the effects of cam-type FAI using finite element analysis (Ng et al., 2012). Their findings reinforce the importance of detailed geometric representation in understanding joint mechanics during various activities. In addition to CT, MRI has emerged as a powerful tool for 3D imaging of the hip joint. The capacity to provide the detailed anatomical information required for 3D quantitative assessment of the femoral head-neck junction using MRI has been demonstrated in the literature (Xia et al., 2015). The use of high-resolution MRI not only facilitates accurate bone segmentation, but also aids in the assessment of cartilage morphology and pathology (Xia et al., 2014). These advances in imaging technology enable researchers to render the complex geometry of the hip joint, which is crucial for developing accurate finite element models.

The development of a finite element model of the hip joint relies heavily on the acquisition of precise geometric data through advanced 3D imaging techniques. The use of individualized geometries derived from CT and MRI improves the accuracy of the models, allowing for better simulation of hip joint mechanics and pathology (Figure 1). Continued advances in imaging technology

and computational methods will continue to play an important role in improving these models for clinical and research applications.

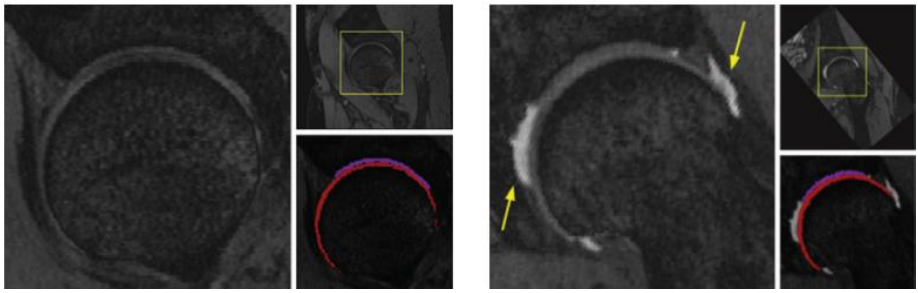

Figure 1: Acquisition of data from MRI or CT images (Xia et al., 2014)

3.2 Determination of Hip Joint Material Properties

Obtaining a FEM of the hip joint requires a comprehensive determination of the material properties of its components, including the femur, acetabulum and various soft tissues. Once these material properties are determined, they are assigned to each part of the 3D modeled hip joint. Thus, each part behaves like the tissue it represents during the simulation. The hip joint is subjected to complex loading conditions during activities such as walking, running and other dynamic movements, making it necessary to accurately characterize the mechanical behavior of its materials.

The mechanical properties of hip joint components can be obtained from various studies using finite element analysis. Xiong et al. highlight the importance of personalized modelling methods to capture changes in hip joint contact stress throughout a gait cycle, noting that conventional models often oversimplify the complex dynamics involved in real-life scenarios (Xiong et al., 2022). This is supported by Park and Nam, who developed a FEM specifically for older women to analyze fracture risk during falls, noting that age and gender significantly influence material properties and stress distributions in the hip joint (Park & Nam, 2020). The surrounding soft tissues also play a vital role in the overall mechanics of the hip joint. Watson et al. emphasize the importance of accurately modeling boundary conditions, including the contributions of ligaments and muscles, to simulate a realistic replica of the mechanical environment of the hip joint (Watson et al., 2017). This is crucial for studying mechanical stress changes in patients with developmental dysplasia of the hip and determining the effects of surgical interventions developed for this disease (Ike et al., 2015).

3.3 Defining Boundary Conditions and Loading Scenarios

The next step after obtaining the FEM of the hip joint is to determine the boundary conditions and loading scenarios that accurately reflect the physiological and pathological states of the joint (Figure 2). The hip joint is subjected to complex loading conditions that are influenced by various factors such as body weight, muscle forces and joint geometry. A thorough understanding of these factors is essential to create a robust FEM that can simulate real-life conditions.

Boundary conditions in FEM are critical as they define how the model interacts with its environment. In their study, Wang et al. found that the distal femur is completely fixed, while the acetabulum and articular cartilage are partially constrained and allow vertical motion in the coronal plane. This simulation led to contact forces approximately three times the body weight, demonstrating the significant loads to which the hip joint is subjected during activities such as walking or running (Wang et al., 2016). Similarly, Xiong et al. emphasized the importance of considering the entire gait cycle in modeling hip joint mechanics and noted that simplified models often ignore the dynamic loading conditions present at various stages of movement (Xiong et al., 2022). This suggests that a more comprehensive approach to boundary conditions that incorporates changing constraints throughout the gait cycle would improve the accuracy of the FEM. Loading scenarios should also reflect the physiological conditions to which the hip joint is exposed. A study shows that during normal walking, the hip joint can withstand contact forces as high as 4-5 times body weight, which is critical for understanding the loading environment of the joint (Correa et al., 2010). Furthermore, Akrami et al. noted that many existing studies have focused on prosthetic analysis rather than the joint itself, and there is a gap in the understanding of natural hip joint mechanics under various loading conditions (Akrami et al., 2018). Incorporating these loading scenarios into FEM is essential to predict joint behavior under both normal and pathological conditions.

In addition to static loading conditions, dynamic factors such as muscle forces and joint stability should also be integrated into the FEM. The contribution of muscles to hip joint contact forces during walking has been extensively studied and muscle activation patterns have been shown to significantly influence joint loading (Correa et al., 2010). Furthermore, the role of soft tissues such as ligaments and the joint capsule in stabilizing the hip joint is also very important. Fetto demonstrated the biomechanical advantages provided by soft tissue stabilizers, which play a crucial role in maintaining joint structure during dynamic

activities (Fetto, 2019). Therefore, a comprehensive FEM should take into account both the bony structures and the surrounding soft tissues to accurately simulate the response of the hip joint to various loading scenarios.

Obtaining a finite element model of the hip joint requires a thorough understanding of boundary conditions and loading scenarios that reflect the complexity of human biomechanics. By integrating the dynamic nature of loading during gait and information from various studies, researchers can create more accurate and clinically relevant FEMs. This approach not only improves the predictive capabilities of the model, but also helps in designing interventions and treatments for hip joint pathologies.

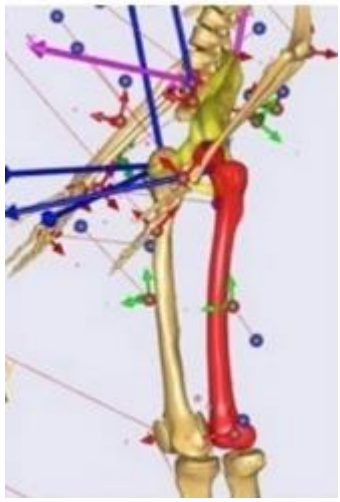

Figure 2: Defining Boundary Conditions (Xiong et al., 2022)

3.4 Mesh Creation

The next step after creating the model and boundary conditions of the hip joint is the mesh generation step (Figure 3). In this step, the bones and soft tissues included in the 3D model are broken down into much smaller pieces that can be analyzed. The hip joint, characterized by its complex geometry and load-bearing functions, is an ideal candidate for FEA due to its structure, which can be accurately obtained from imaging methods such as CT and MRI scans. This feature allows researchers to simulate various mechanical responses under different loading conditions, thus facilitating the study of both normal and pathological conditions of the hip joint (Incze-Bartha et al., 2023).

Mesh generation is a critical step in the finite element modeling process because the quality of the mesh directly affects the accuracy of the simulation

results. Special techniques used in mesh generation enable the creation of complex geometries by optimizing the node distribution necessary to accurately capture the mechanical behavior of the hip joint (Wang et al., 2017). Advances in mesh generation techniques are vital to ensure that FEM accurately reflects the mechanical properties and stress distributions within the hip joint.

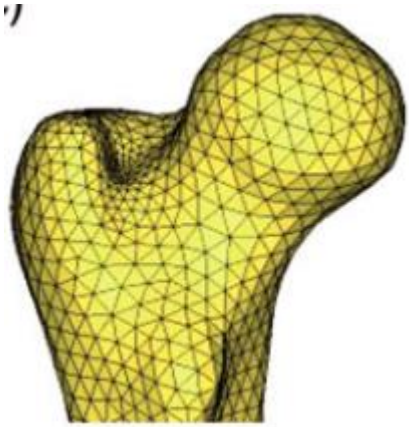

Figure 3: Mesh Creation (Wang et al., 2017)

4. FEM Applications in Hip Joint Pathologies

4.1 Osteoarthritis

The application of the FEM in the study of hip joint pathologies, especially osteoarthritis (OA), has become increasingly important in understanding the mechanical behavior of the hip joint under various conditions. FEM allows the simulation of complex interactions within the hip joint and can provide detailed information on stress distribution, contact pressures and the effects of different pathologies on joint mechanics.

Osteoarthritis is a degenerative joint disease characterized by loss of cartilage tissue and associated joint surface changes. Studies have shown that mechanical loading plays an important role in the onset and progression of OA. Wesseling et al. emphasized that individual-specific geometry significantly affects acetabular contact pressure during gait, which is a critical factor in the development of OA (Wesseling et al., 2019). This is supported by Ng et al. who showed that cam-type FAI leads to high hip joint stresses that are closely linked to cartilage degeneration (Ng et al., 2016). The mechanical loading conditions to which the hip joint is exposed during activities such as walking may exacerbate these stresses and contribute to the progression of OA.

In a study comparing healthy and cam-type FAI hip joints using FEM, the relationship between mechanical loading and cartilage degeneration was demonstrated (Lostado-Lorza et al., 2021). Similarly, Vafaeian et al. emphasized the importance of understanding mechanical behavior in the context of joint pathologies in dysplastic hips (Vafaeian et al., 2017). Collectively, these studies underline the utility of FEM in elucidating the biomechanical factors that contribute to the development and progression of hip OA. The application of FEM in the study of hip joint pathologies, especially osteoarthritis, can provide important insights into the mechanical behavior of the hip joint under various conditions. By simulating the effects of different pathologies and surgical interventions, FEM is a powerful tool to improve our understanding of hip joint mechanics and develop treatment strategies for patients with hip disorders.

4.2 Hip Dysplasia

Hip dysplasia, characterized by abnormal formation of the hip joint, often results in increased mechanical stress on the acetabulum and femoral head. Studies using FEM have shown that patients with acetabular dysplasia have high joint stress that can lead to joint degeneration over time. Sakuma et al. reported a decrease in mechanical stress following rotational acetabular osteotomy, demonstrating that surgical intervention can significantly alter the biomechanical environment of the hip joint (Sakuma et al., 2022). Similarly, Vafaeian et al. investigated the mechanical behavior of dysplastic hips, emphasizing the importance of understanding these dynamics to prevent further complications (Vafaeian et al., 2017).

The effect of joint geometry on stress distribution has been extensively studied. Anderson et al. reported that FEM can be used to predict cartilage contact stresses, which is crucial for understanding the loading conditions to which dysplastic hips are subjected (Anderson et al., 2010). This is important as changes in joint morphology can exacerbate stress concentrations, leading to accelerated cartilage degeneration and subsequent osteoarthritis (Henak et al., 2014).

The role of surgical interventions such as periacetabular osteotomy has also been the focus of FEM studies. Kitamura et al. demonstrated that acetabular correction in the coronal plane can effectively reduce joint contact pressure in dysplastic hips, thus optimizing the postoperative mechanical environment (Kitamura et al., 2022). This demonstrates the importance of personalized FEM analyses in predicting mechanical behavior and planning surgical approaches for patients with developmental dysplasia of the hip (Ike et al., 2015).

4.3 Osteoporosis

Osteoporosis is a condition characterized by decreased bone density and increased risk of fractures, especially in the hip region. The relationship between bone metabolism and joint health is critical in the management of the disease. Bisphosphonates, commonly used to manage osteoporosis, have been shown to improve periprosthetic bone mass and potentially increase the stability of hip implants, thus reducing the risk of postoperative fractures (Bottai et al., 2015; Watanabe et al., 2022). The mechanical effects of these treatments can also be assessed through FEM, providing a better understanding of how interventions may affect joint mechanics and fracture risk.

The role of joint morphology and loading types in hip joint pathologies is another area where FEM is proving invaluable. Research shows that abnormal loading conditions can lead to increased contact pressures and subsequent cartilage degeneration, a precursor to osteoporotic fractures. Studies with FEM can accurately simulate the mechanical environment of the hip joint using person-specific models derived from imaging data and reveal how surgical interventions such as osteotomies can alter stress distributions and potentially reduce fracture risks (Ike et al., 2015).

In summary, FEM is a critical tool in understanding the complex interactions between hip joint pathologies and fracture risks in osteoporotic patients. By integrating clinical data, imaging and biomechanical simulations, researchers can develop more effective strategies to predict and manage hip fractures.

4.4 Femoroacetabular Impingement (FAI)

FAI is a pathology characterized by abnormal contact between the femur and acetabulum, leading to pain and potential joint degeneration. A study using FEM to investigate FAI revealed that cam-type FAI significantly alters the mechanical load on the hip joint and contributes to cartilage degeneration due to abnormal contact pressures during activities such as walking/sitting (Lostado-Lorza et al., 2021). The findings underline the importance of understanding the mechanical effects of FAI, as it will allow the development of treatment strategies aimed at restoring normal joint function and preventing further degeneration. It was also emphasized that the morphology of the lesion and individual-specific loading patterns significantly influence hip joint stresses. These stresses depend not only on morphology but also on other anatomical and biomechanical factors specific to each patient (Ng et al., 2018; Ng et al., 2016). This patient-specific approach is crucial in developing specialized interventions for individuals suffering from FAI.

In addition to mechanical loading, the role of soft tissue structures such as the acetabular labrum is critical in maintaining hip joint stability and function. The role of the labrum in joint mechanics is supported by Locks et al. (2017), who noted that the labrum enhances joint stability and load distribution, which is particularly important in FAI types where labral pathology is common (Locks et al., 2017). Furthermore, the effects of FAI go beyond mechanical loading to include functional consequences. Samaan et al. found that FAI patients exhibit altered joint moment distributions during sitting and standing tasks compared to healthy controls, which may affect their functional performance (Samaan et al., 2016).

4.5 Prosthesis Planning Using FEM

The application of the FEM in the analysis and treatment of hip joint pathologies, especially in surgical and prosthetic planning, has received considerable attention in recent years. FEM has been used to evaluate the mechanical effects of surgical procedures such as periacetabular osteotomy and total hip arthroplasty. Research shows that FEM can effectively predict the mechanical behavior of postoperative hip joints, providing information on stress distributions and potential complications such as prosthesis wear (Ma et al., 2023; Ceddia, 2023). In one study, FEM was used to evaluate wear patterns during gait cycles, highlighting the utility of the method in optimizing prosthesis design (Hidayat, 2023). In addition to surgical planning, FEM has also been applied in the design and evaluation of hip prostheses. The ability to simulate the mechanical environment of the hip joint allows the evaluation of different prosthetic designs under physiologic loading conditions, increasing the reliability and longevity of implants (Kamel, 2018). This is important as the demographics of patients undergoing THA are shifting towards younger individuals and the need for more durable and effective prosthetic solutions (Ceddia, 2023).

5. Limitations of Existing FEM Models for the Hip Joint

Although the FEM has become a very important tool for analyzing the biomechanics of the hip joint, several challenges remain regarding the accuracy and applicability of current FEM models. A major limitation is the reliance on idealized joint geometries, which often fail to capture the complex anatomical variations present in patients. Another challenge is the variability in loading conditions that FEM models must account for. Current models often use static loading conditions that do not accurately reflect the dynamic nature of human movement. The mechanical behavior of the hip joint under changing conditions, such as different stages of the gait cycle, can significantly affect stress

distribution and joint health. Studies have shown that dynamic loading conditions can lead to different stress responses in the hip joint that cannot be revealed by static models (Watson et al., 2017). This limitation is important in the context of joint diseases where understanding the impact of loading on cartilage degeneration is critical (Wesseling et al., 2019).

The accuracy of FEM results is highly dependent on the quality of the input data, especially the segmentation of medical imaging datasets. Incorrect segmentation can lead to inaccurate representations of joint geometry, which in turn affects the predicted mechanical responses (Vafaeian et al., 2017). The difficulty of accurately modeling cartilage layers, which are often difficult to identify from imaging data, further complicates the reliability of FEM simulations (Li et al., 2018).

In conclusion, while FEM has proven to be a valuable tool in understanding hip joint mechanics, significant challenges remain. These include the reliance on geometries, the exclusion of soft tissue structures, the need for dynamic loading conditions, and the accuracy of input data. Solutions to these limitations are critical to increasing the applicability of FEM in clinical settings and improving outcomes for patients with hip joint disorders.

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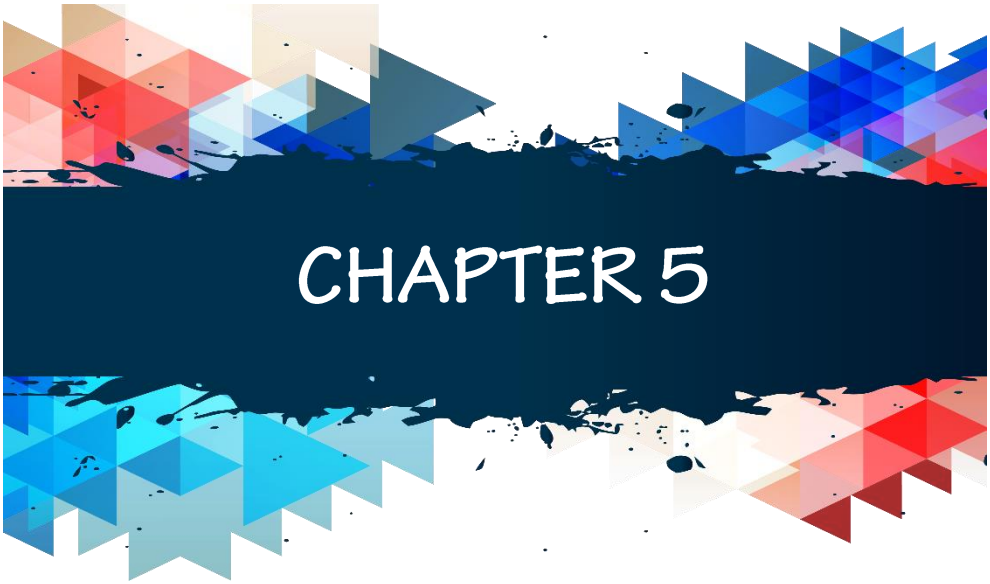

Mistreatment and Violence in Obstetric Care

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Pregnancy and the birth of a child is one of the biggest changes in a woman's life. These important events can cause a woman to be stressed and emotionally vulnerable. Both physical and mental changes can result in the development of complications during pregnancy and childbirth (Kowalska et al., 2022). Therefore, to ensure safe motherhood, all pregnant women should be provided with compassionate and respectful care that is appropriate to their physical and psychological needs in the prenatal, intrapartum and postnatal periods. With this care, women will have a positive and healthy birth experience without being harmed or mistreated in all maternal processes (Oluwaseyi, & Sowunmi, 2024).

"Maltreatment" is the abuse, harm or control of another person. "Obstetric maltreatment" is the disrespectful, harassing-abusive approach to women receiving health care services during pregnancy, childbirth and postnatal period, unnecessary interventions or harmful practices to women (Chervenak et al., 2024).

"Obstetric violence" is the appropriation of women's bodies and reproductive processes by health professionals, violating their privacy, abandonment, providing undignified care, dehumanizing and/or abusive treatment, medicalization and abuse of natural processes, which leads to loss of autonomy and capacity to freely make decisions about their own bodies and sexuality and negatively affects women's quality of life. Maternity care affects women's autonomy, integrity, right to participate in decision-making, and right to self-governance over their bodies and sexuality. (Annborn & Finnbogadóttir, 2022).

Chervenak et al. (2024) reported that "Obstetric maltreatment" is a more comprehensive term than "Obstetric violence" and stated that violence refers to the intentional use of physical force to injure, damage or cause harm to another person, while maltreatment means controlling, harming and abusing another person against their will. They also emphasized that maltreatment can result from systemic problems, lack of training or misunderstandings. They stated that this situation could be interpreted as a deliberate act of violence by health service providers (Chervenak et al., 2024).

Based on the assumption that it is a limited term, the term "obstetric mistreatment" can be used instead of "obstetric violence".

Obstetric Violence in History

The prenatal period is a critical time for both the fetus and the pregnant woman, as many complications can arise during this period and lead to negative health outcomes (Crandall, K. (2021; O'Brien & Rich, 2022). Despite positive

developments in perinatal care, inequalities still persist in reproductive health services. Racial, geographical and social status-based inequalities persist in countries around the world. Today, inequalities in reproductive health care utilization are linked to a history of obstetric violence.

Obstetric violence, together with the oppression and exclusion experienced, shows gender discrimination. It can be said that this situation is not only an individual problem but also a social problem and that there are deep inequalities. The roots of obstetric violence stem from the deep traces of gender inequality in the historical process. When we turn to the past from the present, it can be observed that the control over women's bodies is determined by a male-dominated order.

In the 16th century, women were forced to choose between conversion and family separation in the postpartum period and experienced obstetric violence resulting from religious discrimination. In Papal Rome at the time, Roman Catholic authorities isolated the babies of Jewish women who refused to convert to Christianity through baptism. In the late 18th century, in the governorates of New Spain and Peru, obstetric violence also occurred during the Spanish Empire's colonization of the Americas. During colonization, priests performed caesarean sections on women who were unable to give birth against their will. During this period, the authorities made caesarean sections compulsory and decided that saving the souls of fetuses was a priority over the lives of mothers.

Between the 16th and 19th centuries, reproductive health was influenced by the system of slavery. Obstetric violence was also at the heart of slavery in the United States (US), where black women's reproductive labor (including daily activities such as cooking and laundry, as well as childbearing) was exploited for economic profit. Enslaved women's childbearing was used by their owners to enrich themselves. When enslaved women failed to conceive or bear healthy children, they faced severe penalties. Slave women were also forced to perform hard physical labor during their pregnancies. They were denied care in the postpartum period and deprived of the healing process. During the development of obstetrics and gynecology, enslaved women faced violence resulting from medical interventions. In the pre-war period, physicians in the US developed new medical procedures by continuously experimenting on enslaved women and people marginalized by race, social class and citizenship status. In this process, marginalized individuals were not provided with the same care as other patients.

In the late 19th and 20th centuries, with the rapid medicalization of childbirth, male-dominated obstetricians sought to replace traditional obstetricians and

establish authority over childbirth. This led to the broader phenomenon of gender-based violence. Women were subordinate to their physicians and subjected to unnecessarily aggressive interventions. Women were denied the right to make decisions about their own body and health. In the 19th century, doctors in many regions claimed that middle-class and upper-class white women experienced more pain in childbirth and applied pain relief methods to them. In their study, O'Brien and Rich (2022) reported that to this day, healthcare professionals in the United States provide fewer pain relief treatments to black and Latina women in labor than to white women, and that racial disparities in labor pain management persist.

Historically, forced sterilization has been one of the forms of obstetric violence. In the early 20th century, the so-called "eugenics" movement began to improve the human race. In this context, forced sterilization was carried out on women who were considered hereditarily "unfit" (disabled, poor or racially marginalized). Sterilization abuse was found to continue later in the 20th century, despite the official repeal of eugenics laws. In the 1970s, also in the US, thousands of Native American women were abused and forcibly sterilized in Indian Health Service hospitals. Indian Health Service hospitals are known to have sterilized about a quarter of Indian women of childbearing age in the 1970s. A 1974 court case revealed the forced sterilization of low-income African-American female patients. In 1978, 10 Mexican immigrant women filed a class action lawsuit against Los Angeles County Hospital for forcibly sterilizing Latina patients. In these lawsuits, physicians failed to inform patients about the procedure or provided misleading information. They also forced women to sign consent forms for sterilization during childbirth or after cesarean section while under the influence of heavy sedative drugs. Poor women were told that if they refused sterilization, they would lose social support services or the custody of their children (Sadler et al., 2016; O'Brien & Rich, 2022).

Between 1996 and 2001, 250,000 - 300,000 women were sterilized in Peru. The president of the time stated that these racist practices were a solution to the country's "Indian problem". When historical processes are analyzed, it is seen that obstetric violence is reinforced by factors such as gender, race, poverty, disability and nationality.

In the mid-20th century in Argentina, Brazil and Chile, government regimes and military authorities inflicted sexual violence on detained women. Babies of detained women were confiscated. Thus, obstetric violence was practiced through the abuse of childbearing women and the severing of kinship ties.

Obstetric violence has been observed to continue in the 21st century. Forced separation of mothers and babies after birth in the USA, forced sterilization of women in prison between 2006 and 2013, shackling of women in prison during pregnancy and childbirth, Black and Latina women in prison being subjected to obstetric violence disproportionately more than white women in prison are examples of obstetric violence encountered in the 21st century (O'Brien & Rich, 2022; van Der Waal et al., 2023).

Obstetric violence is a global phenomenon and women in many countries are subjected to mistreatment during maternal care. Today, it has been determined that women are subjected to disrespectful, dehumanizing and non-consensual interventions and abuse during maternal care (O'Brien & Rich, 2022).

The Emergence of Obstetric Violence Awareness

The term obstetric violence was first used and defined in Venezuela. In the definition of obstetric violence, practices that may negatively affect women's quality of life, such as health professionals taking control of women's bodies and reproductive processes, using approaches that are unworthy of human dignity, and depriving women of their autonomy and the right to make free decisions about their sexuality, were emphasized (Sadler et al., 2016; Annborn & Finnbogadóttir, 2022).

Obstetric violence is perpetrated by obstetric health professionals during the care provided to women during pregnancy, childbirth and the postpartum period. Obstetric violence includes, but is not limited to, neglect and abuse, shaming, discrimination, performing procedures without consent, making decisions for the woman, manipulation.

Behaviors towards these forms of violence (Chadwick, 2021; Annborn & Finnbogadóttir, 2022; Van Der et al., 2023; Rusu, Nogueira & Topa, 2024; Oluwaseyi & Sowunmi, 2024):

- Performing procedures without consent; vaginal examination, episiotomy and episiotomy repair (without anesthesia), caesarean section, or fundal pressure, hitting, pinching, punching, shroud shaking to women who refuse these procedures
- Forced or non-consensual tubalization and insertion of an intrauterine device immediately after delivery

- Women being subjected to violence because of their gender and race (most frequently Black women and women living in indigenous communities)
- Timely and ineffective intervention in obstetric emergencies
- Forcing a woman to give birth in the supine position even though there is an environment in which she can give birth in other birth positions
- Preventing early attachment of the newborn to its mother without a medical reason
- Use of labor acceleration techniques even when low-risk and natural birth is possible
- Providing care without taking care of the confidentiality and privacy of the woman's personal information
- Degrading treatment of women
- Lack of equitable care in situations that are out of women's hands, such as low socio-economic status or HIV-positive cases
- Leaving women alone during childbirth, not following up and abandoning them
- Forced detention of women in health facilities without payment of fees for using health services

These interventions may constitute human rights violations (Annborn & Finnbogadóttir, 2022). But human rights are universal and inalienable, and protecting them contributes to positive health outcomes (Gogoi, A., Ravi, T. (2023). International human rights organizations have important roles in monitoring violations of sexual and reproductive health and rights, setting standards and establishing laws. Today, it can be said that the approaches of these organizations to obstetric violence are still at an early stage of development. They focus on international human rights standards on ill-treatment during childbirth, including forced or coerced sterilization, denial or neglect of access to emergency obstetric care. However, many forms of ill-treatment remain unaddressed or undervalued under international human rights law (Khosla et al., 2016; Rusu, Nogueira & Topa, 2024).

Respectful and Sensitive Care in the Prenatal Period

Prenatal care affects women's autonomy, their right to participate in decision-making and their self-governance over their bodies and sexuality. The World Health Organization (WHO) has exposed obstetric violence through an organization called Disrespect and Abuse. The organization Disrespect and Abuse advocates for women's rights during childbirth. It emphasizes that care should be provided without discrimination, harm, physical, mental or verbal abuse or harassment, especially during labor and delivery. WHO reports that women are exposed to obstetric violence during the birth process and that this problem should therefore be addressed as a priority, comprehensively assessed, prevented or eliminated altogether. In order to achieve this, it emphasizes the need for increased efforts by all nations (Annborn & Finnbogadóttir, 2022).

The Centers for Disease Control and Prevention (CDC) (2023) reported that Black, Hispanic and multiracial mothers are most likely to experience mistreatment at birth. It also reported that women with no insurance or public insurance were more likely to be mistreated during childbirth than those with private insurance. Approximately 29% of women experienced discrimination during childbirth. These data suggest that women from certain racial and ethnic minority groups may be more likely to have adverse health experiences during pregnancy and childbirth that affect quality of care and health outcomes (Davis, 2019; CDC, 2023).

During maternal care, health professionals may in some cases exercise power of control and absolute obedience over women. Such use of power can lead to the subordination of women's identity and sexist attitudes. Thus, this authority over women is legitimized and women's self-determination is prevented and their right to dignity can be taken away (Rusu, Nogueira & Topa, 2024).

On the issue of over-medicalization of childbirth, WHO recommends that health facility managers and health personnel review protocols and emphasizes that the necessity of certain practices should continue to be questioned and investigated. It also advocates respecting women's autonomy when undertaking interventions (Sadler et al., 2016; Espinosa et al., 2022; Rusu, Nogueira & Topa, 2024).

To promote safe motherhood during pregnancy and childbirth, all pregnant women should receive respectful and sensitive care during labor and at the time of birth. Respectful care at birth can provide quality midwifery care and reduce maternal mortality. WHO recommends respectful maternity care at birth. Respectful care includes protecting the dignity, privacy and confidentiality of the woman at birth, not harming or mistreating her, ensuring her informed

participation in decisions during the birth process and at the time of birth, and providing continuous midwifery care support (WHO, 2018).

As Van Der Waal et al. report in their study, in 2019, the "What Women Want Campaign" was organized and reached a total of 1.2 million women in 114 countries. In this campaign, women were asked what their only demand was for quality reproductive health services. Women responded "Respect and dignity during care". This answer shows that in terms of reproductive health globally, women most want obstetric violence to be eliminated (Van Der Waal et al., 2023).

Conclusion

Obstetric violence is a multifaceted complex condition that requires a multidisciplinary approach. It is vital to take international and national joint studies and decisions to prevent obstetric violence. It is important that relevant organizations in all countries of the world address the legal dimension of obstetric violence and develop legislation. Access to maternal care services should be ensured and legal barriers should be eliminated. Initiatives that address obstetric violence and succeed in making lasting change should be identified and a roadmap should be created. It is important to ensure the participation of women's working groups and civil society organizations in the design, planning, implementation and evaluation of obstetric care services.

Health authorities in countries should provide all women with unbiased information about evidence-based interventions used in obstetrics. It is important for both women and health professionals to report exposure to obstetric maltreatment and to develop a system where this can be done. It is important to include obstetric violence, human rights in obstetric care, reproductive rights in the curricula of all educational institutions that train health professionals and to emphasize gender-based issues (Rusu, Nogueira & Topa, 2024).

In summary, countries and policy makers should work to prevent potential mistreatment of women receiving obstetric care in general. Healthcare providers should promote professional care that is impartial, respectful and women-centered. An ethical framework for all obstetric care providers should be developed and systemic change efforts should be initiated to prevent mistreatment or obstetric violence (Chervenak et al., 2024).

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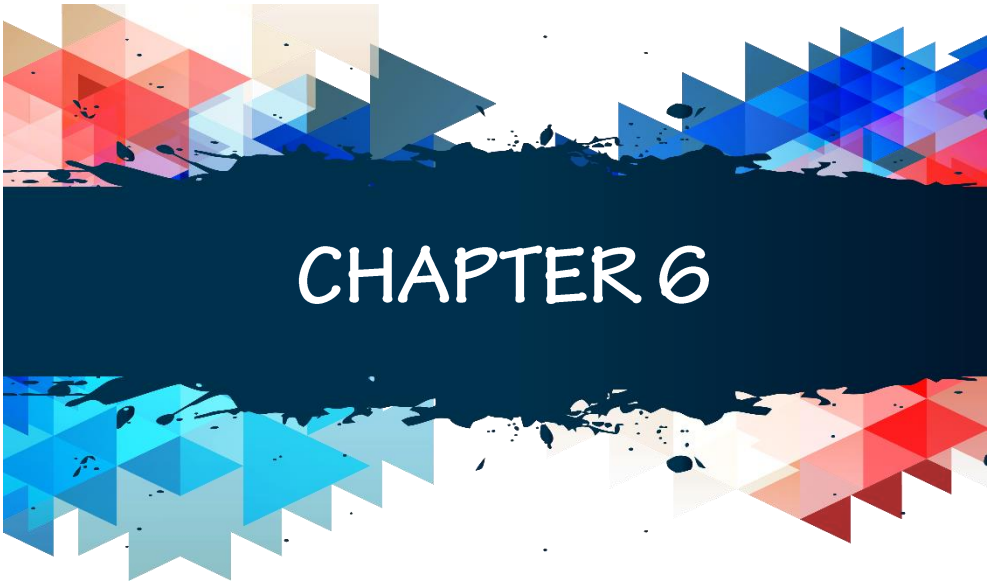

Use of Cryotherapy on Endodontics

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1. Introduction and Background

The word cryotherapy, formed by combining the words cryo and therapy, describes a treatment that aims to reduce pain by lowering the temperature of the target tissue 1. Lowering the temperature by drawing heat from the high-temperature tissue to the low-temperature object causes vasoconstriction in the tissue, causing tissue metabolism, microvascular permeability and nerve conduction to slow down 2. The magnitude of the temperature change and biophysical changes in tissues depends on the difference between the temperature of the object and the application of cold or heat, the duration of exposure, the thermal conductivity of the tissues, and the type of agent used to apply the heat or cold 1, 2.

Cryotherapy is frequently used in medicine in areas such as sports injuries, relieving musculoskeletal pain, treating muscle spasms, and slowing/stopping the growth of premalignant lesions 3, 4.

It has been stated that applying cryotherapy after various surgeries involving hernia, gynecology operations and orthopedics shortens the healing period by reducing edema and inflammation 5,6. It is also thought that cryotherapy can reduce the need for medication because it reduces post-operative pain 5.

2. Mechanism of action of cryotherapy

Cryotherapy has three main physiological tissue effects: vascular, neurological and tissue metabolism 6, 7. When cold is applied to the tissue for more than 15 minutes, first vasoconstriction occurs in the vessels, followed by vasodilation and vasoconstriction again. Finally, the resulting vasoconstriction reduces vascular permeability, thus reducing exudate and serum outflow from the tissue, thus reducing postoperative edema 7-9. The analgesic effect of cold application is an analgesic effect the release of chemical mediators of pain and the slower spread of neural signals 10, 11. Cold application may stimulate the release of neuroactive agents such as endorphins, which may initiate the analgesic effect. Endorphins bind to opioid receptors in the medullary dorsal horn, thus inhibiting the nociceptive transmission of impulses to the central nervous system. In addition, cold application may lower the activation threshold of special nerve endings (tissue nociceptors) that are activated after tissue injury, which may produce the local anesthetic effect described as cold-induced neurapraxia 12, 13. Cryotherapy reduces tissue blood flow and cell metabolism by more than 50%. Thus, it slows down the rate of biochemical reactions by reducing the production of free radicals in the tissues and reduces the rate of oxygen consumption. Thus, it prevents tissue hypoxia and further tissue injury 9, 13.

The local physiological effect mechanisms of cryotherapy have also been investigated. Cold application basically removes heat from the tissues and causes the temperature to drop. When the temperature drops, vasoconstriction occurs, and the formation of edema is restricted. Vasoconstriction also slows down the cell metabolism, reduces the oxygen demand of the cells and limits the production of free radicals in the tissues. It has been reported that the number of inflammatory enzymes increases with increasing temperature, applying cold locally to the skin has been shown to change the pain threshold and reduce pain 14. Activation of pain receptors called thermoreceptors, which have temperature-sensitive nerve endings that are activated by changes in tissue temperature, by cryotherapy may block nociception in the spinal cord 12, 13.

Despite all this, the time required for cooling, how long the tissue remains cold after the cooling agent is applied, and how far beyond the area where the cooling agent is applied it provides cooling are still not adequately explained 15.

3. Use of Cryotherapy in Dentistry

In dentistry, cryotherapy has been reported to be effective in the treatment of intraoral excisional surgical procedures, tooth extraction, implant applications, and temporomandibular joint disorders associated with arthritis 16. The decrease in skin temperature during cold application causes an increase in the pain threshold. However, the optimal temperature for the target tissue has not yet been determined to 17. In the latest systematic review published in 2018, which evaluated the results of studies investigating the effect of cold application on pain, swelling and trismus after tooth extraction, it was reported that cryotherapy may have a small additional benefit in reducing pain after third molar surgery and is not effective on swelling and trismus 17.

4. Cryotherapy Applications in Endodontics

4.1. Effect of Cryotherapy on Pain in Endodontic Treatment

Intracanal cryotherapy in the root canal is recommended as a simple and cost-effective technique for the management of postoperative pain after endodontic treatment 18-20.

One way to apply cryotherapy to inflamed periradicular tissues is to dilate the root canal system and then irrigate the root canal with a cold agent. This procedure has been shown to be more successful when a negative-pressure irrigation activation system, such as the EndoVac (Kerr Endo, Orange Country, CA, USA) system, is used 21.

Vera et al. shed light on cryotherapy in the endodontic field 22. Vera et al. found that intracanal irrigation with cold saline caused a temperature decrease of more than 10 degrees on the root surface, and this effect lasted for 4 minutes 22. They hypothesized that this decrease in root surface temperature could have a local anti-inflammatory and analgesic effect on the root surface, inspiring further studies on cryotherapy in the periapical tissue and its surroundings 18,19, 23-25. There is no certainty about the most appropriate dosage for cryotherapy, as it varies depending on the nature of the tissue. In most studies, the duration of intracanal cryotherapy was kept as 5 minutes. Since Vera et al. reported that 5 minutes of irrigation of root canals with 2.5 ℃ cold saline solution reduced the external root surface temperature, similar application times were also used by various authors 22-25. In 2016, cryotherapy was first used clinically by Keskin et al. to minimize postoperative pain after single-session root canal treatment in endodontics. Statistically significant lower pain levels were found in the intracanal cryotherapy group compared to the control group 23. Vieyra et al. evaluated the reduction in postoperative pain after single-session root canal treatment using three different irrigation regimens at different temperatures. A total of 240 patients with vital teeth requiring conventional root canal treatment were selected for the study and after root canal preparation, the final irrigation was performed using 17%EDTA and 10 mL cold saline solution at 4 °C, 2.5 °C or room temperature. Pain level was assessed using VAS. According to the data obtained from the study, no statistically significant difference was found in the degree of pain or duration of pain between the 4℃ and 2.5℃ groups. However, the patient group irrigated with EDTA at room temperature showed significantly higher postoperative pain than the other groups 25.

It is known that in cases of irreversible pulpitis, the inflammation is limited to the inside of the pulp and that pulp removal provides relief to the patient. Therefore, the direct effect of cryotherapy on teeth with irreversible pulpitis has not yet been determined. Bazaid et al. compared the effect of cryotherapy in reducing postoperative pain between teeth with and without apical periodontitis and irreversible pulpitis. A random distribution was made among 40 patients with irreversible pulpitis. The patients were randomly divided into two groups according to the temperature of the final irrigation solution used; the control group using room temperature saline and the experimental group using cold saline. Then, the groups were divided into two subgroups according to the pulp diagnosis as irreversible pulpitis with apical periodontitis or irreversible pulpitis without apical periodontitis. As a result of this study, it was reported that the use of intracanal cryotherapy was effective in reducing postoperative pain in patients

with irreversible pulpitis and apical periodontitis, but it did not affect patients with irreversible pulpitis without apical periodontitis 26. Jain et al., also recommended intracanal cryotherapy only to reduce post-operative pain in symptomatic irreversible pulpitis with apical periodontitis 20. In a case report, it was shown that cryotherapy could also be applied to control bleeding in vital pulp treatments, and the tooth remained vital, asymptomatic and functional for 12-18 months 21. However, more clinical studies are needed to determine the long-term prognosis of cryotherapy in vital pulp treatments 27.

4.2. Effect of Cryotherapy on Inferior Alveolar Block

Topçuoğlu et al. reported that intraoral cryotherapy application before the procedure increased the effect of inferior alveolar nerve blockade, especially in teeth with symptomatic irreversible pulpitis 28.

4.3. Effect of Cryotherapy on Fracture Resistance of Teeth Previously Treated with Endodontics

Thermal changes occurring in the tooth structure cause mechanical stress in dental tissues. The magnitude of the stress occurring in the tooth structure varies depending on the tooth geometry, the temperature difference between the tooth and the environment, and the age of the tooth. It is known that mechanical stress, which will cause a temperature change in the tooth structure, causes structural deformation in the form of tensile stress in the enamel and compressive stress in the dentin in the first second of its application to the tooth 29,30. Another study examined the effect of cold application on the fracture resistance of teeth. When the results were examined, it was concluded that cryotherapy application significantly reduced the fracture resistance of teeth 31. Another study examined the effect of cold application on the fracture resistance of teeth. When the results were examined, it was concluded that cryotherapy application significantly reduced the fracture resistance of teeth 31. In this study, body temperature was excluded and as a result, a significant decrease in fracture resistance was shown in teeth treated with cryotherapy. Therefore, extraoral cold application instead of intracanal cooling can be recommended as an alternative technique for postoperative pain control. However, it should be remembered that in vitro studies does not provide real clinical conditions 31.

4.4. Antimicrobial Activity of Cryotherapy

Another basic goal of root canal treatment is to reduce the number of microorganisms in the root canal. For this purpose, the canal is chemomechanically instrumented 32, 33. A study examining the effect of

cryotherapy after NaOCl irrigation on the number of microorganisms in the canal showed that the cryotherapy group had fewer microorganisms than the control group 34.

5. Result

Cryotherapy is a frequently used application in medicine and dentistry from past to present. In cases diagnosed with symptomatic apical periodontitis in dentistry, it has been shown in many publications that the application of rinsing the root canal with cooled saline in the final irrigation reduces postoperative pain. It is reported that it provides bleeding control in vital pulp treatments. It has also been supported by a study that allows deep anesthesia. Cryotherapy provides the advantages of being easy to apply and cheap 35. It is also a non-pharmacological method with a low risk of side effects in postoperative pain control 36. Studies supporting that it reduces the microbial load in root canals show that its use in endodontics is promising. Although there are many studies evaluating the effect of cryotherapy on postoperative pain, there are not enough studies in the literature regarding its other effects. Its effects on these issues should be evaluated with further studies

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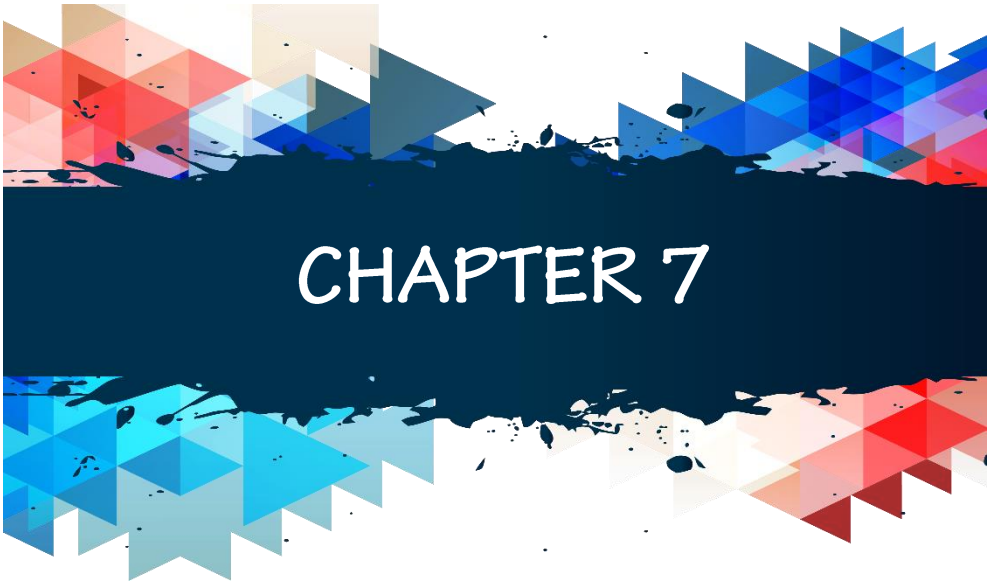

Aging Skeletal System

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In recent years, both population size and average life expectancy have significantly increased in developed countries, and this trend is expected to continue. However, as these extended lifespans are not strongly supported by natural selection, healthy life expectancy has not increased at the same rate (Kontis et al., 2017; Oeppen & Vaupel, 2002). This disparity has led to a higher prevalence of age-related diseases and multiple chronic conditions within the elderly population, creating a substantial disease burden in advanced ages and challenging individuals' capacity to cope with these difficulties. Among these health issues are cancer, cardiovascular diseases, kidney and neurodegenerative diseases, metabolic dysfunction, frailty syndrome, pulmonary fibrosis, osteoarthritis, and osteoporosis (Niccoli & Partridge, 2012).

Osteoporosis, often defined as “porous bone,” is one of the most common aging-related diseases characterized by a reduction in skeletal integrity and bone strength. Bone strength is determined by both bone mineral density and overall bone quality, so declines in these factors can increase fracture risk, exacerbating age-related morbidity (Rubin, 2005). Although various oral and intravenous options are considered safe and effective for the prevention or treatment of osteoporosis, a significant portion of individuals with osteoporosis do not receive treatment. In fact, the proportion of individuals receiving appropriate treatment following a hip fracture has decreased in recent years, a trend attributed to concerns over rare but serious side effects. Reluctance to initiate or continue treatment contributes to the prevalence of osteoporosis and increases fracture incidence among elderly individuals (Lai et al., 2010; Tu et al., 2018).

Bone is a specialized tissue that serves competing functions throughout life. For instance, it must be light enough to permit movement yet strong enough to withstand trauma. In elderly individuals, major traumas most commonly occur due to falls. When the applied force exceeds bone strength, a structural failure known as a fracture occurs. Therefore, optimizing bone strength is critical in reducing fracture risk. Bone strength depends partially on the amount of bone accrued during growth, but it is also a composite of bone mass, matrix and mineral composition, as well as macro- and micro-architectural features, including trabecular connectivity and cortical porosity (Rubin, 2005).

The primary determinants of bone strength throughout an individual’s lifespan include genetic predispositions, levels of sex steroids such as estrogen, nutrition, physical activity, and vitamin D. Conversely, harmful habits, various diseases, and pharmacological agents like glucocorticoids can damage bone integrity. An example underscoring the importance of estrogen in skeletal health is the significant decline in estrogen levels during menopause, which is associated with

a marked increase in bone resorption. Additionally, the aging process itself accelerates bone loss in both male and female populations. The pathophysiological mechanisms underlying skeletal aging are as complex as those determining bone strength and fragility. Another growing concern among the elderly population is polypharmacy, which heightens the risk of hip fractures. Historically, treatment approaches for osteoporosis and other age-related diseases have often followed a "one drug per disease" strategy. However, this approach has increased the risk of adverse drug interactions in older adults. Recently, a new treatment perspective has gained importance, addressing these conditions collectively within the therapeutic process (Lai et al., 2010). This text summarizes current insights into osteoporosis incidence, assessment and intervention strategies, and mechanisms related to the aging process of bone tissue.

EPIDEMİOLOGY OF OSTEOPOROSIS AND FRACTURES

Osteoporosis is a consequence of the aging process that directly affects bone strength and quality (Khandelwal & Lane, 2023). The prevalence of osteoporosis in Turkey is notably significant among adults aged 50 and older. Recent studies indicate that approximately 25% of individuals over the age of 50 have been diagnosed with osteoporosis. The rate of osteoporosis is particularly higher among women. According to data from the Turkish Osteoporosis Society in 2017, the prevalence of osteoporosis in individuals aged 50 and older is approximately 33% in women and about 7% in men. The prevalence of osteopenia is also substantial, recorded at 52% in women and 35% in men. In terms of fracture risks associated with osteoporosis, hip and spinal fractures are particularly prominent, with an observed increase in these risks as individuals age (Aydın, 2019; Tuzun et al., 2012)

ASSESSMENT OF SKELETAL FRAGILITY

The most effective method for assessing skeletal fragility is to examine the incidence of fragility fractures. Fragility fractures occur as a result of low-energy trauma, such as standing or falls from low heights. Fractures in the hip, spine, and distal forearm are particularly important predictors of subsequent fracture risk. Notably, vertebral fractures identified incidentally through radiological examinations are also regarded as a hidden indicator of bone fragility.

Bone loss and fracture risk increase due to various factors, including age, lifestyle factors such as smoking and alcohol consumption, endocrine disorders such as hyperparathyroidism and hypercortisolism, genetic diseases like cystic fibrosis, and the use of medications such as glucocorticoids or anticonvulsants (Bouxsein, 2005; Cosman et al., 2014).

Various clinical tools have been developed to identify individuals at high risk of fractures. The Fracture Risk Assessment Tool (FRAX) and the Garvan Institute's fracture risk calculator are tools designed to estimate the risk of hip and major osteoporotic fractures. FRAX is one of the most widely used tools in clinical practice; however, the Garvan calculator offers additional benefits for patients with a predisposition to recurrent falls and fractures.

These tools can predict the likelihood of hip fractures and major osteoporotic fractures. Notably, the Fracture Risk Assessment Tool (FRAX) and the Garvan Institute's bone fracture risk calculator are widely used in clinical settings for this purpose. While FRAX is predominantly preferred, the Garvan calculator offers additional advantages for patients experiencing recurrent falls and fractures (Kanis et al., 2008; Van den Bergh et al., 2010).

Dual-Energy X-Ray Absorptiometry (DXA) is widely used to measure bone mineral density (BMD) by using low-dose X-rays. Low BMD is a significant indicator of increased risk for fragility fractures, making these measurements particularly valuable for postmenopausal women and men over the age of 50. BMD is evaluated using a "T-score," which indicates the deviation of an individual's bone density from the young adult mean. According to the World Health Organization, T-score classifications are as follows:

- T-score of -1.0 and above: "normal"
- T-score between -1.0 and -2.5: "osteopenic"
- T-score of -2.5 and below: "osteoporotic"

Additionally, DXA devices can provide detailed assessments of structural changes by analyzing fractures or deformities in the spine. This measurement is essential for early identification of individuals prone to fragility fractures. Moreover, it is estimated that around one-third of postmenopausal women and 50-80% of men diagnosed with osteoporosis may have an undiagnosed metabolic bone disease, highlighting the importance of laboratory testing in assessing skeletal health. These tests offer detailed information on mineral levels and bone structure, helping to identify potential fracture risk factors (Ebeling et al., 2022; Sindel & Gula, 2015)

ASSESSMENT OF FRAILTY AND SARCOPENIA

Sarcopenia is characterized by a progressive decline in muscle mass and functional capacity associated with aging. It is significantly linked to numerous adverse health outcomes, including increased risks of falls, fractures, and mortality. Diagnosing sarcopenia requires evaluating muscle mass, muscle strength, and physical performance. While various tools exist for these assessments in research settings, financial constraints in clinical practice often hinder their widespread implementation. Additionally, body mass index (BMI) and circumferential measurements are sometimes used to assess sarcopenia, though they are not considered entirely reliable. In contrast, whole-body DXA (dual-energy X-ray absorptiometry) scans provide a more dependable method for estimating lean muscle mass (Cooper et al., 2013; Cruz-Jentoft et al., 2010).

Furthermore, gait speed, sit-to-stand time and the Timed Up and Go (TUG) test are straightforward clinical tools that yield valuable insights into physical performance. In the TUG test, an individual rises from a seated position, walks three meters, and returns, with the time taken recorded. Grip strength, measured with a calibrated dynamometer, is also an important clinical parameter (Cooper et al., 2013).

Although fracture risk assessment tools like the FRAX calculator are commonly used, they may underestimate fracture risk among older adults, as they fail to adequately consider frailty and sarcopenia-related factors. The FRAiL calculator, designed to evaluate hip fracture risk in nursing home residents, is a newer tool that incorporates physical performance and muscle function indicators to estimate the likelihood of hip fracture within two years. This approach enables a more comprehensive assessment of fracture risk, helping to inform more accurate care planning for older adults (Kanis et al., 2008).

When assessing fall risk, a history of falls within the past year is particularly relevant, as it correlates with an increased risk of future falls. Additionally, cognitive impairment, sensory deficits, and polypharmacy are recognized as critical predictors that heighten the likelihood of falls and hip fractures (Seki Öz, & Çömlekçi 2023; Tanıl et al., 2014).

AGE-RELATED ALTERATIONS IN BONE MINERAL DENSITY, MICROARCHITECTURE, MATERİAL PROPERTİES, AND FRACTURE RISK

In recent decades, substantial progress has been made in understanding the patterns of change in bone mineral density (BMD) and other measurable

components influencing bone strength. Clinically, bone health is most often evaluated through area-based BMD measurements using dual-energy X-ray absorptiometry (DXA) due to its widespread availability. Consequently, research tends to focus on bone mass and BMD (Sindel & Gula, 2015). However, given DXA's limitations, recent studies increasingly prefer quantitative computed tomography (QCT), which offers more detailed volumetric BMD measurements and allows for differentiation between trabecular and cortical bone regions. Longitudinal studies utilizing these tools have shown a lifetime trabecular bone loss of approximately 45% in men and 55% in women, and cortical bone loss of roughly 18% in men and 25% in women (Riggs et al., 2004).

Bone mass and BMD alone are insufficient for accurately determining fracture risk. For example, older adults with similar femoral neck T-scores measured by DXA exhibit markedly higher fracture risk than younger individuals (Farr et al., 2014). Beyond aging, elevated fracture risk independent of BMD is observed in patients with type 2 diabetes undergoing glucocorticoid therapy (Van Staa et al., 2003). This highlights the importance of assessing bone quality. Indeed, variations in bone material properties and microstructure in individuals with type 2 diabetes can impact fracture risk. Through high-resolution peripheral quantitative computed tomography (HR-pQCT), detailed examination of bone microarchitecture has become possible, enhancing understanding of the effects of aging on BMD-independent factors (Nicks et al., 2012; Söyleme, 2020).

A study comparing young and older individuals with similar areal BMD levels measured by DXA found significantly higher cortical porosity in the distal radius of older individuals, despite similar trabecular microarchitecture (Nicks et al., 2012). This suggests that bone quality may serve as an independent factor contributing to increased fracture risk in the elderly.

AGE-RELATED CHANGES IN BONE REMODELING

The skeleton is a highly active organ that undergoes continuous renewal throughout life, involving the removal of old and damaged bone tissue by osteoclasts and the formation of new bone matrix by osteoblasts. Osteoclasts and osteoblasts operate in a spatially and temporally coordinated manner. This coordination is maintained by various local and systemic factors released by osteocytes and other cell types, thus establishing the unique structure and architecture of the skeleton.

At the cellular level, bone remodeling occurs in a three-phase process. In the initial phase, osteoclasts resorb old or damaged bone tissue, a process known as "resorption." In the second phase, mononuclear cells arrive to occupy this

resorbed area, referred to as the "reversal" phase. Finally, in the third phase, osteoblasts fill the resorbed area by forming new bone tissue. This remodeling process relies on complex interactions among cells in the bone microenvironment. Microscopically, these remodeling cycles are constantly occurring throughout the skeleton to adjust bone mass, size, and shape in response to mechanical demands, provide protection against stress or injury, and repair micro-damage accumulated over time.

However, in midlife for women and later in life for men, this balanced remodeling process becomes disrupted. During this period, bone formation fails to keep up with increased resorption, leading to a net loss of bone mass (Ağar, 2020; Steiniche & Eriksen, 1999).

AGE-RELATED DETERIORATION OF BONE STRUCTURE AND INTEGRITY

With aging, the natural imbalance in bone remodeling leads to declines in bone mass and structural integrity in both sexes. If this detrimental bone balance is not corrected, bone degradation continues along trabecular, endocortical, and intracortical surfaces, eventually resulting in an osteoporotic skeleton characteristic of advanced age. Features of osteoporotic bone include reduced trabecular connectivity, thinning or complete loss of trabecular structures, endocortical bone loss leading to cortical thinning, and an increase in cortical porosity due to enhanced Haversian remodeling. These changes demonstrate the adverse effects of osteoporosis on bone health and the associated increase in fracture risk (Seeman, 2013).

A significant portion of this bone loss is due to age-related deficiencies in osteoblast-mediated bone formation. For example, the average trabecular wall thickness, an indicator of osteoblastic activity, shows a marked decline with age in both men and women. This highlights the negative impact of aging on bone health and the factors contributing to osteoporosis development. Biochemical markers of bone formation also show age-related patterns. In men, these markers steadily decrease with age, whereas in postmenopausal women, elevated levels indicate a high rate of bone turnover. This phenomenon reflects the complex interactions between osteoclasts, which resorb bone, and osteoblasts, which form new bone. However, in postmenopausal women, bone loss occurs at a faster rate than bone formation, creating a negative bone balance. Thus, aging is associated with impairments in bone formation in both sexes (Eriksen, 1986; Seeman, 2013).

The adult skeleton consists of approximately 20% trabecular bone and 80% cortical bone. Since trabecular bone loss generally occurs faster than cortical bone

loss, bone degradation initially affects the trabecular compartment more significantly, while cortical bone loss accelerates over time. Age-related cortical bone loss contributes to the increased incidence of fractures, especially non-vertebral fractures, in the elderly population. Additionally, bone loss in specific skeletal regions (such as the femoral neck and distal forearm) raises fracture risk in these areas. Overall, age-related deterioration in both trabecular and cortical bone leads to a decrease in bone quality and strength, increasing fracture risk in older adults and suggesting that such events may become inevitable with advanced age(Nalla et al., 2006; Zebaze et al., 2010).

PATHOGENESIS OF SKELETAL AGING: FUNDAMENTAL MECHANISMS OF AGING

The pathogenesis of skeletal aging involves complex interactions between biological aging processes and bone health. The geriatric population frequently encounters not just one age-related disease but multiple coexisting morbidities, complicating treatment strategies. Aging impacts not only individual disease development but also the interactions among these conditions. This clustering tendency of age-associated diseases exacerbates health complications and increases the complexity of care needs. Therefore, a more profound understanding of aging-related biological processes across tissues and systems is essential for strategies aimed at slowing or halting the progression of age-related diseases (Khosla et al., 2022; López-Otín et al., 2013).

The primary objective in the field of geroscience is to prolong the healthy lifespan while reducing the years spent with multiple illnesses. Fundamental aging mechanisms, observed across various tissues such as bone, lead to functional decline. These mechanisms include genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, cellular senescence, mitochondrial dysfunction, deregulated nutrient sensing, stem cell exhaustion, and altered intercellular communication. These characteristics of aging directly impact healthy lifespan. Research in animal models, for example, has demonstrated that targeting these mechanisms can delay the onset or alleviate the progression of multiple age-associated diseases (López-Otín et al., 2013).

Bone aging reflects these fundamental biological mechanisms, causing cellular damage within various bone cell types and contributing to universal stress responses. Long-lived osteocytes, in particular, are directly affected by the aging process. The antagonistic hallmarks of aging, such as deregulated nutrient sensing, mitochondrial dysfunction, and cellular senescence, initially evolved as mechanisms to limit cellular damage; however, they become detrimental as aging

progresses. For instance, senescent cells accumulate in the bone microenvironment, releasing pro-inflammatory cytokines and chemokines that increase bone resorption or promote the differentiation of stem cells into adipocytes, thus exacerbating skeletal aging (Collado et al., 2007; Farr & Khosla, 2019).

In conclusion, targeting fundamental mechanisms of aging may offer new therapeutic strategies for preventing age-related bone diseases. Approaches aimed at reducing cellular senescence, for instance, hold potential for osteoporosis prevention and healthy lifespan extension. Aging accelerates bone loss in both genders, disrupting the balance between bone formation and resorption and leading to increased bone marrow adiposity, along with osteoblast and osteocyte apoptosis.

MANAGEMENT OF SKELETAL FRAGILITY

Strategies aimed at reducing the incidence of age-related fractures focus not only on slowing the decline in bone density but also on managing chronic conditions that may exacerbate skeletal fragility and sarcopenia. Therefore, "osteoporosis treatment" should be tailored to each patient's individual goals, taking into account their current health status and psychosocial factors. Additionally, improving access to community resources—such as geriatric-friendly fitness centers, transportation services, and affordable housing options—can significantly enhance the effectiveness of management strategies (Coll et al., 2021; Nuti et al., 2019).

EXERCISE-BASED STRATEGIES FOR BONE DENSITY IMPROVEMENT AND FALL RISK REDUCTION

Numerous studies have shown that exercise programs focusing on strengthening core muscles and improving balance are effective in reducing the risk of fallsstance training that includes weight-bearing and moderate-to-high intensity exercises over 12–18 months can significantly improve bone mineral density (BMD). In this contexin supervised multimodal programs has also been observed to enhance both BMD and physical performance (Atalay, 2015; Erzeybek, 2012; Howe et al., 2011; Sherrington et al., 2019).

Exercise programs for older adults should be safe, suseing adapted to individual characteristics. For example, low-intensity walking, sitting, and standing exercises should be graduallto individual tolerance levels.

Yoga, which has gained popularity in recent years, shows potential to improve balance and reduce the risk of falls in older adults when practiced moderately

(Youkhana et al., 2016). Additionally, there is moderate evidence that Tai Chi enhances balance, reduces fall risk, and supports bone health, particular adults and women with osteoarthritis (Okuyan Birimoğlu & Bilgili, 2017).

EXTRA-SKELETAL BENEFITS OF OSTEOPOROSIS TREATMENT

Osteoporosis treatments offer various benefits beyond bone health, impacting areas such as cardiovascular function, neurological health, and general physical well-being. For instance, vitamin D and calcium supplementation not only strengthen bones but also enhance muscle function, reducing fall risk and supporting mobility in elderly populations. Some osteoporosis treatments, like certain bisphosphonates, have been linked to improved cardiovascular outcomes and immune function, highlighting the potential for a more comprehensive approach to managing osteoporosis (Reid et al., 2020)(43).

Research on the extra-skeletal effects of osteoporosis medications has highlighted significant benefits, particularly in frail elderly individuals where these effects are essential for guiding treatment decisions. In postmenopausal women, studies have shown that zoledronic acid infusions administered every 18 months may reduce the incidence of certain cancers, especially breast cancer, in women at risk for osteopenia. Additionally, a study on denosumab found that three years of treatment was associated with increases in lean body mass and grip strength, indicating a positive impact on overall physical performance (Bonnet et al., 2023; Reid et al., 2020). These findings underscore the importance of considering non-skeletal benefits when choosing osteoporosis treatments, contributing to a more holistic approach to elderly health care.

CONCLUSION

As a result of global developments, people are living longer, but this extended lifespan does not necessarily come with improved health outcomes. Osteoporosis, a prevalent health issue linked to aging, is characterized by a reduction in bone strength and an increased risk of fragility fractures. In Turkey, as in many parts of the world, the prevalence of osteoporosis among individuals aged 50 and older remains a significant concern. The National Osteoporosis Foundation recommends bone mineral density (BMD) measurements for women aged 65 and older and men aged 70 and older to reduce this risk. Furthermore, individuals with clinical risk factors, as well as postmenopausal women and men over 50, should also be evaluated.

Bone mineral density measurement is a crucial method for determining the risk of fragility fractures. Low bone density increases the likelihood of such fractures. Techniques like DXA scans provide insights into bone health, while laboratory tests, including those measuring vitamin D, calcium, and phosphorus levels, as well as biomarkers of bone turnover, play a complementary role. The aging population presents significant challenges, including an increase in age-related diseases and chronic morbidities. Bone health is notably impacted by aging, with both women and men experiencing increased bone loss and imbalances in bone formation.

In conclusion, the early diagnosis and management of osteoporosis are critical to reducing fracture risk. Evaluations tailored to individuals' health conditions provide essential information for preventing and treating osteoporosis, contributing to healthier aging. Therefore, it is essential for both individuals and healthcare professionals to be aware of osteoporosis. Research into new strategies for treating osteoporosis and other age-related diseases is accelerating. These innovative approaches aim to improve the quality of life for individuals and minimize the health issues associated with aging.

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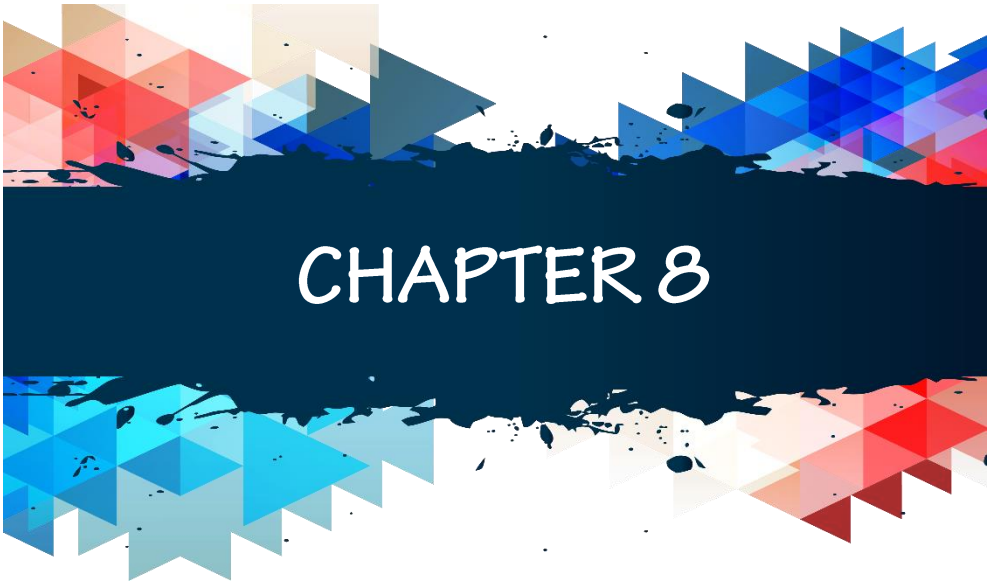

Bone Fractures Non-Union and Novel Therapeutic Strategies

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BONE FRACTURES AND HEALING PROCESS

Bones have a hierarchical structure required to bear mechanical loading and stand against fractures. Numerous substitutes of bones provide mechanical stability and have plasticity to avoid bone damage. Even so, bone fractures are widely seen, and bone healing is such a challenging issue (Fazzalari, 2011). There are several reasons for bone fractures, such as surgeries, tumors, osteoporosis, osteoarthritis, and various pathologies (Munmun & Witt-Enderby, 2021; Tanrıkulu & Gönen, 2017). Bone fractures affect all individuals including mostly elderly people worldwide. High-intensity energy traumas correspond to 85% of bone fractures in young adults, mostly during sports activities. Bone repair widely could be achieved by surgical intervention, but the post-surgical healing, bone regeneration, and load-bearing capacity do not heal as it is before (Naveiro et al., 2023).

Bone regeneration requires a complex collaboration of mechanisms, classified into three phases; inflammatory, repair, and remodeling (Elhawary et al., 2021). The inflammatory phase begins immediately after the fracture of a bone, and due to the tear of surrounding vessels blood influx to the fracture site causes a hematoma. The inflammatory cells accumulated in the hematoma, release cytokines which trigger the inflammatory cascade beginning. includes inflammatory cytokines to the fracture site. Initially, polymorphonuclear neutrophils (PMN) reach the area, following monocytes and macrophages infiltrating the fracture site (Kovtun et al., 2018). These macrophages transform into the M1 phenotype and start to release pro-inflammatory cytokines. The adaptive immune response begins with the arrival of lymphocytes. During this, the already presented PMNs and macrophages start to clear the apoptotic cells and residues which is a sign of the ending of the inflammatory phase (Mountziaris & Mikos, 2008). Through the end of the inflammatory phase, the release of inflammatory cytokines flow to the site decreases. Additionally, immune cells begin to leave the tissue. Macrophages polarized into M2 phenotype and begin to release anti-inflammatory cytokines such as IL-4, IL-10, and IL-13. The communication of bone-derived mesenchymal stem cells and immune cells stimulates angiogenesis and osteogenesis (Frade et al., 2023). BMP/TGF-β/SMAD signaling pathway activation is the starter of osteogenesis, while repair and remodeling stages are ready to act (Chen et al., 2012). Osteoblasts the major cells of bone structure begin to differentiate by P38/MAPK signaling. This cascade leads to alkaline phosphates (ALP) and osteocalcin expression. During these processes, cytoplasmic calcium increase has a key role in callus formation. The increase of calcium stimulates the gene expressions of critical growth factors

such as insulin-like growth factor (IGF), vascular endothelial growth factor (VEGF), transforming growth factor beta (TGFβ), bone morphogenic proteins (BMP2, and BMP4). These factors are the major markers of bone growth and regeneration (Hutchings et al., 2020).

The repair phase of bone fractures comprises bony and hard callus formation. The callus formation consists of two main ossification stages (Bigham-Sadegh & Oryan, 2014). Intramembranous ossification initially begins in the periosteum. The already presented mesenchymal stromal stem cells differentiate into osteoprogenitor cells and form the hard callus. Endochondral ossification takes action in the endosteum and bone marrow. Here, bone marrow mesenchymal stem cells differentiate into chondrocytes and release the cartilaginous matrix. The chondrocytes after hypertrophic differentiation, mineralize the surrounding matrix to form the cartilaginous callus. Following the apoptosis of the chondrocytes, osteoblasts invade the area. The cartilaginous matrix is formed into the bone matrix by osteoblasts. During endochondral ossification, the bony callus turns into a hard callus (Galea et al., 2020).

Following callus formation, the last phase of beno regeneration begins. The remodeling phase is the renewal of the damaged bone tissue. The balance between the osteoblasts and osteoclasts is provided. Lamellar bone deposition and resorption at the end from the repaired bone tissue (Maruyama et al., 2020; Wildemann et al., 2021).

NON-UNION OF BONE FRACTURES

The ideal conditions for bone regeneration where the above-mentioned healing phases are provided, maintain the complete regeneration of the bone fracture. However, in some cases, bone healing is not wholly complete, and the union of the fracture site and host bone is not always improved. This type of non-healing is called non-union. There are several reasons for non-union fractures including biological and mechanical conditions. Non-union fractures cause increased pain, elongated immobilization, disabilities, and psycho-social insufficiency (Wildemann et al., 2021). Due to developmental surgical methods, stabilization techniques, and material fixation, the gold standard method for non-union fracture healing is autograft application. Autograft is now the widely used method in large or non-union bone defects. However this method has such drawbacks, pain, hematoma, or decreased load-bearing ability (Buza & Einhorn, 2016) (Schmidt vd., 2021, Haeusner vd., 2023). Bone allografts are alternatives to autografts but may cause unexpected immune responses and high costs (Muller vd., 2013). Over the past decade, synthetic biomaterials have gained attraction in

the healing of bone fractures non-union. However, these materials cannot maintain mechanical stability. So metallic alloys are suggested to improve load-bearing capacity (Matassi vd., 2013). Due to the promising fixation of bone fragments and mechanical stability, metallic alloys are unnatural and cannot adapt to physiological conditions (Fu vd., 2011). Overall, there are still urgently needed techniques and substitutes in bone tissue engineering to overcome these challenges.

NOVEL THERAPIES FOR NON-UNION BONE FRACTURES

In recent years, based on biomaterials technology, several biological approaches have been evaluated for robust non-union bone regeneration with promising outcomes. There are multiple strategies comprised of mesenchymal stromal cell (MSCs) therapy, BMP, and VEGF treatment. The pre-osteoblastic MSCs can maintain bone regeneration during the healing process. Novel studies based on animal experiments claimed that MSCs therapy offers promising results in non-union bone fractures (Schlundt et al., 2018). MSCs could be applied in different ways such as intravenous, locally or through a composite biomaterial. MSCs act as osteoblast precursors in the fracture site and facilitate bone healing. One of another promising approaches that has gained attract recently is the BMP administration. BMPs are mostly applied via biomaterials. The BMP family members BMP7 and BMP2 are FDA-approved agents for bone fracture treatments (Whitty et al., 2022).

Angiogenesis is an indispensable factor for bone regeneration due to the increased need for nutritional supply during regeneration. Thus, stimulating the angiogenetic factors is essential. VEGF is a well-known vessel formation stimulating factor and providing VEGF activation is important in bone regeneration. In experimental studies, direct application or controlled release of VEGF enhanced the union of critical-sized bone fractures (Hu & Olsen, 2016; Keramaris et al., 2008; Menger et al., 2022).

Nanotechnology also offers promising advantages in bone healing and non-union bone fracture regeneration. Polymer-based technologies with three-dimensional printing approaches have great attention in tissue engineering applications (Aykora & Uzun, 2024). Due to numerous therapeutic approaches non-union bone fractures still have a challenge in orthopedics. There is still an urgent need for the development of efficient therapies to foster bone regeneration and fill large bone gaps.

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CHAPTER 9

Protective Effects of Juglone on Rat Testis Exposed to Electric Field

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1. Introduction

Electrical events in the environment affect non-living and living things (e.g. electrical devices) is doing. Effect on non-living and living things Electrical Fields; Electric field, Magnetic field, and Electromagnetic field it is divided into three. These Electric Fields; when electrical energy is produced, the energy from transmission lines or cables or when shipped by air or when deployed or in electrical appliances are formed when used. Use of electrical energy; in our modern life Since it is an integral part of fields are everywhere around us. Humans have high exposure to various artificial electric fields in our modern society. There has been a relationship between exposure to electric fields and different disorders or their treatment affecting various organs. Electric fields are generated by the clinical devices used in medical therapies during treatment, and electric fields cure different pains. In addition, electric fields commence behavior, humoral, or cellular responses [1].

An electromagnetic field (EM field or EMF) is a physical field that electrically charged objects produced. The behavior of charged objects near the field is affected by EMFs. The EMF is indefinitely expanded through space, determining electromagnetic interaction. One of the four fundamental natural forces is EMF, the others are weak interaction, strong interaction, and gravitation. One can regard the EMF combined magnetic and electric fields. Modern electrical devices and power lines are increasingly used adversely affecting public health, and encouraging to pay remarkable attention to chronic exposure to EMF. spermatogenesis is adversely affected by exposure to EMF by the Leydig and Sertoli cells. 50 Hz magnetic fields cytostatically and cytotoxically change the mice's differentiating spermatogonia. There is little information on the impact of EMF on the seminiferous tubules' boundary tissue cytoarchitecture, with some crucial functions, including transport and mechanical support of nutrients to sperm and spermatozoa discharge by keeping pressure on the tubules [2, 3, 4].

Antioxidants potentially could prevent excess ROS formation and enable the repair of cellular damage. Juglone (5-hydroxyl-1,4-naphthoquinone) is a phenolic compound found in walnuts. A variety of benefits have been attributed to juglone including anti-inflammatory, anticancer, antiplatelet, antioxidant, vasodilator, neuroprotective, and antineoplastic properties. Free oxygen radicals, such as hydroxyl radicals and superoxide anions are directly scavenged by Juglone. Endogenous antioxidant enzymes can be influenced indirectly by Luteolin protecting cells against the expression of proteins involved in apoptosis and oxidative damage. There is a partial relationship between the juglone's protective

role and inflammatory cytokines' inhibition. Juglone safely protects against cellular toxicity [5, 6, 7, 8, 9].

Few reports are related to the impact of EF radiation and the juglone's antioxidant activity regarding the testicular tissue. Both the efficacy of juglone administration for the decrease of harmful effects and the potential adverse effects of EF on testis tissues were studied.

2. Materials and Methods

2.1. Experimental Groups and Animals

This study was on young Wistar-Albino rats (n = 24, 250–300 g). Süleyman Demirel University Experimental Animals Research Unit (decision numbered 21438139-267) submitted the ethical permission. We kept all animals in the Experimental Animal Laboratory under standard conditions during the experiment. We randomly divided rats into three groups as EF + Juglone (EF + JUG), EF, and Control. We applied a 21 h/day electric field to the EF + JUG and EF groups for 30 days. gavage method was used to give a juglone (JUG) antioxidant substance, plus the application of an electric field to the EF + JUG group. We formed the experimental groups as follows. The first group is the control group containing rats without electric field gavage and exposure (8 rats). The second group is the EF group which is the electric field exposure group (8 rats). The third group is the EF + JUG group containing 8 rats exposed to 1 mL 300 ppm antioxidant juglone (5-hydroxy-1,4-naphthoquinone) and an electric field based on the gavage method.

2.2. Creation of EF

The methodology which Anderson et al. used was followed for the AC 50 Hz which was used in the study [9]. We performed the experiment at Süleyman Demirel University Experimental Animals Research Unit. Electronics and Communication Engineering faculty members at Süleyman Demirel University took the power measurement, and IEEE standards were adopted

Basic parallel plate exposure setup limitations and design are described in [10]. Cell phones and other devices affecting the environment were not included in the room. On the other hand, a control group was placed in a similar room, but far away from the electric field. Measured data of electric field (V/m) were obtained for both exposed and control groups.

We cared for and fed the Groups under the same environmental conditions. We kept rats in an environment with humidity of 55–60%, at a fixed temperature

of 20 ± 2 ◦C, and with a 12/12 h light/dark cycle. We used a homogeneous and uniform electric field.

The 1 × 0.5 m surface is completely smoothed. A fully conductive plate is placed horizontally and shaped using a 50 Hz, 5000 V AC power transformer to ensure a sufficiently smooth surface, the ends of which are pressed into the center of the plate without piercing the plate and connected to bolts made of stainless steel.

The plate dimensions were chosen to be large enough to give a sufficiently uniform electric field. The cage volume was 40 × 50 × 20 cm3 (W × L × H). The selected panel was very large with respect to the studied subject as compared to the used and studied materials. sufficiently smooth electric field lines can be provided with the plates of this dimension. İf the area between the plates is fairly smooth, the corner effects will be ignored.

We have used the term "Smooth" for the noiseless situation. All types of parallel plate setups used in the present study have a major problem known as the corner effect. There are moderately straight electric field lines in the central region of the plates bending at the corners.

Therefore, we chose relatively large plates chosen relative to the test area, with physically rounded plates' corners. such experiments should not have curved electric field lines. For this reason, it was necessary to be sure that there were no bent electric field lines. We used the instrumentation and measurement methodology during the experimental period to monitor this "smoothness"[11]. We calculated the cage sizes for the longevity and health of the animals. The cages were 50 cm wide, with the glass or plastic irrigation apparatus not to disturb the homogeneous lines of the electric field. We cleaned the cages every day and monitored the humidity and temperature of the environment not to disturb the applied electric field's homogeneity.

2.3. Histopathological Examinations

We fixed dissected right testes in formalin solution 10% for 14 days. Then, we dehydrated the tissues with graded alcohols and cleared them using xylene (Sigma-Aldrich). We then impregnated the samples with paraffin (Merck, Darmstadt, Germany). We cut the sections transversely with a rotary microtome for stereology at 20 μm and for immunohistochemical and histopathological examination at 7 μm. We stained the sections for histopathological and stereological analyses with eosin and hematoxylin (H & E) [12]. We passed the prepared materials quickly passed through distilled water through xylol and

alcohol series and closed them with entellan. We performed the histopathological observation in the prepared materials examined with a light microscope (Leica DM 500, Wetzler, Germany)

3. Results

The control group seemed to have normal seminiferous epithelium, Leydig cells, interstitial connective tissue, and seminiferous tubules. There were vacuoles, areas without separation, and spermatogonia of spermatogenic cells from the wall of the tubule in the EF group's seminiferous tubules. However, we detected a few necrotic seminiferous tubules. In addition, the seminiferous tubules had an irregular shape and the seminiferous tubules separated occasionally from each other. Damage was observed in Leydig cells and the interstitial area in the EF group. The EF+juglone group had more regular interstitial areas. The EF + juglone group had anomalies, but fewer than those of the EF group. Spermatogenic cells were found to be separated from the tubule wall related to the epithelium disruption. In absence of spermatogonia, some vacuoles had irregularly shaped seminiferous tubules and regions.

Table 1. Damage severity of groups in testis tissue

Groups	Damage severity
EF	+++
EF + Juglone	++
Control	-

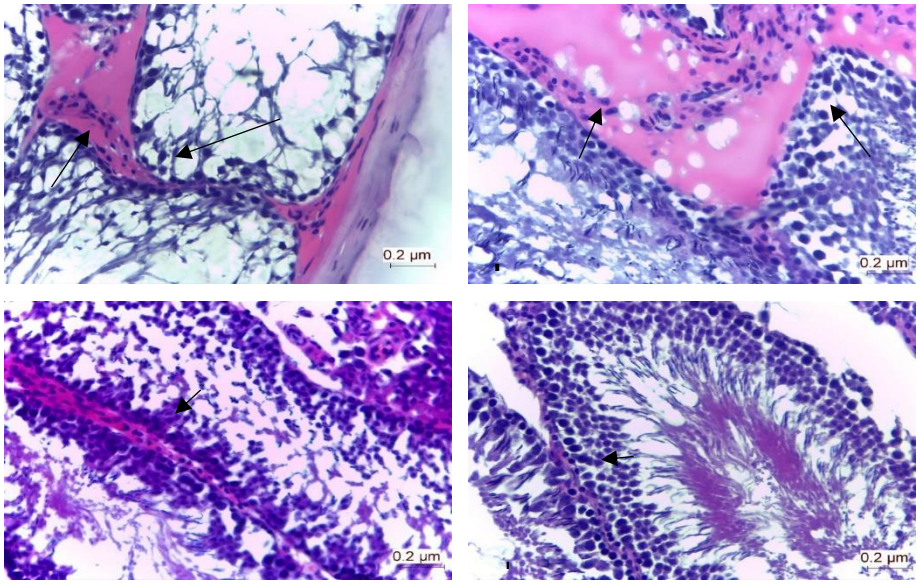

Figure 1 a. Decrease in the number of spermatogenic cells and separation of these cells from the tubule wall, EF group, H&E, X 40; **b.** Degeneration and edema in the interstitial region, EF group, H&E, X 40; **c.** Normal seminiferous tubules and normal interstitial region, Control group, H&E, X 40; **d.** Separation and mild damage to the seminiferous tubules, near-normal spermatogenic cells, EF + Juglone group, H&E, X 40.

4. Discussion

Increasing cases of male infertility are due to lifestyle and environmental factors. Electric emission and electromagnetic radiation with different frequencies biologically and genetically affecting humans result from fast technological advancement. Devices such as power lines, cell phones, and monitors lead to electromagnetic radiation as a main source of exposure. Different studies find that radiation on the male reproductive system's physiological factors such as perm parameters (viability, morphology, and motility), genomic instability, and metabolism leads to detrimental consequences. Since one cannot rule out the nonthermal and thermal interaction of nonionizing radiations with biological tissues, reactive oxygen species generation is emphasized in most studies. Redox equilibrium is altered by the oxidative stress disrupting normal functioning and morphology of sperms and declining total antioxidant capacity [13].

Over the past decades, public attention has been attracted to a possible relationship between man-made electric field and their undesirable health effects.

The aim of this study was to investigate the histopathological effects of intermittent (8 hours on/16 hours off) exposure to the electric field with horizontal low frequency on the rat testis. The exposed group was continually exposed to a 50 Hz horizontal electric field for 8 hours/day in the experiment. Several histological alterations, such as the seminiferous epithelium's necrosis, focal tubular atrophy, and degeneration were found in the exposed group's testes, but not in the control group's ones [1]. In this study, we obtained similar results in the EF group.

Khaki et al. [2] reported that the percentage of apoptotic cells was reduced after administrating extract of Ocimum basilicum (1.5 g/kg body weight) compared to the control group. Percentage of the apoptotic cells significantly increased exposure to 50 Hz of EMF. Administration of 50 Hz of EMF with extract of O. basilicum (1.5 g/kg body weight), significantly decreased the percentage of apoptotic cells from 18.12±1.05 to 10.05±0.01 in spermatogonia, and primary spermatocytes, from 20.11±0.05 to 9.05±0.05, and vein congestion from 8±0.03 to 0.5 ±0.01. These results show that O. basilicum protected against apoptosis caused by EMF. The negative impacts of the electric field were found to slightly decrease in the EF+juglone group due to the antioxidant activity of juglans in this study.

They randomly divided Wistar albino rats into four groups: EMF luteolin, control, and EMF + luteolin. The EMF group reduced the number of primary spermatocytes, Leydig cells, and spermatids to the control group. The EMF + luteolin group had a significantly higher number of primary spermatocytes, Leydig cells, and spermatids than the EMF group. Sperm morphology was also affected by the EMF. Repeated exposure of rat testis to 900 MHz EMF changed testicular tissue and the deleterious effects of EMF were significantly reduced by the antioxidant, luteolin[14]. We found similar results in the groups.

They divided adult male albino rats into six groups: rosemary group (receiving 5 mg/kg b.wt rosemary extract), control group, EMF (4 h) group (exposed to the magnetic field (50 Hz and 5.4 kV per meter) for 4 h), EMF (2 h) + rosemary group (receiving extract and magnetic field for 2 h), EMF (2 h) group (exposed to the magnetic field (5.4 kV and 50 Hz per meter) for 2 h), and EMF (4 h) + rosemary group (extract and magnetic field for 4 h). Their resutls showed the effect of rosemary leaves' ethanolic extract on pathogenic bacteria. Histological results found the inhibiting effect of the rosemary extract on the electromagnetic fields' destructive effect on testicular tissue. This research finds several useful effects of rosemary's ethanolic extract in supporting individuals ecologically contaminated with EMF [15]. In our study, juglone produced similar effects.

Exposure to electric fields causes edema, bleeding, interstitial fibrosis, the proliferation of Leydig cells, basal membrane thickening, seminiferous tubule degeneration, vascular congestion, testicular tissue tubular necrosis, and atrophy [16]. As a result, the spermatogenic epithelial layer desquamated in seminiferous tubules [17], increasing the process of destruction in cells producing steroids and changing the status of the histohaematic barrier [18]. Our results are consistent with the above-mentioned study's results: reduction of epithelial thickness, partial breaks, and basal lamina of the seminiferous tubule, interstitial area congestion and edema, a reducing interstitial connective tissue, seminiferous tubule epithelium vacuolization in the EF exposed group. In addition, serious damage in testicular tissue was observed. Morphometric examination showed the diameter of the seminiferous tubule, tunica albuginea thickness, and decreasing Leydig cell count. The present study finds serious damage to EF in testicular tissue. On the contrary, Juglone was found to provide partial protection against this damage. The use of a higher Juglone dose may be more protective against testicular damage caused by the EF. Also, our findings show the similarity of the Juglone group's morphology to that of the control group.

The study revealed apparent structural alterations in the testes, such as significantly decreasing the diameter of seminiferous tubules and the germinal epithelium's height, seminiferous tubules' irregular shape, germ cells disorganization, immature germ cells desquamations, giant multinucleated cells formation, and significantly expanding interstitium due to application of MR in utero. The transmission electron microscopy showed irregularities of the basement membrane in seminiferous tubules, the cytoplasm vacuolation, and organelles adversely affected in Sertoli cells, Leydig cells, germ cells, endothelial and peritubular cells. The adjacent Sertoli cells had often incomplete tight junctions, and experimental animals had more necrotizing germ cells than the controls. MR-exposed animals showed increasing germ cell necrotizations proved using a Fluoro-Jade C method, and the decline of the proliferation of germ cells was confirmed using the analysis of proliferating cell nuclear antigen. Their results showed the adverse effect of prenatal exposure to MR on postnatal testicular growth among rats [19]. Regions without spermatogonia and separation of spermatogenic cells from the tubule wall were found in the EF group's seminiferous tubules but few necrotic seminiferous tubules were found in our study.

They randomly divided 32 male Wistar Albino rats into four groups each with eight animals such as EF, EF + RES, RES, and control. The testes' histopathological examination showed a decrease of germ cells in the

seminiferous epithelium with interstitial tissue's vascular and edema congestion. The immunohistochemical examination increased the number of apoptotic cells. RES partially improved histopathological, immunohistochemical, and biochemical, findings in the EF + RES group. These results clearly showed damage in rat testis through EF. The damage caused by EF can be improved by RES [20]. We obtained similar results in this study.

To study the non-time-varying electric field's effect due to direct current transmission lines on testosterone synthesis, we continually exposed male ICR mice (24 h/d) to 56.3 ± 1.4 kV/m static electric field. Results showed no significant change in testicular oxidative stress indicators and serum testosterone level on the 7th day after ceasing the exposure of 28 d and on the 3rd day of exposure, The mitochondrial structure in Leydig cells could be damaged by testicular oxidative stress, reducing the cholesterol transport rate from the cytoplasm to mitochondria [21]. In this study, the damage was observed in the interstitial area and Leydig cells in the EF group. In the EF+juglone group, the interstitial area was found to be more regular.

The effect of multiple or single exposures to EMF's different doses from mobile phones on the hippocampus and testis structure in adult albino Wistar rats was studied. Spermatogenic cells had vacuolations with some abnormalities of sperm structure. There were intercellular vacuolations in the Testis, significantly decreasing seminiferous and tubules germinal epithelium thickness and significantly increasing the mean area percent of caspase-3 reaction and collagen fibers. semen analysis showed several sperm abnormalities [22]. The present showed vacuoles, regions without separation, and spermatogonia of spermatogenic cells from the tubule wall in the EF group's seminiferous tubules but detected few necrotic seminiferous tubules.

The study aimed to investigate whether the effects of a nanosecond pulsed electric field, without thermal effects, change the gene expression and molecular processes of Leydig TM3 cells permanently. We exposed the cells to an average electric field (an electric field of 14 kV/cm, 60 ns pulse width, and 80 quasi-rectangular shape pulses). We recorded the putative disturbances for 24 h. Exposure to the nanosecond pulsed electric field caused a 70% reduction in cell adhesion, a loss of microvilli, and an increase in cell diameter by 19%. the phosphatidylserine nonapoptotic externalization was found in some cells with pores in the plasma membrane. Based on the Microarray's transcriptome analysis, the gene expression involved in DNA repair, cell proliferation, oxidative phosphorylation, and the plasma membrane proteins overexpression was negatively transiently affected. It is concluded that the TM3 cells' physiology and

gene expression are affected by transiently nanosecond pulsed electric field, with cellular responses' noticeable heterogeneity[23]. This study found damage in Leydig cells and the interstitial area in the EF group. the EF+juglone group had more regular interstitial areas.

5. Conclusions

We think in the research that EF reduces sperm count and motility affecting gonadal functions. The results will contribute to both engineering and medicine fields and also contribute to this anomaly treatment. In the engineering field, electrical equipment and cables could be used healthily at workplaces and homes. They will also help determine the limits of exposure set in different countries. Due to the failure of the Juglone dose used in this study to give a complete protective effect on all testicular damage parameters as an antioxidant, using higher doses of Juglone in further studies is believed to be more protective against EF damage.

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A Beginner's Guide to Bioinformatics: Multiomics Data and Biological Databases for Drug Discovery

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Multi-omics data is critical in drug discovery since it provides a comprehensive un-derstanding of complex biological systems and aids in the identification of therapeutic targets. Researchers can analyze genomics, transcriptomics, proteomics, and metabolom-ics, as well as other molecular levels, and combine multi-omics data (Li, Yin, Zhao, & Sun, 2021; Nassar, Raddassi, & Wu, 2021). This approach gives rise to a more complete and understanding signature of how diseases work and how drugs can affect or cure them.

One of the best-gained accelerations about multi-omics data is that information from differing -omics fields can be used together or combined. This integration leads research-ers to find new or possible biomarkers, predict how well a drug can cure, determine types of cancer, and classify patients to get personalized treatment (Nassar et al., 2021). For instance, multi-omics data has been used in the field of personalized medicine to predict how drugs will work and to build up treatment strategies, especially for patients suffering from breast cancer (Khan & Shedole, 2022). By evaluating personal gene expression profiles and molecular signatures, researchers can find new or potentially therapy-specific drug targets for better treatments (Shrestha et al., 2021).

Using the obtained multi-omics data has also led to drug discovery via identifying new or candidate therapeutic targets. The Connectivity Map is an example of a web-based platform that presents options for using multiomics data for drug discovery and development. The Connectivity Map resource has enhanced drug discovery by identifying links between small molecules, diseases, and drugs by analyzing gene expression profiles of cells treated with bioactive small molecules or therapeutic agents (Lamb et al., 2006). In precision medicine, treatments are tailored to patients' molecular profiles; thus, multi-omics data is instrumental in drug discovery. The obtained data can help researchers understand disease mechanisms more clearly, find biomarkers, and develop targeted therapies with lower side effects (Shrestha et al., 2021).

Machine learning and deep learning technologies can be used to for medical prediction using multiomics data. Deep learning is a machine-learning process that has received considerable attention in a variety of fields. It is distinguished by its capacity to extract high-level abstractions from data automatically, allowing it to discover complex and nonintuitive links inside datasets (B. Wen et al., 2020). Multi-omics data combined with deep learning algorithms can predict drug combination effects and design novel biomedical nanoparticles (Gao et al., 2022).

Databases	URL	Data Type	Reference
STRING-db	https://string-db.org/	Protein-Protein Interactions	(Szklarczyk et al., 2023)
Reactome	https://reactome.org/	Pathways and Reactions in Human Biology	(Milacic et al., 2024)
KEGG	https://www.genome.jp/kegg/	Pathways, Genomic Information	(Kanehisa, Furumichi, Sato, Kawashima, & Ishiguro-Watanabe, 2023)
BioGRID	https://thebiogrid.org/	Protein and Genetic Interactions	(Oughtred et al., 2021)
IntAct	https://www.ebi.ac.uk/intact/	Protein Interactions	(del Toro et al., 2022)
neXtProt	https://www.nextprot.org/	Human Protein-centric Information	(Zahn-Zabal et al., 2019)
DrugBank	https://www.drugbank.ca/	Drug and Drug Target Information	(Knox et al., 2024)
ChEMBL	https://www.ebi.ac.uk/chembl/	Bioactive Molecule Information	(Davies et al., 2015; Jupp et al., 2014; Mendez et al., 2019)
STITCH	http://stitch.embl.de/	Chemical-Protein Interaction Networks	(Szklarczyk et al., 2016)
PubChem	https://pubchem.ncbi.nlm.nih.gov/	Chemical Molecules and Compounds	(Kim et al., 2023)
BindingDB	https://www.bindingdb.org/bind/index.jsp	Protein-Ligand Binding Affinities	(Gilson et al., 2016)
TTD	https://idrblab.net/ttd/	Drug-Target Information	(Y. Zhou et al., 2024)

Table 1. Commonly used databases to integrate different data types

The integration of multi-omics data is crucial for the development of new drugs; this gives researchers the ability to examine intricate biological systems from various angles, identify therapeutic targets, forecast drug responses, and develop novel drugs. Drug discovery and development could advance even more by incorporating machine-deep learning algorithms and multi-omics data (Gao et al., 2022). Researchers can accelerate the development of new drugs and enhance patient outcomes with the power of multi-omics data.

Graph databases have also been used to integrate heterogeneous biological data (Yoon, Kim, & Kim, 2017). The use of graph databases allows the analysis of complex biological relations, which in turn presents researchers with a powerful tool for data integration and analysis. Besides, in addition to facilitating data integration, biological databases also aid in the prediction of drug-target interactions (DTIs). Online biological databases such as KEGG, DrugBank, ChEMBL, and STITCH store and maintain information related to known drugs and drug-target interactions, providing valuable data for computational methodologies to predict DTIs (Nair, 2018).

Data-driven drug development relies on biological databases for drug, target, and interaction information (Kadowaki, Wheelock, Hattori, Goto, & Kanehisa, 2006). These databases contain a wealth of biological data for drug discovery and development research. Drug targets and pathways are important features of biological databases. The Therapeutic Target Database (TTD) is an example of a database that provides drug, target, and drug-targeted pathway information. Researchers need this information to understand drug mechanisms and therapeutic uses; however, some databases may not cover all clinical trial drugs and drug-targeted pathways (Yang et al., 2016).

Biological databases are not limited to drug-target interactions but cover a wide range of research topics. For instance, the comprehensive peptide research database Peptipedia supports machine learning techniques that demonstrate how biological databases can be used to assist a wide range of research approaches and sectors (Quiroz et al., 2021). It's crucial to remember that integrating biological databases is indeed difficult since data redundancy, duplication, and the necessity for tools specifically for validation have all been noted as critical problems (Kaisar, El-Attar, Abdelkader, & Elmashad, 2022). To ensure their dependability and value in data-driven drug development, researchers always attempt to improve the quality and security of biological databases. They are also essential for data-driven drug development because they offer useful details on medicines, targets, and their relationships with each other (Kadowaki et al., 2006). These databases serve diverse research approaches and domains by facilitating data integration, analysis, and prediction of drug-target interactions. However, to guarantee the dependability and correctness of the data produced by these databases, issues with database integration and validation need to be resolved.

Navigating Biological Databases

In order to utilize biological data for scientific study, those who are new to the field need to acquire a solid understanding of fundamental concepts and information related to databases, including different types of databases and the methods for accessing them.

The NCBI reference sequences (RefSeq) database is considered a crucial resource composed of a collection of non-repetitive sequences that include genomes, transcripts, and proteins. The dataset encompasses a diverse array of organisms and offers thorough and current information regarding sequence characteristics. The fact that the data in RefSeq records comes from numerous sources improves its accuracy and dependability (Pruitt, Tatusova, & Maglott, 2007).

Another significant resource is the Universal Protein Knowledgebase (UniProt) which serves as a centralized repository for protein sequences and comprehensive functional annotations. The integration of the Swiss-Prot and TrEMBL databases results in the creation of a comprehensive and extensively annotated knowledge base for protein sequences. UniProt additionally provides non-redundant sequence databases to facilitate efficient searching and offers a range of query interfaces to enhance accessibility to the data (Apweiler, 2004).

The European Nucleotide Archive (ENA) is a notable database and responsible for the collection and curation of nucleic acid sequences and associated data, which are essential components of the enduring scientific knowledge base (Cochrane et al., 2012). The archive provides a comprehensive data repository that facilitates the integration of diverse nucleotide sequences. ENA also intends to implement Compressed Reference-oriented Alignment Map (CRAM) data compression technology to improve the efficiency of data storage and retrieval processes. Besides, the RT-qPCR Primer Database, GenBank, and the National Center for Biotechnology Information (NCBI) Gene Database are important biological data sources in addition to the databases mentioned above (Antonaros et al., 2019; Forsman et al., 2010). Those provide specialized information and tools to support diverse research objectives, including primer design and DNA sequence analysis.

Biological databases are of paramount importance in the fields of bioinformatics and scientific inquiry. These platforms offer researchers the opportunity to access a vast amount of biological data, enabling the processes of data mining, analysis, and discovery. Researchers are strongly urged to actively participate in the sharing of data and make use of these available resources in

order to further their research endeavors and make valuable contributions to the existing body of knowledge within their respective fields.

Ensuring Data Quality and Relevance

Bioinformatics is mostly about analyzing and making sense of big biological datasets, like gene expression data, protein interaction networks, and whole genome sequencing data, but not just those. Several processes have been suggested and put into place to improve the accuracy and reliability of the information; thus, the analyzed biological data should be accurate and useful.

To ensure the accuracy and relevance of data, interoperability is essential. Interoperability technology and reward mechanisms help the bioscience community create an open culture of "data commoning" (Sansone et al., 2012). Integrating data is crucial in bioinformatics research. Data from different sources and at different levels of detail can provide valuable insights and aid information extractio. However, advanced data fusion methods must be studied and developed to ensure the accuracy and reliability of insights from combined data sources (West & Ali, 2016).

Along with data quality, bioinformatics researchers must also consider biological data relevance. Bioinformatics methods are more reliable when there are replicates of accurate experimental data. However, extensive experimental data is sometimes unavailable, making simulated data essential for methodology development and evaluation. Computational bioinformatics approaches aim to create models that better represent biological processes than experimental methods. This is done to prepare for massive biological data (Sandve & Greiff, 2022).

Bioinformatics research ensures the quality and relevance of biological data by establishing protocols for data documentation and sharing, facilitating system compatibility, advancing data integration techniques, and using empirical and simulated data for method refinement and evaluation.

Database name	Key features	Data type	URL	Reference
GenBank	Sequenced nucleotides, annotations, and references	Genomics	https://www.ncbi.nlm.nih.gov/genbank/	(Benson et al., 2012)
RefSeq	Curated annotations, standardized identifiers, and links to other databases	Genomics, transcriptomics	https://www.ncbi.nlm.nih.gov/refseq/	(Brister, Akoadjei, Bao, & Blinkova, 2015; O'Leary et al., 2016; Tatusova et al., 2016)
UniProtKB	Curated annotations, standardized identifiers, and links to other databases	Proteomics	https://www.uniprot.org/help/uniprotkb	(Apweiler, 2004)
Protein Data Bank, PDB	Atomic coordinates, annotations, and references	Structural biology	https://www.rcsb.org/	(Burley et al., 2023)
KEGG	Graphical maps of pathways, links to other databases, and tools for data analysis	Genomics, transcriptomics, proteomics, metabolomics	https://www.genome.jp/kegg/	(Kanehisa et al., 2023)
GO	Standardized vocabulary for describing gene function	Genomics	https://geneontology.org/	(Ashburner et al., 2000)
Ensembl	Genes, transcripts, regulatory elements, and other genomic features	Genomics, transcriptomics	https://www.ensembl.org/index.html	(Martin et al., 2023)
NCBI Taxonomy	Scientific names, taxonomic ranks, and links to other databases	Genomics	https://www.ncbi.nlm.nih.gov/taxonomy	(Schoch et al., 2020)
ArrayExpress	Graphical maps of pathways, links to other databases, and tools for data	Transcriptomics	https://www.ebi.ac.uk/arrayexpress/	(Parkinson et al., 2011)

	analysis			
Gene Expression Omnibus	Graphical maps of pathways, links to other databases, and tools for data analysis	Transcriptomics	https://www.ncbi.nlm.nih.gov/geo/	(Barrett et al., 2012)
Human Protein Atlas (HPA)	Protein expression data, protein structure data, and other proteomic information	Proteomics	https://www.proteinatlas.org/	(Uhlen et al., 2010)
miRBase	MicroRNA data, microRNA expression data, and other microRNA information	Transcriptomics	https://mirbase.org/	(Kozomara, Birgaoanu, & Griffiths-Jones, 2019)
InterPro	Protein domain and functional site annotations, links to other databases, and tools for data analysis	Proteomics	https://www.ebi.ac.uk/interpro/	(Blum et al., 2021; Jones et al., 2014)
NextProt	Protein sequences, annotations, and references, links to other databases, and tools for data analysis	Proteomics	https://www.nextprot.org/	(Zahn-Zabal et al., 2019)
OMIM	Human gene and genetic disorder information, links to other databases, and tools for data analysis	Genomics	https://omim.org/	(Amberger, Bocchini, Scott, & Hamosh, 2019)
ClinVar (Clinical Variant)	Human genetic variant and phenotype information, links to other databases, and tools for data analysis	Genomics	https://www.ncbi.nlm.nih.gov/clinvar/	(Landrum et al., 2014)

Table 2. Commonly used biological databases.From Data to Insights

The process of gaining entry to biological databases and retrieving biological data holds significant importance for researchers. Multiple databases are accessible that offer maintained and non-duplicated collections of genomes, transcripts, and proteins. The NCBI Reference Sequences (RefSeq) database is a highly utilized and widely used database (Pruitt et al., 2007). The collection contains sequences from prokaryotic, eukaryotic, and viral genomes. The RefSeq records provide a complete picture of the sequence and its characteristics by combining data from multiple sources. The database contains coding areas, conserved domains, tRNAs, and variations.

UniProt stores protein sequences and functional annotations. The Swiss-Prot, TrEMBL, and PIR protein databases are combined to create a comprehensive and thoroughly annotated protein sequence knowledge base. UniProt's non-redundant sequence databases simplify searches. The databases are available online or in various file formats (The UniProt Consortium, 2007).

The European Nucleotide Archive (ENA) is a major repository for nucleic acid sequences and related data (Cochrane et al., 2012). The permanent scientific archive relies on ENA to collect, maintain, and present enormous sequencing data. With a programmable interface for accessing and managing integrated data, the system centralizes data.

Researchers have the opportunity to utilize web-based interfaces or tools that facilitate the retrieval of data from remote database servers (A. Moftah, M. Maatuk, & White, 2018). Users can efficiently and accurately search for biological entities using these technologies.

Biological databases are crucial to biological research since they provide easy access to a wealth of information (Bader, 2006). These tools play an important role in enabling the processes of data mining, analysis, and the identification of novel therapeutic approaches for genetic disorders (Villalba & Matte, 2021).

RefSeq, UniProt, and ENA provide carefully curated sequences and annotations without redundancy. Web-based interfaces, tools, and R packages allow scholars to study the obtained data. The utilization of databases and technologies plays a significant role in the progression of biological research and the acquisition of novel insights within the discipline. Based on the statistical programming language R, the Bioconductor project provides a diverse set of interoperable packages for high-throughput genetic and bioinformatics analysis (Huber et al., 2015).

The process of preparing biological data for analysis in bioinformatics investigations includes multiple phases aimed at ensuring data quality and reducing complexity. Managing missing data is important in research since this can occur for a number of reasons, such as a mistake in an experiment or an unexpected response from a gene (Yi & Latch, 2022).

In addition, to effectively handle the issue of missing data, it is imperative to accurately account for the presence of heterogeneity in gene expression research. It is important to be integrated into preprocessing techniques for biological data to enhance its informational value. To obtain normalized data that accurately represents metabolite concentrations, various data preparation techniques, including centering, scaling, and transformations, are implemented (Van Den Berg, Hoefsloot, Westerhuis, Smilde, & Van Der Werf, 2006). The aforementioned clean data can thereafter be utilized as input for subsequent investigations.

In the realm of bioinformatics studies, the process of readying biological data for analysis encompasses several key tasks, namely the handling of missing data, the consideration of heterogeneity, and the assurance of data quality through the implementation of preprocessing and sample preparation methodologies. The steps mentioned are very important for making sure that biological data analysis is accurate, worthy, and reliable (Fowler, San Lucas, & Scheet, 2019).

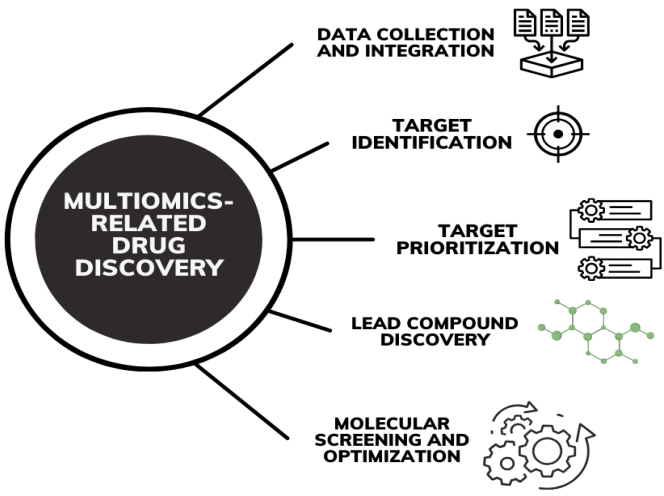

Figure 1. Multiomics data usage steps for drug discovery

Integrating Multiomics Data

Integrating multi-omics data stands for putting together different types of biological data, making sense of how they are connected, and making data presentation easier. This procedure is critical for acquiring a full understanding of complex biological systems and deriving useful insights from the enormous gene and protein lists generated by high-throughput genomic research (Huang, Sherman, & Lempicki, 2009). However, analyzing and visualizing multi-set crossings, which are critical for comprehending complicated relations, can be difficult and inefficient. To overcome this, researchers developed theoretical frameworks and methods for quickly computing the statistical distributions and accurate probabilities of multi-set intersections. Scalable approaches and software packages were put in service for visualizing multi-set intersections, and the statistics were also associated with them (M. Wang, Zhao, & Zhang, 2015). Additionally, bioinformatics assistance and packages such as DAVID and ToppGene Suite are accessible and provide tools for gene list enrichment analysis, functional annotation, and candidate gene prioritization (J. Chen, Bardes, Aronow, & Jegga, 2009; Huang et al., 2009).

Visualization is critical in the analysis and comprehension of omics-scale data because it allows for the creation of clear and integrated representations that reveal biological insights (Gehlenborg et al., 2010). Multiomics data integration provides a variety of applications in biomedical research, including tailored complicated disease therapy and drug and drug target discovery. However, suitable models and databases that can handle the heterogeneity of the data and allow for quick retrieval of essential information are required for the integration and analysis of multiomics data (Thapa & Ali, 2021). In general, putting together multi-omics data means combining, analyzing, and displaying different types of biological data to fully understand complex biological systems and comprehend important highlights.

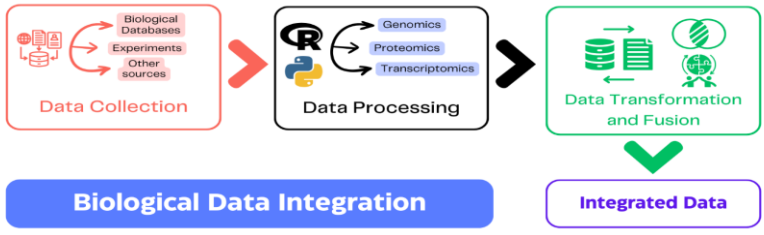

Figure 2. Biological data integration steps

Target and Biomarker Identification

Finding drug targets and biomarkers is an important step in the process of making new drugs and personalized medicine. The powerful methods of proteomics and metabolomics are used in these processes. Proteomics, which is the large-scale study of proteins, has been widely used to reveal possible drug targets. Mass spectrometry-based proteomics is a practical tool in this area by making it possible to find and measure thousands of proteins in complicated samples, which in turn leads the scientists to understand more about how proteins interact with each other, how organelles are organized, and how proteins behave (Aebersold & Mann, 2003). By searching the proteome, researchers can discover proteins that are dysregulated in various diseases that can be utilized as drug targets.

On the other hand, metabolomics studies how metabolites behave in biological processes and has become a useful tool for discovering biomarkers (Johnson, Ivanisevic, & Siuzdak, 2016). Metabolomic analyses can uncover small changes and differences in biological pathways, triggering them to explain how different physiological states and diseases work. By comparing the metabolomic profiles of healthy people and patients, researchers can find specific metabolites that are linked to diseases and may be used as biomarkers for diagnosis, prognosis, and treatment reactions. Both proteomics and metabolomics deal with high-tech tools and computer-based methods. Mass spectrometry is widely used in proteomics since it is necessary to identify and measure proteins. New developments in informatics and analytical tools have made it more efficiently possible to analyze the obtained data via metabolomic analyses. In both areas, computational integration of different sources of knowledge is also important since it is easier to comprehend complex data and encounter possible drug targets (X. Wang, Yang, Sun, & Zhang, 2012).

Overall, proteomics and metabolomics are useful tools for discovering new drug targets and biomarkers that are in progress. Proteomics shows how proteins become out of balance in diseases, while metabolomics shows how metabolic processes vary in diseases. By using these methods together, researchers can learn more about how diseases work, and combining multi-omics data builds up different types of biological data, figuring out how they are related and how the data are linked together. This process is essential for understanding complex biological systems and getting useful information from large lists of genes and proteins from high-throughput genomic experiments. If the multiomics data are combined, the biological themes and relations can be understood more deeply. It is hard and not scalable to analyze and show multi-set linkages, which are

important for understanding complex connections. To overcome this, researchers have come up with theoretical frameworks and methods for quickly figuring out the statistical distributions and accurate probabilities of multi-set intersections (M. Wang et al., 2015).

Molecular Design and Virtual Drug Screening

Drug development approaches include the use of molecular drug docking and virtual drug screening. While molecular drug docking is used to anticipate the binding affinity and orientation of a small molecule drug candidate with its target protein, virtual drug screening uses computer approaches to find prospective drug candidates from huge chemical libraries. AutoDock4 is a popular program for virtual drug screening and molecular docking that takes into account the receptor's limited flexibility. It has undergone thorough testing and validation, including cross-docking studies employing flexible sidechains and redocking experiments (Morris et al., 2009).

Finding prospective therapeutic candidates with high binding affinity to the target protein is the aim of virtual drug screening. Several methods, like pharmacophore-based docking and machine learning-based classification models, can be used to accomplish this. Pharmacophore models can improve the precision and dependability of molecular docking-based virtual screening (Dong, Deng, & Xiao, 2011). The prediction accuracy of active chemicals in docking conformations can be improved by machine learning techniques (Berishvili, Voronkov, Radchenko, & Palyulin, 2018). Molecular drug docking is the process of figuring out how and where a small molecule drug candidate will bind to its target protein. This can be done by utilizing different docking techniques, such as AutoDock (Rauf, Zubair, & Azhar, 2015). Covalent docking is another important part of molecular drug docking. It involves creating a covalent link between the drug candidate and the target protein (Scholz, Knorr, Hamacher, & Schmidt, 2015).

Molecular drug docking and virtual drug screening are crucial methods for developing new drugs. To determine possible drug candidates and forecast their affinities for binding with target proteins, computational approaches are used. They can be more accurate and reliable with the help of pharmacophore-based docking and machine learning-based categorization models, among other things. These methods have been used in many aspects of drug discovery and development, which has sped up and made drug development more affordable.

The studies of molecular design can be categorized into three primary domains, which serve as focal points for novice researchers. The three primary

stages in the drug discovery process include target identification and validation, lead compound identification, and lead compound optimization. By utilizing multisomics data, it is possible to gain insights into the involvement of specific molecules or genes in the pathogenesis of diseases (Anzenbacher & Zanger, 2012). The comprehension of the arrangement and operation of specific molecules enables the development of lead compounds capable of engaging with said molecules (Grant, 2009). The optimization of lead compounds aims to enhance their efficacy, minimize toxicity, and improve their pharmacokinetic and pharmacodynamic properties (Singh, 2006). An additional advantage of multi-omics data lies in its potential to offer insights into the metabolic processes of compounds (Ma, O. Kiesewetter, Lang, Gu, & Chen, 2010).

Various tools are employed by researchers to identify drug targets through the analysis of multiomics data. Despite the continuous growth in the number of available tools, it is imperative for researchers interested in investigating drug discovery and development using multi-omics data to familiarize themselves with the commonly used open-source tools. Table 3 provides a summary of widely recognized tools that play a significant role in the process of drug target identification.

The identification of lead compounds in the field of drug discovery is a critical stage that necessitates the utilization of a range of tools and techniques to screen libraries of compounds and determine potential chemical indicators or initial targets for drug development efforts. Various techniques and procedures have been utilized to achieve this objective, encompassing whole-organism phenotype screening, in vitro model technology, pharmacophore modeling, high-throughput screening (HTS) assays, virtual screening, and structure-based approaches. The primary objective of these tools is to enhance the probability of identifying lead compounds that exhibit success, minimize the rates of drug attrition, and expedite the overall drug discovery process (Lin, 2022).

The process of identifying lead compounds in drug discovery consists of the application of a wide range of tools and technologies. These include whole-organism phenotype screening, pharmacophore modeling, high-throughput screening assays, virtual screening, structure-based approaches, metabolite identification, and toxicology assays. The mentioned tools collectively aim to enhance the efficiency and rate of success in identifying lead compounds, thereby ultimately facilitating the acceleration of the drug discovery process.

New researchers may encounter a term regarding ADMET analysis. The term in question refers to a fundamental aspect of the drug discovery procedure, which

centers on comprehending the Absorption, Distribution, Metabolism, Excretion, and Toxicity characteristics of a prospective drug compound. The significance of conducting this testing cannot be overstated, as the efficacy of a compound against a specific target alone does not guarantee success in subsequent phases of drug development due to the potential negative impact of inadequate ADMET properties (Pantaleão, Fernandes, Gonçalves, Maltarollo, & Honorio, 2022).

ADMET analysis refers to a set of procedures that examine the absorption, distribution, metabolism, excretion, and toxicity of a substance. Each letter in the acronym represents one of these processes (Muscifa, Sumaryada, Ambarsari, & Wahyudi, 2022).

The process of absorption refers to the uptake of drugs or molecules into the tissues and cells from the bloodstream. Insufficient absorption of a drug can impede its ability to attain a therapeutically effective concentration within the target tissue (Patel, Kumar, Rawal, Thaker, & Pandya, 2020). The utilization of in vitro and in vivo analysis yields valuable insights into this particular process.

Distribution is a crucial aspect that requires assessment in the drug discovery process through the utilization of multiomics data. This procedure explains the mechanisms by which a drug diffuses throughout the various compartments of the body and ultimately reaches its intended site of action (Penner, Xu, & Prakash, 2012). To achieve therapeutic efficacy, a drug needs to reach a sufficiently elevated concentration at its intended site within the body, while conversely minimizing its accumulation in non-target tissues to mitigate potential adverse reactions. The utilization of animal studies and nuclear medicine methodologies is of significant importance for this analysis.

Every molecule that enters the human body will undergo metabolism at some point. Therefore, acquiring knowledge regarding the metabolic pathways of a drug is of utmost significance (Amado, Woodley, Cristiano, & O'Neill, 2022. The metabolism of drugs occurs predominantly through enzymatic processes in the hepatic system. The comprehension of a drug's metabolism facilitates the anticipation of its potential interactions with other pharmaceutical substances. Both in vitro and in vivo studies offer valuable insights into the metabolic aspects of ADMET analysis as well as the in silico studies (Zhang et al., 2022).

The removal of drugs from the human body is ultimately determined by the process of excretion. The human body possesses mechanisms that prevent the accumulation of chemicals, drugs, and foreign inorganic/organic substances within its tissues. The elimination of drugs or their metabolites from the body typically occurs via renal excretion (urine) or fecal excretion (stool). Animal

studies are conducted to ascertain the primary pathway of excretion and the half-life of a drug (Luo et al., 2010).

Toxicity testing is the final step in the ADMET analysis. Toxicity testing is critical given that the lower the toxicity, the less harm to the patients. A drug must be safe at its therapeutic dose, and understanding toxicity is critical for calculating the therapeutic index (the toxic dose to effective dose ratio). Toxicity is assessed using in vitro and in vivo tests, as well as in silico methods such as QSAR (Quantitative Structure-Activity Relationship) modeling (Strikwold et al., 2017)

Category	**Tool**	**Description**	**For more information**	**References**
Genomics	NCBI BLAST	Aligns sequences to find regions of similarity, identifying gene variations related to diseases.	https://blast.ncbi.nlm.nih.gov/Blast.cgi	**(Boratyn et al., 2012)**
Genomics	Ensembl Genome Browser	Enables users access to genomic data and annotations, which are helpful in locating genetic targets.	https://www.ensembl.org	**(McLaren et al., 2016)**
Transcriptomics	Cufflinks	Utilizes the RNA-Seq data analysis to discover novel genes and comprehend gene expression levels, frequently in relation to various diseases or therapies.	https://cole-trapnell-lab.github.io/cufflinks	**(Trapnell et al., 2013)**
Transcriptomics	DESeq2	A statistical package for determining differential expression in digital gene expression data using a model based on the negative binomial distribution.	https://bioconductor.org/packages/devel/bioc/manuals/DESeq2/man/DESeq2.pdf	**(Love, Huber, & Anders, 2014)**
Metabolomics	MetaboAnalyst	A comprehensive	https://www.metaboanalyst.ca/	**(Pang,**

		tool for metabolomic data analysis, including pathway analysis and biomarker identification.		**Chong, Li, & Xia, 2020)**
Databases for Target Identification	ChEMBL	A database of bioactive drug-like small molecules; it includes compound bioactivity data against drug targets.	https://www.ebi.ac.uk/chembl/	**(Davies et al., 2015; Mendez et al., 2019)**
Databases for Target Identification	DrugBank	Provides detailed drug data, including chemical, pharmacological, and pharmaceutical data with comprehensive drug target information.	https://drugbank.com/	**(Knox et al., 2024)**
Databases for Target Identification	Human Protein Atlas	Contains information on protein expression across various tissues and organs in the human body, which is useful for identifying potential targets.	https://www.proteinatlas.org/	**(Uhlen et al., 2010)**

Table 3. The tools commonly used for multiomics data associated drug target identification

The process of drug target validation holds significant importance in guaranteeing the effectiveness and safety of prospective pharmaceuticals. Initially, it is crucial to identify the direct involvement of the target in the disease process (Finan et al., 2017). This involves determining the link between the target and the disease, which can be accomplished through a range of experimental and computational techniques. Moreover, it is critical to demonstrate the impact of target modification on disease biomarkers and endpoints, as this constitutes a crucial facet of target validation (Kremmidiotis & Lavranos, 2005). This step entails demonstrating the effect of modifying the target on the disease, thereby demonstrating the target's relevance in the disease process.

Drug targets must be thoroughly investigated, the relationship between the target and the disease must be established, the effects of target perturbation on disease biomarkers and endpoints must be shown, and sophisticated experimental technology platforms must be used. The successful development of safe and effective medications depends on following these procedures (X.-D. Zhou et al., 2022).

The process of structure-based drug design (SBDD) involves various essential stages, which encompass the retrieval and preparation of protein structures, the preparation of a library of ligands, docking, and the structural modification of the most promising compound to facilitate the design of novel compounds (Ibrahim et al., 2020). The utilization of SBDD methods, such as molecular docking simulations and de novo drug design, plays a crucial role in contemporary medicinal chemistry. These methods facilitate the expedited and cost-effective identification of potential lead compounds, surpassing the capabilities of conventional approaches (Batool, Ahmad, & Choi, 2019). Moreover, the determination of ligand-binding sites on the protein surface plays a pivotal role in structure-based drug design (Evteev, Ereshchenko, & Ivanenkov, 2023).

The SBDD pipeline incorporates a range of computational and experimental methodologies, such as docking algorithms, homology modeling, and X-ray crystallography, with the aim of facilitating the logical development of potential drug candidates (Aggarwal & Koes, 2020). In addition, the procedure encompasses the assessment of the desired framework and the essential inquiries to contemplate when selecting an approach for the discovery and evaluation of drug leads (Anderson, 2003).

Furthermore, the application of Structure-Based Drug Design (SBDD) has been utilized to effectively target and combat specific therapeutic requirements. For instance, it has been employed in the creation of inhibitors for the main protease of the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), thereby emphasizing its pivotal contribution in tackling evolving health crises (Nandi, Kumar, & Saxena, 2024). The utilization of this technique has also been observed in the development of pharmaceuticals for neurological disorders, highlighting its adaptability in tackling various medical conditions (Aarthy, Panwar, Selvaraj, & Singh, 2017).

The utilization of computational methods, machine learning, and deep learning to integrate and analyze intricate biological and chemical data has led to the emergence of virtual screening for drug discovery with multi-omics data. This approach shows great potential in the field. The significance of virtual screening

in modern drug discovery is underscored by the potential of multi-omics data in identifying therapeutic targets, addressing biomedical objectives, and advancing precision medicine.

The utilization of multiomics data in virtual screening for drug discovery has emerged as a crucial technique in the field of pharmacogenomics. This approach has gained significance, particularly in light of the growing number of pharmaceutical targets resulting from the sequencing of the human genome (Bissantz, Folkers, & Rognan, 2000). The use of multiomics data in early stages of drug discovery projects has been highlighted as a promising approach (Prasasty & Istyastono, 2019). In addition, the utilization of deep learning-based techniques for integrating multiomics data has demonstrated promise in the field of biomedicine, particularly in the domain of drug discovery (Y. Wen et al., 2023).

Virtual screening, particularly molecular docking, has been recognized as a valuable tool for the identification of bioactive compounds in the context of drug discovery, complementing high throughput screening (HTS) (Rudrapal & Chetia, 2020). Furthermore, computer-assisted virtual screening has been recognized for reducing the cost and time required for drug discovery, with in silico approaches becoming more efficient and cost-effective (Zucca, Scutera, & Savoia, 2013). Furthermore, by predicting molecular properties, machine-learning algorithms have shown promising results in virtual screening for automated drug discovery (Korotcov, Tkachenko, Russo, & Ekins, 2017).

Challenges

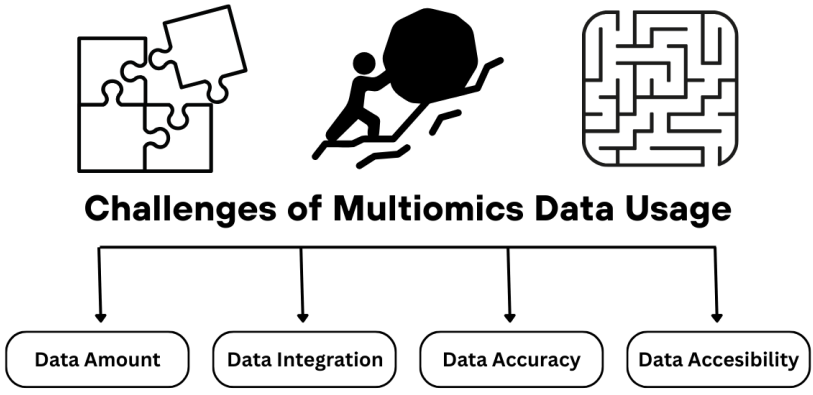

Figure 3. Challenges of Multi-omics Data Usage

By giving researchers access to a wealth of knowledge on pharmaceuticals, drug targets, and drug activities, biological databases serve a critical role in drug discovery and development (Wishart et al., 2008). However, leveraging these databases comes with several difficulties. The enormous amount of data included in these databases presents one of the key difficulties. It may be challenging for researchers to sort through and extract pertinent facts from this volume of data. Additionally, it is difficult to stay up to speed on the most recent information because the material in these databases is always being updated and increased.

The integration of various datasets from multiple sources is another issue. Biological databases contain genomic, proteomics, and chemical data. Integrating numerous data sources and finding trends can be difficult. Chem2Bio2RDF, which links data from systems chemistry, biology, and chemogenomics, is one way that this problem has been partly solved (B. Chen et al., 2010).

Furthermore, it is essential to guarantee the reliability and correctness of the data in biological databases. Even though the data has been curated and validated, mistakes and inconsistencies might still happen. To assure the data's accuracy, researchers must critically assess it and cross-reference it with information from other trustworthy sources.

The usability and accessibility of these databases provide another difficulty. Although many biological databases are readily accessible, using them efficiently and extracting the necessary information may necessitate specific knowledge and abilities. To enable effective data retrieval and analysis, user-friendly interfaces and strong search features are crucial (Wishart et al., 2008).

Biological databases offer significant knowledge for drug discovery and development, but there are still several difficulties that researchers must overcome. The integration of many types of data, the volume and complexity of the data, its quality and dependability, and the database's accessibility and usefulness are a few of these. These issues must be resolved to fully utilize biological databases in advancing drug research and development.

Biological databases and bioinformatics are essential tools in drug discovery's fast-changing, data-driven world. This overview informs beginners on how to navigate this evolving landscape and use multi-omics data for drug development.

Our journey has aimed to guide how to simplify the complexity, from the basics of biological databases to the art of retrieving data and putting it all together. This review tends to enable beginners to navigate the multi-omics world. Target identification, biomarker discovery, and chemical screening have

been facilitated for beginners. Integration barriers and emphasized ethical problems in this emerging industry have been pointed out with beginner-friendly solutions. This review provides insights into the trends and breakthroughs that will build up bioinformatics in drug discovery as technology advances. Interdisciplinary collaboration is the foundation of scientific advancement, and this review encourages all researchers, regardless of their field, to embrace it. This trip strengthens the biological-computational interface, advancing medication development and improving patient care.

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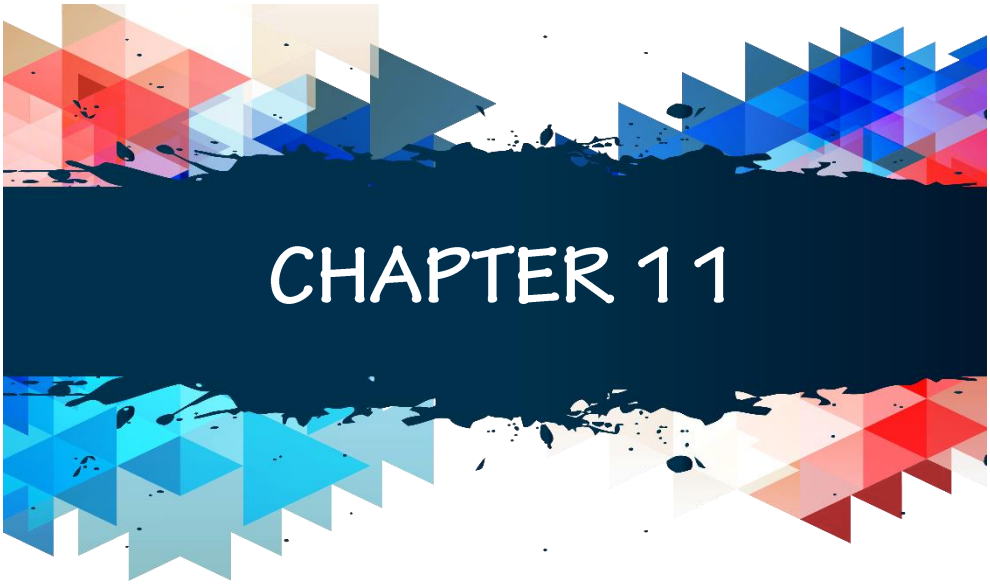

The Role of the Hypothalamus in the Regulation of Food Intake

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Introduction

Nutrition and metabolic events are of great importance for the survival of living organisms (Mayer 1953). Regulation of food intake constitutes one of the most complex regulation mechanisms in the organism (Valassi 2008). The most important neural centres regulating the amount of food to be taken into the body and appetite are located in the brain. The brain is the known coordinator of feeding behaviour and the hypothalamus is the part of the central nervous system where feeding-related processes are controlled (Mayer 1953).

The paraventricular nucleus (PVN), ventromedial nucleus (VMN), dorsomedial nucleus (DMN), arcuate nucleus (ARC) and lateral hypothalamic area (LHA) in the hypothalamus play a role in the regulation of food intake (Conn 2010; Meister 2007). The ARC is the energy nucleus of the hypothalamus involved in the regulation of food intake and energy metabolism and in the perception and evaluation of energy signals. ARC-derived neurone groups are called orexigenic (increasing food intake) and anorexigenic (decreasing food intake). There are many orexigenic and anorexigenic molecules involved in energy balance and central and peripheral control of nutrition. Orexigenic peptides are neuropeptide Y (NPY), agouti related peptide (AgRP), ghrelin, orexin A and B, melanin concentrating hormone (MCH). Anorexigenic peptides are leptin, cocaine amphetamine-related peptide (CART), cholecystokinin (CCS), glucagon-like peptide-1 (GLP-1) (Valassi et al. 2008).

The LHA acts as a hunger centre. The LHA is the feeding centre containing glucose-sensitive neurons evoked through hypoglycaemia and plays an important role in hypoglycaemia-induced hyperphagia. LHA lesions cause hypophagia and weight loss. VMN is known as the satiety centre (Guyton & Hall 2013). It has been identified as an important target region for leptin, which plays a role on the hypothalamus to inhibit food intake. Lesions in the VMN or paraventricular nucleus (PVN) lead to hyperphagia and obesity syndromes. These centres have neuronal connections with each other as well as with other regions of the brain. Stimuli transmitted from the periphery via the nucleus tractus soliterius (NTS) can be delivered directly to their centres. These areas are also associated with developed cortical neurons (Kandel 2000).

Ventromedial Nucleus (VMN)

Neurons of the ventromedial nucleus (VMN), which regulates feeding, are located in the dorsomedial aspect of the nucleus and this area is reciprocally associated with the dorsomedial hypothalamic nucleus. Neurons of the VMN are sensitive to glucose and some respond by increasing glucose (Conn 2010). VMN

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Hyperglycaemia, which occurs with the completion of eating behaviour, is believed to inhibit the feeding centre while activating the VMN (Mayer and Thoma 1967). Electrical stimulation of this area causes a feeling of satiety, leading to the rejection of even most appetising foods (aphagia). Destruction of this area in humans and animals causes gluttony and excessive weight gain. It has been reported that when VMNs are stimulated by food intake, they induce a feeling of satiety and stop food intake through α-adrenergic receptors and obesity develops following weight gain in their lesions (Morley 1987).

Paraventricular Nucleus (PVN)

The paraventricular nucleus (PVN) is adjacent to the upper part of the 3rd ventricle in the anterior hypothalamus and is located dorsal to the medial area of the hypothalamus (Conn MP 2010). The PVN is the main site of corticotropin-releasing hormone (CRH) and thyrotropin-releasing hormone (TRH) secretion. It contains numerous neuronal pathways involved in energy balance that converge in the PVN. The PVN receives nutrient signals from NPY/AgRP and POMC/CART neurons in the ARC. POMC-derived α-melanocyte stimulating hormone (α-MSH) and the appetite-stimulating peptides orexins and galaninine have large projections from NPY neurons. Thus, the PVN plays an important role in the integration of nutritional signals in the thyroid and hypothalamic-pituitary axis. The PVN projects to many regions in the brainstem, such as the nucleus tractus solitarius (NTS), which receives vagal nerve messages from the gastrointestinal tract (Ruggiero et al. 1990). The most effective hormone whose production and secretion increases in anorexigenic neurones under the effect of leptin is α- MSH. The extensions of these neurons reach the PVN in the hypothalamus and by stimulation of the α-MSH receptor melanocortin 4 receptor (MC4R), food intake decreases while energy expenditure increases (Abizaid 2008). Lesions in the PVN lead to hyperphagia and obesity syndromes (Satoh et al., 1997).

Dorsomedial Nucleus (DMN)

The dorsomedial nucleus was identified in the hypothalamus in 1963 by Bernardis et al. through electrophysiological lesion studies. DMN has extensive connections with other medial hypothalamic nuclei and lateral hypothalamus. This nucleus functions to integrate and process information. It is important in the regulation of food intake by circadian rhythm. The DMN receives signals from the SCN, which regulates circadian rhythm in the hypothalamus (Saper et al. 2005). Dorsomedial nucleus lesions have been shown to cause hypophagia (under-eating) and decreased body weight (Jeong et al., 2017).

Arcuate Nucleus (ARC)

The arcuate nucleus (ARC) is located adjacent to the 3rd ventricle and above the median eminence. The ARC is the energy nucleus of the hypothalamus involved in the regulation of food intake and energy metabolism and in the perception and evaluation of energy signals (Van et al., 2014). ARC-derived neurone groups are called orexigenic (increasing food intake) and anorexigenic (decreasing food intake). The ARC contains a population of neurones expressing NPY, AgRP and proopiomelanocortin (POMC), a precursor of α-melanin stimulating hormone known to suppress appetite (Valassi et al. 2008). Of these, NPY/AgRP neurones are orexigenic, whereas POMC/CART neurones are anorexigenic.

Stimulation of POMC neurones decreases food intake and increases energy expenditure. Activation of NPY-AgRP neurons increases food intake and decreases energy expenditure. These neurons are major targets for the effects of various appetite-regulating hormones such as leptin, insulin, cholecystokinin (CCK) and ghrelin (Guyton & Hall 2013). In the hypothalamus, NPY/AgRP neurones are suppressed by the hormone leptin, whereas POMC/CART neurones are positively stimulated. NPY/AgRP and POMC/CART neurones project from the ARC to other regions of the hypothalamus such as PVN, DMN, VMN and LHA (Ahima et al. 2000).

Table 1. Feeding-regulating peptides in the brain

Anoreksijenik peptide	Source Area	Orexigenic peptide	Source Area
α-Melanocyte Stimulating Hormone (α-MSH)	PVN	Neuropeptide Y	ARC
Leptin	ARC	Agouti-Related Protein	ARC, PVN
Cholecystokinin	PVN	Melanin-Concentrating Hormone	LHA
Seratonin	ARC	Orexin Neurons A ve B	LHA
Kolesistokinin (CCK)	PVN	Ghrelin	ARC
Cocaine And Amphetamine-Regulating Transcript	ARC	Galanin	ARC, PVN
Pro-Opiomelanocortin (POMC)	ARC		

AGRP, agouti-related protein; ARC, arcuate nucleus; CART, cocaine and amphetamine-regulating transcript; CCK, cholecystokinin; CRH, corticotropin-releasing hormone; GALP, galanin-like peptide; GHRH, growth hormone-releasing hormone; LH, lateral hypothalamus; MCH, melanin-concentrating hormone; NPY, neuropeptide Y; NTS, nucleus tractus solitarius; Pe, periventricular nucleus; POMC, pro-opiomelanocortin; PYY, peptide YY; PVN, paraventricular nucleus; SCN, suprachiasmatic nucleus; SON, supraoptic (Shioda ve ark. 2008).

Lateral Hypothalamic Area (LHA)

The lateral nuclei of the hypothalamus serve as the hunger centre (Guyton & Hall 2013). The LHA is the feeding centre containing glucose-sensitive neurons evoked through hypoglycaemia and plays an important role in hypoglycaemia-evoked hyperphagia (Bernardis et al. 1996). The lateral hypothalamic area (LHA) is a subcortical brain region that exerts control over motivated behaviour, feeding and energy balance across species. Recent single-cell sequencing studies have identified at least 30 different LHA neuron types. Some of these affect certain aspects of energy homeostasis; however, the functions of many LHA cell types remain unclear (Rossi 2023). LHA and VMN are mutually related in the regulation of food intake and energy metabolism. In addition to the regulation of food intake, LHA and VMN control frequent inter-meal eating behaviour and the

adjustment of the number of meals and meal size in food intake (Meguid et al. 1997).

LHA cells project directly to the autonomic and motor systems of the cerebral cortex, spinal cord (Funahashi et al. 2003) and brainstem. LHA neurons have two distinct groups of neuronal cells containing hypocreatine/orexin and MCH that regulate feeding behaviour. In the human brain and rodents, MCH and orexins are found in the perifornical area, LHA and zona incerta. Neurochemical peptides have received more attention with the discovery of leptin, which projects along neurons in the ARC and VMN, leading to food intake and body weight-reducing effects. Leptin directly affected cytosolic Ca+2 concentration in a simple neuron isolated from the ARC, VMN and LHA. Leptin inhibits glucose-sensitive ARC and LHA neurones and stimulates glucose-sensitive VMN neurones by increasing leptin receptors in these cells. Increased MCH mRNA levels increase food intake when leptin levels fall rapidly in the fasting state and during intracerebroventricular administration of MCH. Increased or central administration of peptides such as CART in the LHA decreases food intake (Rossi 2022).

Preproorexin mRNA is localised in the hypothalamus, especially in the lateral and posterior hypothalamic areas. Orexins cause protein kinase- C-mediated Ca+2 entry in the LHA, stimulated by Ca+2 decrease in spinal neurons, Ca+2 increases in A10 dopaminergic neurons (Smarta and Jerman 2002). Ventral tegmental area (VTA) dopamine cells innervate through ghrelin-sensitive orexin neurons in the LHA and ghrelin directly increases DA release (Abizaid 2009). Changes in neuroendocrine mediators in the gastrointestinal tract and peripheral areas affecting fat stores play a role in the regulation of food intake. Vagal fibres from the gastrointestinal tract combine with projections through the NTS and LHA and exert a combined effect on food intake. A drop in blood glucose level may signal the onset of food intake. The LHA contains glucose-sensitive neurons that are activated via glucopenia, and thus, in a short time, favourably regulate feeding and energy expenditure. Some or all of the orexin neurones may be glucose-sensitive neurones or may receive stimulus projections from glucose-sensitive neurones (Subramanian and Ravichandran 2022).

LHA and VMN are also the reward-punishment centre in the brain (Guyton & Hall 2013). The reward mechanism in the brain is mediated by the DA receptor D2R. D2R deficiency in the striatum is associated with decreased metabolic activation in the prefrontal cortex (PFC) orbitofrontal cortex (OFC). OFC plays

a role in food recognition and impulse control. When the OFC is dysfunctional, it is unable to control dominant impulses, leading to aggressive behaviour in food craving. In humans, the level of DA in the dorsal striatum is decreased in relation to the pleasure derived from eating pleasant foods. A low number of D2Rs in the striatum causes hyperphagia. D2R receptor deficiency reduces the sensitivity of the feeling of satisfaction occurring in the nucleus accumbens and as a result, obese individuals consume more food to cope with the lack of DA signals (Volkow et al. 2011).

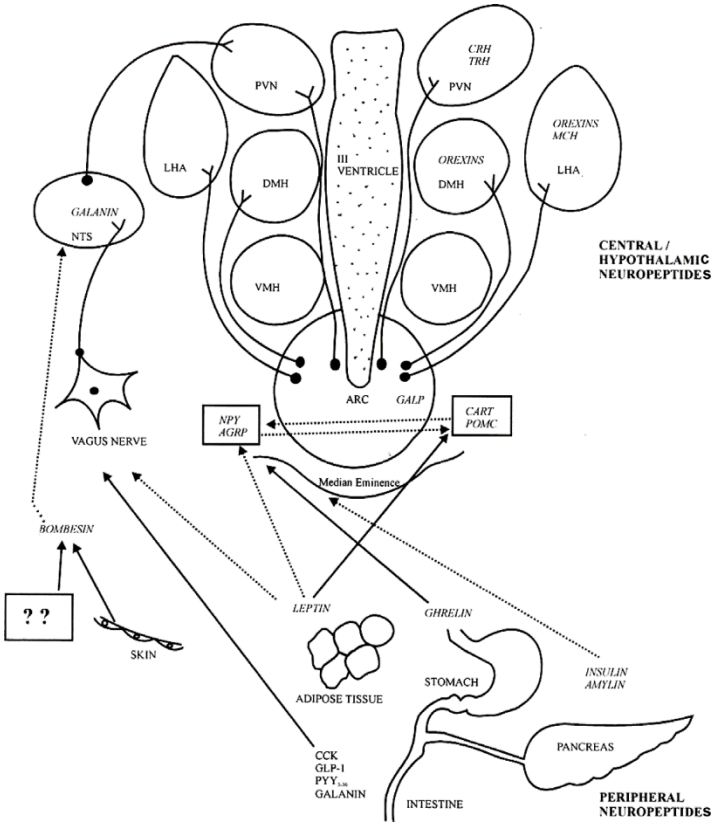

Fig. 1. Chart showing the interrelationship between the various appetite-regulating neuropeptides and their main sites of origin and action. The superior part of the diagram shows the relative position of the hypothalamic nuclei involved in the regulation of appetite, as seen in the coronal part of the hypothalamus. The bottom part shows the peripheral organs and the neuropeptides secreted by them. The solid lines indicate an excitatory effect and the dotted line indicates the inhibitory effect of the peripheral neuropeptide on the hypothalamic nuclei/neuropeptides. Experimental studies have shown that lesions in the medial part of the hypothalamus are associated with a hyperphagia, whereas lesions in the lateral part of the hypothalamus lead to loss of appetite. Abbreviations PVN- Paraventricular nucleus: LHA - Lateral Hypothalamic Area: DMH - Dorsomedial hypothalamus: VMH - VMH: Ventromedial Hypothalamus: ARC - Arcuate Nucleus: NTS - Tractus Solitarius Nucleus: ?? - Unknown origin of the neuropeptide Bombesin. (Arora 2006)

Orexigenic Molecules

Neuropeptide Y (NPY)

NPY is a neurotransmitter containing 36 amino acids and produced in the central nervous system which was first discovered in the pig brain by Tatemoto and Mutt in 1982. NPY is located in the hippocampus, amygdala, ARC, PVN, SCN, median eminence (ME), VMN, and LHA regions of the hypothalamus which are located in the part of the hypothalamus adjacent to the 3rd ventricle. In the ARC, NPY neurons project to the PVN and LHA regions of the hypothalamus (Williams et al. 2000). NPY functions in many signalling pathways via G protein-coupled receptors. NPY has six G-protein coupled receptors; Y1, Y2, Y3, Y4, Y5 and Y6. All these receptors bind to G protein and inhibit adenylyl cyclase. NPY1 and NPY5 receptors are associated with appetite stimulation. Whereas NPY2 and NPY4 receptors lead to appetite suppression. Y5 receptors, to which NPY binds to stimulate food intake, are found at higher levels in the LHA compared to other areas (Williams et al. 2000). NPY is a potent orexigenic peptide that stimulates food intake. Chronic administration of NPY in experimental animals leads to obesity with hyperglycaemia and hyperinsulinaemia (Conn 2010).

Agouti Related Peptide (AgRP)

AgRP is a 132 amino acid orexigenic peptide involved in the regulation of nutrition and body weight. AgRP is released exclusively from the ARC of the hypothalamus in the brain and all AgRP-producing neurones are co-secreted with NPY. PVN, DMN and LHA project to various hypothalamic areas. In the hypothalamus, AgRP has an important function in the ARC in food intake and food-seeking behaviour. Leptin inhibits the release of AgRP. AgRP is an effective antagonist of MC-3 and MC-4 receptors of melanocortin receptors involved in energy balance control and affects food intake. Increased amount of AgRP has been shown to cause obesity (Arora 2006).

Ghrelin

Ghrelin is a 28 amino acid orexigenic peptide released from the gastric enteroendocrine (Lee et al 2002), which has been primarily identified as an endogenous ligand for the growth hormone secretagogue receptor (GHSR). Ghrelin is released from a number of neurones in the hypothalamus located between the LHA, ARC, VMN, DMN and PVN. This region in the hypothalamus is intertwined with extensions from the SCN. Ghrelin is also synthesised from placenta, kidney, heart, thyroid and leyding cells. It plays a remarkable role in GH-released and orexigenic effects, prolactin secretion under the control of

adrenocorticotropic hormone (ACTH), glucose and lipid metabolism, gastric motility, acid secretion, cardiac function, sleep and reproduction (Valassi et al. 2008). The hormone ghrelin has been shown to be a major regulator of feeding behaviour in both humans and some rodents. It exerts its appetitive effect via NPY and AgRP in the ARC in the hypothalamus. The level of ghrelin in plasma increased during fasting and decreased during postprandial periods. Therefore, ghrelin is shown as an appetite-increasing or food intake triggering hormone. As in other physiological effects of ghrelin, it uses GHSRs in its appetite-enhancing effect. The hormone realises this appetite-increasing effect through the NPY molecule in the hypothalamus. GHSR is located in the hypothalamus-pituitary unit, especially on NPY and GHRH neurons. The effect of ghrelin is mediated by inhibition of POMC neurons and enhancement of NPY/AGRP pathways in a manner opposite to leptin (Yanagi et al. 2018).

Orexin A and B

In 1998, two precursor peptides of orexin, orexin A (OXA) and orexin B (OXB), were identified. These two small peptides of 33 and 28 amino acids in length, orexin A and B, also called hypocretin 1 and 2, originate from the same precursor protein called preproorexin (Arora 2006). The effects of orexins are mediated by G protein-coupled receptors called OX1R and OX2R. In fasted rats, OXA increases the coupling of orexin receptors in the hypothalamus with Gq, Gs and Go proteins, while decreasing their coupling with Gi (Karteris et al. 2005). OXA elicits many prominent orexigenic effects by combining with OXB. PFA, LHA and DMN neurones are also expressed in the hypothalamic area including the NTS and projections adjacent to the hypothalamic nucleus. These receptors are specifically expressed in neurons localised around the LHA. Preproorexin is most abundant in the lateral and posterior hypothalamus. In starvation, preproorexin increases the amounts of orexin A and B mRNA. Intracerebroventricular injection of orexin-A or its administration to LHA results in a rapid increase in food intake (Willams et al. 2000). Preproorexin-expressing neurons in the LHA have projections in a wide area from the cortex to the upper part of the spinal cord. These projections involve the hypothalamus and other structures in the regulation of feeding. ARC and NTS are some of these areas. The NTS receives vagal afferent stimuli from the intestine and transmits them to the LHA (Cai et al. 2002). The interrelationship of the LHA with many different parts of the brain is shown to be the place where information about eating behaviour is collected. Therefore, destruction of LHA neurons in mammals results in hypophagia, indicating that the neuronal pathways that provide the balance of eating behaviour are disrupted. The observation of orexin nerve

endings in the ARC and PVN in the hypothalamus suggests that the regulation of eating behaviour also occurs in these regions. Apart from the hypothalamus, orexin nerve endings are observed in the medial structures of the thalamus in the cerebral cortex, circumventricular organs (subfornical organ and area postrema), limbic system (hippocampus, amygdala, septum, indusium griseum) and brainstem (LC and raphe nucleus). In addition, 30% of orexin neurons are activated by insulin-induced hypoglycaemia, indicating that plasma glucose level affects the activity of orexin neurons (Williams et al. 2000). Orexin peptides and their receptors may play a role in various regulatory systems including neuroendocrine, temperature control and cardiovascular, as well as regulation of energy homeostasis and nutrient intake (Subramanian and Ravichandran 2022).

Melanin-Concentrating Hormone (MCH)

MCH is a cyclic neuropeptide containing 19 amino acids. The cell bodies of MCH-containing neurons arise mainly from the LHA and zona incerta, known as the feeding center. There are two types of receptors for MCH signals in the brain, called G protein-coupled melanin-concentrating hormone 1 receptor (MCH-1R) and melanin-concentrating hormone 2 receptor (MCH-2R). These receptors project widely, especially in the brain regions hippocampus, amygdala and cerebral cortex (Arora 2006). With the infusion of MCH in rats, significant hyperphagia begins and body weight increases. MCH-damaged rats (knockout) are resistant to diet-induced obesity due to increased energy expenditure and locomotive activation. In Ob/Ob leptin-deficient mice, MCH mRNA levels were increased and food intake was increased. Administration of leptin reduces MCH expression and thus food intake decreases (Valassi et al. 2008).

Anorexigenic Molecules

Leptin

Discovered in 1994 by Friedman and colleagues, leptin is a 16 kDa polypeptide hormone (Zhang et al. 1994). Initially thought to be synthesised from white adipose tissue, it has now been found to be synthesised from placenta, skeletal muscle, stomach, mammalian epithelial cells and anterior pituitary. The main role of leptin in the body is to regulate food intake and energy metabolism and prevent the development of obesity through a negative "feedback" effect on the brain (especially the hypothalamus). In addition, regulation of metabolism and body weight maintenance (Robert et al. 2011), sexual development and reproduction, regulation of gastrointestinal functions, sympathetic nervous system activation have been found to have very important roles. Leptin is found in circulation in proportion to body fat mass and passes to the central nervous

system in proportion to plasma levels. Leptin receptors are divided into two groups as long receptors (OB-Rb) and short receptors (OB-Ra). OB-Rb is mostly located in the ARC, PVN, DMN and LHA of the hypothalamus and has an important role in feeding (Arora 2006).

When the amount of adipose tissue increases, adipocytes increase leptin production, leptin travels to the brain, crosses the blood-brain barrier by facilitated diffusion and binds to OB-R at various sites in the hypothalamus, particularly in the POMC neurons of the ARC and PVN. Stimulation of OB-R in these hypothalamic nuclei initiates several effects that reduce fat storage. Leptin regulates energy intake or expenditure by binding to its specific receptors in the hypothalamus to alter the expression of several neuropeptides that regulate neuroendocrine function. Leptin directly affects two important neurones in the ARC. It affects the anorexigenic CART/POMC neuropeptides that decrease food intake and the orexigenic NPY/AgRP neuropeptides that increase food intake.

Leptin suppresses CART/POMC neurons in starvation and re-enhances NPY/AgRP expression. Leptin may also regulate short-term energy intake and modulation of meal size according to changes in energy balance. With a negative energy balance, low leptin signalling inhibits the action of POMC/CART neurons and increases NPY/AgRP release, activating the anabolic cycle and inhibiting the catabolic cycle, with a decrease in energy expenditure and an increase in meal size. Genetic absence of leptin or leptin receptor is associated with hyperphagia and many types of obesity (Valassi 2008). Leptin may also reduce food intake by coupling with NA projections in the brain. An inverse relationship emerges between leptin activity and NA. Increased leptin decreases NA activity to suppress eating behaviour, whereas in the absence of leptin, increased NA release results in increased food intake.

Proopiomelanocortin (POMC)

POMC is the precursor of several molecules including α-MSH, the main regulator of energy balance. The anorexigenic effect of melanocortin is mediated by two receptors, MC3R and MC4R, which are abundant in the brain and especially in the ARC. These receptors are found in the DMN, LHA and ARC of the hypothalamus. More than 5% increase in body weight due to involuntary overeating behaviour in rats has been observed to increase POMC expression. The key role of the melanocortinergic system as a mediator of anorexigenic signalling makes the use of agonist or antagonist molecules promising for the treatment of eating disorders. Due to the long-term anorexigenic effects of these

molecules, MC4R analogues are suitable candidates as antiobesity drugs (Arora 2006).

Cocaine Amphetamine Related Peptide (CART)

CART is a relatively new neuropeptide consisting of 102 amino acids with anorexigenic effect (Arora 2006). CART peptides are localised in specific areas of the hypothalamus, including the PVN, DMN, ARC, LHA and perifornical regions. CART mRNA in the PVN is controlled by neurones containing vasopressin and corticotropin-releasing factor. ICV dose-dependent administration of CART was found to markedly reduce food intake (Valassi et al. 2008). In the ARC, CART neurones are almost completely co-localised with POMC neurones. In starved animals, there is a decrease in CART mRNA in the ARC (Arora et al. 2006).

Cholecystokinin (CCK)

Cholecystokinin (CCK) is an important endogenous peptide found in the gastrointestinal tract and brain (Arora 2006). Cholecystokinin plays an important role in many physiological functions such as pancreatic secretion and stimulation, gallbladder contraction, intestinal motility, memory enhancement and inhibition of gastric motility. CCK receptors are G-protein coupled receptors. CCK has a direct effect on food intake through activation of CCK-A receptors on vagal afferent neurones. Furthermore, endogenous CCK reduces the meal size in the intake of different types of food. Indeed, intravenous infusion of CCK-33 reduced the size of a single food meal as well as the degree of hunger after a meal in humans (Valassi 2008).

Glucagon-Like Peptide-1 (GLP-1)

GLP-1 is another gut hormone released in response to food intake. It suppresses glucagon secretion and delays gastric emptying, while increasing the stimulation of glucose-induced insulin synthesis and secretion (Arora 2006). Furthermore, glucagon infusion decreases food intake and body weight in rats. Preproglucagon gene synthesis is restricted to NTS, neurons in the brain stem nucleus and pancreatic α-cells, and intestinal L-cells. Recent studies have shown a significant dose-dependent decrease in the amount of GLP-1 in both lean and obese subjects at desired caloric intake. Intravenous infusion of exendin-4, a long-acting GLP agonist, reduced fasting and postprandial glucose levels in healthy volunteers, as well as a 21% reduction in daily food intake. It is commercially available as a combination therapy to improve glycaemic control in patients with type 2 diabetes mellitus treated with known oral antidiabetic drugs (Valassi et al. 2008).

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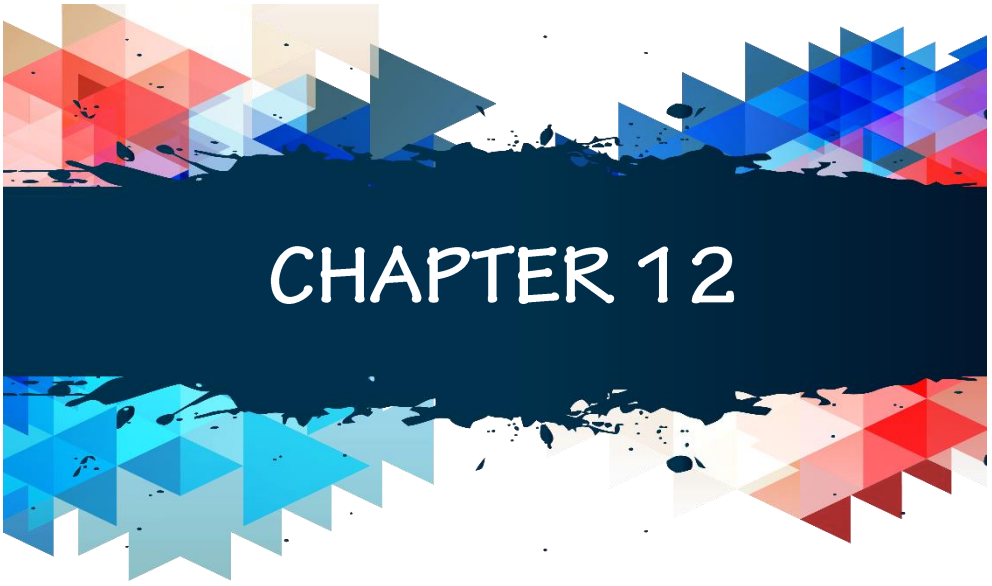

Animal Models of Alzheimer's Disease for Experimental Research

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Introduction

With the increase in the elderly population in the world, there is also an increase in age-related diseases. One of the most common of these diseases is Alzheimer's disease (AD). AD is characterized by memory impairment in daily life functions. According to epidemiologic studies, the number of patients is expected to be over 100 million in 2050. The most prominent feature of the disease at diagnosis is a decrease in cognitive capacity. In addition, behavioral disorders, difficulties in early diagnosis and treatment, classical misinformation, care and caregiver burden and cost, treatment difficulties and cost affect all countries of the world. Early and accurate diagnosis of AD has become mandatory in our aging society. Experimental animal models have been very useful in understanding physiopathological mechanisms in neurodegenerative diseases. AD animal models in the field of learning-memory have helped to understand how the damaged brain functions. Experimental Alzheimer's models are frequently used to develop treatment strategies. In this chapter, experimental AD models, how these models are applied and their contribution to the elucidation of physiopathological mechanisms are described.

Alzheimer's Disease

AD was first described as a type of dementia by German psychiatrist Alois Alzheimer in 1906. A middle-aged female patient with memory and progressive loss of cognitive abilities was followed up for 5 years. After her death, an autopsy showed neurofibrillary tangles and senile plaques in the neocortex and hippocampus. After this case was reported, the term AD was coined as a form of early dementia (Kandel et al. 2013).

AD is defined as premature ageing of the brain. It usually starts in middle age and progresses rapidly, leading to excessive loss of mental power similar to that of very old age. These patients usually require continuous care within a few years after the onset of the disease (Guyton and Hall 2013).

Epidemiological studies indicate that AD is not due to a single factor. Neuronal and central vascular disorders due to aging play a very important role in development of the disease. In addition, head traumas, viral infections and metabolic lesions increase the risk of AD. In some cases, genetic predisposition has a significant contribution (Uzbay 2003).

AD is considered as a form of progressive and irreversible dementia. It is characterised by progressive memory impairment and decreased cognitive performance. Noncognitive symptoms such as depression, aggression and

psychosis may also accompany AD. Early degeneration of cholinergic system nicotinic neurons, amyloid plaque deposition, neurofibrillary tangles, neuroinflammation and white matter loss are observed in brain tissue (Lecanu et al. 2013).

Physiopathology of Alzheimer's Disease

AD is known to affect a large number of people worldwide. It is characterized by progressive memory loss at cognitive level. The formation of extracellular amyloid plaques and amyloid angiopathy in the brain by amyloid beta (Aβ) peptides represents an important step in pathogenesis of the disease (Ştefănescu et al. 2019).

AD is a neurodegenerative disorder and causes cognitive decline and behavioural changes. In older adults, it is the most common cause of dementia. AD is characterised by the Aβ plaques in the cerebrum and the assembly of neurofibrillary tangles (NFT). Aβ deposition leads to formation of NFT and consequent neurodegeneration. Aβ plaques and tau protein tangles in the brain results in the death of brain cells and shrinkage of brain tissue. There is no definitive aetiology for AD, but it appears to be linked to various factors such as genetics, environment and lifestyle. Although no definitive cure has been found, some treatments have been proven to help manage symptoms and improve patients' quality of life (Ivraghi et al. 2023).

The pathophysiology of AD is multifactorial. AD is characterized by macroscopic and microscopic structural changes and dysfunction of the neuronal axis in the brain. Macroscopically, AD is associated with brain atrophy with enlargement of the lateral ventricle. At the microscopic level, AD has been associated with plaques, synaptic degeneration, and neuron loss, especially in the hippocampus and temporal cortex. The disease begins with Aβ plaque production triggered by risk factors. It continues with NFT formation due to hyperphosphorylation of tau protein (Trillo et al. 2013).

Causes of Aβ peptide overaccumulation in the pathogenesis of AD:

1) All known mutations linked to AD increase Aβ peptide production.

2) Patients with trisomy 21 may show neurologic features of AD in middle age with APP gene mutation.

3) Patients with abnormal genes controlling the production of apolipoprotein E (Apo E) have rapid amyloid deposition and this greatly increases the risk of AD.

4) Transgenic mice producing human APP show learning and memory impairments due to the accumulation of amyloid plaques.

5) The formation of anti-amyloid antibodies in humans with AD may alleviate the disease process (Guyton and Hall 2013).

The pathophysiology of AD is multifactorial. AD is a neurodegenerative disease characterized by macroscopic and microscopic structural changes and dysfunction of the neuronal axis in the brain. Macroscopically, AD is associated with brain atrophy with enlargement of the lateral ventricle. At the microscopic level, AD has been associated with plaques, synaptic degeneration, and neuron loss, especially in the hippocampus and temporal cortex. The disease begins with Aβ plaque production triggered by risk factors. It continues with NFT formation due to hyperphosphorylation of tau protein (Trillo et al. 2013).

The clinical features of AD are memory impairment, impaired language function, visual and spatial disturbances. Motor and sensory abnormalities, gait disturbances and seizures are not seen until the late stages of the disease. In AD, there is also a loss of neurons in the part of the limbic pathway that manages memory processes. This loss of memory function is devastating. . AD is a progressive and fatal disease. AD causes inability to perform daily activities and behavioral disorders in the late stages of the disease (Guyton ve Hall 2013).

Drug development for AD has not been very successful. Drug manufacturers have focused on the loss of cholinergic neurotranmission. Tacrine was introduced in the early 1990s and has shown some effect in clinical trials. However, its therapeutic use caused liver toxicity. Later, tacrine was replaced by asthylcholinetransferase inhibitors such as rivastigmine, galantamine, donepezil, which provide controversial therapeutic benefits without liver toxicity. The N-methyl-aspartate (NMDA) antagonist memantine has been of little benefit in the treatment of AD. Drugs such as the Aβ plaque formation inhibitor tramiprozate, the γ-secretase modulator tarenflurbil, the antihistamine latrepirdin and more recently the monoclonal antibodies bapinuzumab and solanezumab have been tested in clinical trials. These compounds have been reported to show significant efficacy in transgenic AD models (Lecanu et al. 2013).

Experimental Animal Models of Alzheimer's Disease

Experimental animal models are experimental constructs performed on one species to investigate a phenomenon occurring in another species and help to identify treatments for a disease (Kumar et al. 2013). Animal models should reflect a phenotype as similar as possible to the disease and contribute

significantly to the development of treatment options (Lecanu and Papadapoulos 2013).

Effective modeling strategies are needed to better understand the underlying mechanisms in AD. Most clinical trials and research towards better treatment of AD fail due to the inability of the animal models investigated to accurately imitation the actual AD pathology. Most current AD models have been developed based on mutations found in familial form of AD, which accounts for less than 5% of AD incidence. Furthermore, research faces further challenges due to the complexities found in the aetiology of sporadic AD, which accounts for 95% of total AD (Ulaganathan & Pitchaimani 2023).

Over the last 20 years, AD transgenic models based on the known genetic origin of familial form of AD have contributed significantly to our understanding of molecular mechanisms involved in the onset and progression of AD. These models have been widely used in the development of AD treatment. In many clinical trials, it has been reported that the flaws in new treatment modalities are that use of AD genetic models does not accurately reflect characteristics of AD in humans. Non transgenic animal models should be used for the sporadic form, which reflects 95% of AD (Lecanu and Papadapoulos 2013).

Although a wide variety of animal models have been developed to imitation some specific features of AD pathology, none of these animal models fully represent the cognitive and memory impairment or biochemical and histopathological abnormalities behold in AD patients. Therefore, the development of an ideal AD model is vital to fully understand the pathology of the disease (Van Groen 2005).

AD animal models have been very useful in understanding psychological and physiological basis. Animal models about learning and memory have helped to understand how damaged and normal brain functioning occurs (Table 1) (Narwal et al. 2012).

Rats have a wide range of behavioral traits and can be well characterized behaviorally. They also have fine and precise motor coordination. Rats show little stress and are skilled in maze tests. AD models provide more accurate results in evaluating cognitive outcomes at the behavioral level (Do Carmo 2013).

Table 1: Animal models of Alzheimer's disease for experimental research

Animal models of Alzheimer's disease for experimental research	
I. Transgenic animal models	Transgenic models of Aβ accumulation
	Transgenic models of Tau protein
II. Non-transgenic animal models	Aβ peptide-associated AD model
	Tau protein-associated model of AD
	Experimental model of aluminum toxicity
	Experimental model of AD induced by ovariectomy
	Experimental models of AD due to loss of cholinergic function
	Experimental AD model induced by intracerebroventricular injection of streptozotocin
	Experimental model of AD induced by bilateral carotid artery
	Experimental AD model induced by sodium azide

Transgenic animal models

Several transgenic mouse models have been established to study AD physiopathology via overexpression of presenilins and amyloid precursor protein (APP). Even none of the transgenic AD models accurately replicate the human condition, the unique to study similar pathological processes in living animals has provided valuable insights into disease mechanisms and the possibility to test therapeutic approaches. Experimental AD models have a role in understanding the relationship between Aβ and tau pathologies as well as the role of soluble Aβ oligomers in disease pathogenesis. Data from various AD models show that the onset of Aβ deposits are related to the level of soluble Aβ1-42 peptide. In AD transgenic models, Aβ1-42 has been reported to effect early neuronal death as well as synaptic impairments. Studies in transgenic AD animal models indicate that synaptic changes precede neuronal deficits, in line with the hypothesis that synaptic impairment is one of the earliest occurrences in AD pathogenesis. In addition, transgenic AD models also support that Aβ may interact with tau to

accelerate NFT formation. Transgenic AD models may allow the development of the evaluation of potential therapeutic targets (Schaeffer et al. 2011)

Several mouse transgenic models carry a mutated human tau gene that develops neurofibrillary degeneration. Tau overproduction leads to tau hyperphosphorylation and development of NFT as reported in AD. Behavioral analyses and maze tests have highlighted impairments in sensorimotor and reflex responses, as well as progressive cognitive decline in spatial navigation. These impairments are associated with progressive accumulation of NFT, insoluble tau complexes and axonal damage extending to spinal cord and brainstem. The rat AD model about progressive NFT in the cortex contains unmutated human truncated tau protein. Cortical neurofibrillary degeneration is observed in 9 months rats (Do Carmo, 2013).

The AD animal model, the 3 × Tg-AD mouse, has many of the features observed in AD pathology. It also shows cognitive changes in memory and learning tasks. 3 × Tg-AD is better able to show amyloid β (Aβ) and neurofibrillary tangles (NFT), two hallmarks of AD. Therefore, 3 × Tg-AD is widely used in AD pathogenesis research and new drug development of AD (Tian et al. 2023).

Rats more closely resemble humans than mice physiologically, genetically and morphologically. Their large body and brain size facilitate drug administration, microdialysis, cerebrospinal fluid sampling, in vivo electrophysiology, neurosurgery and brain imaging procedures. Regarding AD modeling, similar to humans, rats contain 6 isoforms of tau protein. There is also good similarity between human and rat Apo E aminoacid sequences (73.9% with Apo E4, 73.5% Apo E3). Rats have more precise motor coordination and exhibit rich behavioral traits compared to mice. Rats are more skilled in Morris water maze experiments. AD rat models provide more accurate assessments of cognitive outcomes. Importantly, some transgenic rat models have been reported to show features that more closely resemble human diseases compared to mice carrying the same transgenes (Do Carmo 2013).

Non-transgenic animal models

Aβ peptide-associated AD model

Transgenic animal models are invaluable for investigating mechanism underlying the pathogenesis of AD. But overproduction of AD-related genes can lead to errors in interpreting findings from these studies. Presenilin 1 (PS1) and APP overproduction may affect memory, synaptic function and CNS

development. To bran the specific effects of Aβ from the other effects of overproduced APP and PS1, disease models in which Aβ alone is responsible are used. Synthetically produced Aβ oligomers are injected directly into hippocampus and ventricles of animals to model acute Aβ model. The infusion is ensured through cannulas in order to ensure direct delivery of Aβ to site of administration and to avoid problems with blood brain barrier. Aβ can be effective in impairment of associative and reference memory. Aβ is generally considered to be a peptide with a direct role in memory disorders. Another advantage of using an acute Aβ rat model compared to developing a transgenic animal model is the lower cost. However, the disadvantages of using the Aβ infusion model are;

I. These features of AD, senile plaques, NFT and neuronal loss, cannot be reproduced;

II. Aβ preparation and interleaved trial efficacy tests require very careful methodological standardization;

III. It does not allow testing of treatment protocol on disease progression;

IV. Since the animals were cannulated beforehand, it may cause problems in behavioral studies (cannulas may fall off, multiple injections may cause stress in the animal, it may become difficult to follow the animals over time) (Puzzo et al. 2014).

The intrahippocampal injection of Aβ 1–42 typify one of the most useful animal AD models. Aβ 1-42 is often used to create an AD experimental model. Aβ peptide is solubilised and incubated at 37°C for 72 hours to form fibrillar structures. Aβ peptide is injected bilaterally (2.2 nmol/10 µl) into the hippocampus in the AD model (Figure 1) (Ozen Koca et al. 2023).

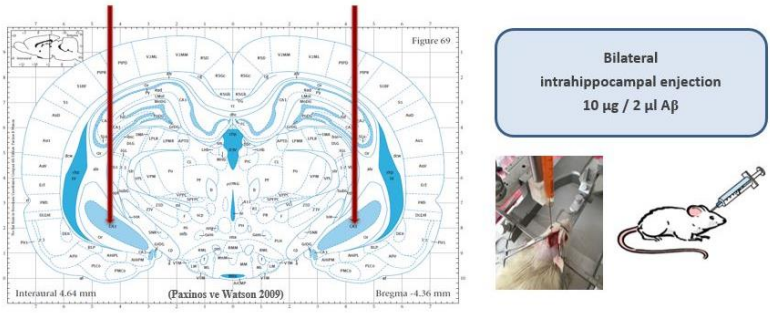

Figure 1. Aβ peptide-associated AD model and intrahippocampal injection of Aβ 1–42 (*Ozen Koca et al. 2023*).

Tau protein-associated model of AD

Extracellular soluble tau has been discovered in high amounts in cerebrospinal fluid in both healthy individuals and AD patients, and effect of tau oligomers on synaptic function has been tested. Similar to Aβ, tau oligomers impair long-term potentiation (LTP). The effects of tau oligomer infusion into the dorsal hippocampus on memory were also investigated. Consistent with the LTP results, oligomeric tau infusion impaired memory in mice. Several studies have shown that acute exposure models can be used as an alternative to investigate AD pathogenesis and drug administration (Puzzo et al. 2014).

Experimental model of aluminum toxicity

Aluminum (Al) toxicity results from disruption of the metal homeostasis such as calcium (Ca), magnesium and iron, resulting in formation of ROS. Al significantly inhibits functions of mitochondrial complex I- III-IV, severely limiting the amount of ATP produced through oxidative phosphorylation. Al toxicity usually manifests as a Ca^{2+}-mediated excitotoxicity. Interestingly, apoptosis plays an important role in neuronal loss in Al-induced excitotoxicity. Al-ATP causes excitotoxicity induced by glutamate hyperstimulation by potentiating the activation of ionotropic glutamate receptors, thereby elevating neuronal tau levels and forming NFT. Studies have shown that oral administration of Al for 90 days (50 mg/kg) in rats results in cognitive impairment and slowed locomotor activity. It also increased catalase and decreased glutathione, acetylcholine (ACh). Increased neuronal loss was shown histopathologically (Chavan et al. 2023).

Experimental model of AD induced by ovariectomy

In studies, the decrease in estrogen and progesterone in menopause was found to be a significant risk factor for the development of AD in women (Green et al. 2004). It has been shown that the age at which AD disease is observed is earlier in women who enter early menopause and in women with low estrogen levels after menopause (Schupf et al. 2008). This suggests that the decrease in the amount of estrogen after menopause may lead to AD. Phosphorylated tau protein and Aβ peptide, two important biochemical markers of Alzheimer's disease, were found to be inversely correlated with estrogen levels. It was shown that the estrogen level in the cerebrospinal fluid of AD patients was lower than in controls of the same age (Schonknecht et al. 2001).

In this AD model, female rats are ovariectomised to reduce estrogen. Ovarectomy is defined as the surgical removal of the ovaries. While the reduction

of endogenous estrogen by this method markedly increased the Aβ level in rodent brains, it was observed that this hormone decreased Aβ accumulation in the AD mouse model (Petanceska et al. 2000). Consistent with this finding, various cell culture studies have revealed that estrogen reduces Aβ production by increasing non-amyloidogenic cleavage of APP by α secretase (Vincent et al. 2000).

Experimental model of AD due to loss of cholinergic function

There is a positive correlation between neuronal damage in the acetylcholine and cholinergic system and dementia and AD. Degeneration of basal forebrain cholinergic neurons occurs in the early stages of AD and is closely associated with cognitive function deficits. In the light of this information, we tried to apply models in which acute or chronic cholinergic damage was induced in the brains of experimental animals by various methods to mimic the symptoms of AD, especially cognitive function deficits. Acute electrocoagulation of cholinergic neurons, fimbria/fornix cross-cutting, administration of nonspecific excitotoxins or cholinergic system-specific toxins such as cholinotoxin significantly reduced cholinergic activity and mimicked the cholinergic system-related symptoms of AD. Chronic intracerebroventricular infusion of quinolic acid into the lateral ventricle in rats results in many neurodegenerative disease symptoms, including the symptoms of AD related to cholinergic damage. In models based on cholinergic dysfunction, senile plaques and neurofibrillary tangles observed in AD patients do not occur (Uzbay 2003).

AD model induced by intracerebroventricular injection of streptozotocin

Streptozotocin (STZ) is an alkylating agent that resembles some properties of nitrosoureas. Lannert and Hoyer developed the STZ animal model in 1998. Neuroinflammation of brain was identified as the main risk factors in STZ-induced AD rat model. Through the production of cytotoxic products and related pro-inflammatory mediators during neuroinflammation, these free radicals also contribute to neuronal loss. According to reports, AChE activity increased and ACh decreased in the brains of STZ-induced rats. In a previous study, STZ (2 mg/kg) was induced in rats for 3-4 months. This caused a decrease in α-secretase activities and an increase in cerebral Aβ-42, β-secretase and COX-2 (Chavan et al. 2023).

Experimental model of AD induced by bilateral carotid artery

Although this model was initially proposed for vascular dementia, it was thought to be suitable for FH with slowly developing cerebral blood flow reduction. In this model, chronic cerebral hypoperfusion is induced by ligation of

both carotid arteries with or without a subclavian artery. More impairment in spatial memory was observed in older rats than in younger rats when performed with the subclavian artery. It was observed that spatial memory impairments could be prevented by reperfusion 1-2 weeks after occlusion and hippocampal neurons could be saved, but no reversal was observed after 3 weeks. When this model was established in aged rats (10 months old), extracellular Aβ accumulation was observed in the cortex after 10 weeks. This finding suggested that decreased cerebral blood flow may contribute to Aβ formation. Although there is no significant difference between young and old in terms of memory impairment in this model, neuronal damage occurs later in young people (Weinstock 2004).

Experimental AD model induced by sodium azide

Sodium azide (NaN_3) administration has been shown to cause mitochondrial dysfunction and inhibit cytochrome oxidase (Chavan et al. 2023). Oxidative stress and neurodegeneration in AD are associated with dysfunction in the mitochondrial electron system, especially cytochrome oxidase. When sodium azide, a selective cytochrome oxidase inhibitor, was administered to rats with the help of an osmotic mini pump, it was shown that spatial memory was impaired (Weinstock 2004).

Sodium azide administered to rats intraperitoneally at a single dose per day (5 mg/kg) for 14 days caused a decrease in learning-memory due to mitochondrial dysfunction, ROS and antioxidant imbalance which may be due to decreased antioxidant levels. Histopathological neuronal loss has been shown. Since its first discovery, cytochrome oxidase has been known to have an important role in mitochondrial function and aerobic energy metabolism in AD patients. Decreased cytochrome oxidase activity in the mitochondrial electron transport chain has been identified as the main deleterious effect of sodium azide (Chavan et al. 2023).

Conclusion

AD is becoming an important health problem with increase in elderly population. Fort his reason, studies for AD diagnosis and treatment need to be improved. In humans, experimental animal models are helpful in elucidating the pathophysiology of AD and discovering new treatment options. In the experimental models of AD, various therapeutic agents can be tested for the treatment of cognitive impairment through various learning and memory tests. In conclusion, more studies in animal models using new techniques may lead to the development of effective treatment strategies for AD, for which there is no definitive cure.

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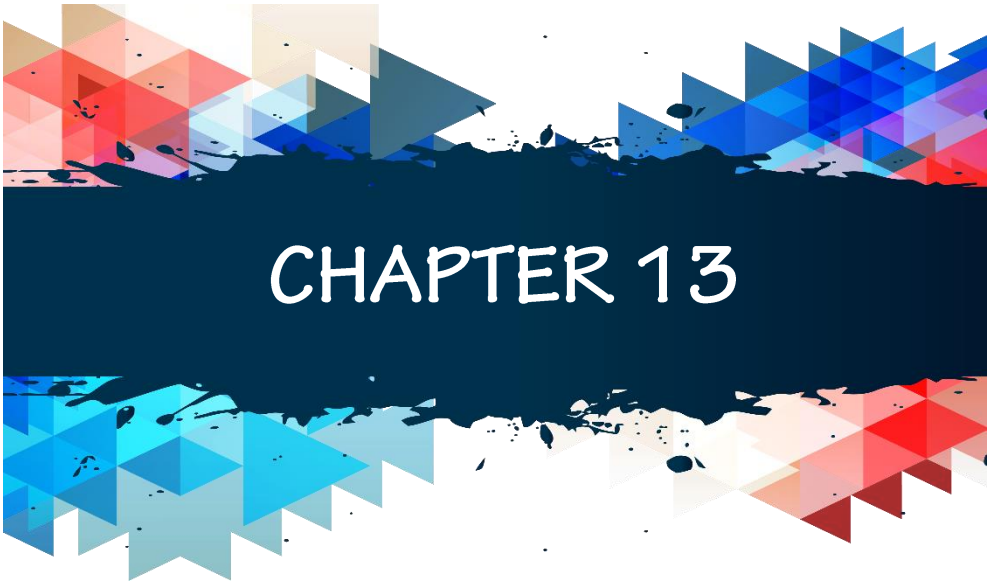

Physiological Effects of Dopamine

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Dopamine (Figure 1), a monoamine neurotransmitter effective in regulating hemodynamic imbalances and consisting of a catechol ringand ethylamine group, exhibits inotropic, chronotropic, and vasopressor effects depending on the dose (Eisenhofer et al., 1997, Deerfield, 2017, Pfizer, 2021; Elsevier, 2024, Tesoro et al., 2023). Indications include bradycardia, cardiac arrest, cardiopulmonary resuscitation, heart failure, shock, and hypotension. Pheochromocytoma,ventricular fibrillation,and ventricular tachycardia are contraindications (Elsevier, 2024).

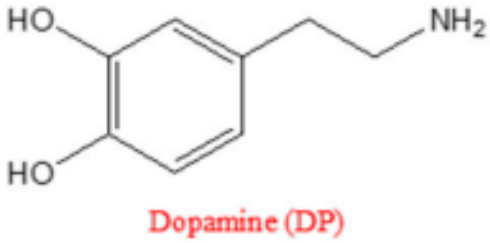

Figure 1. Chemical structure of dopamine (Tesoro et al., 2021).

While most dopamine is from tyrosine, some are synthesized from phenylalanine (Klein et al., 2019). Following the conversion of tyrosinetolevodopa(L-DOPA) via the tyrosine hydroxylase enzyme, the synthesized dopamine is transported from the cytosol to synaptic vesicles by a vesicular monoaminetransporter(VMAT2) and stored there until it is released into the synapticcleft (Figure 2).The acidic environment of the synaptic vesicle lumen also stabilizes dopamine by preventing its oxidation (Guillot and Miller, 2009; Klein et al., 2019, Xu and Yang, 2022). Cytosolic dopamine is degradedbycatechol-o-methyltransferase(COMT) or monoamine oxidase(MAO) in neurons or glial cells to form homovanillic acid (HVA) or oxidized to form metabolites (DOPAL and DOPAC) and hydrogen peroxide (H2O2) (Xu and Yang, 2022).

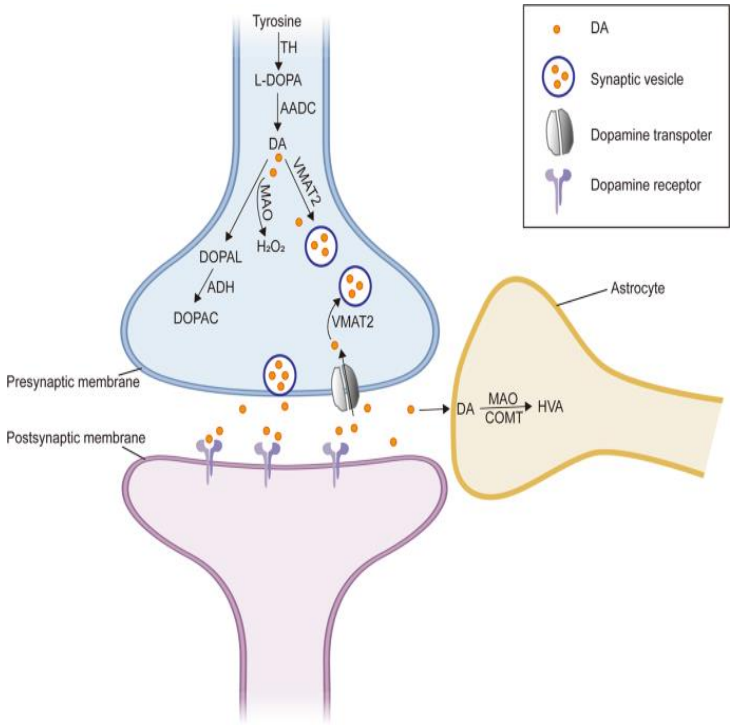

Figure 2. Dopamine synthesis (Xu and Yang, 2022).

Dopamine neurotransmission is of two types: phasic and tonic (Figure 3). Dopamine phasic transmission is triggered by action potentials reaching the dopaminergic neuron synapse, which causes a rapid and transient dopamine release in the synaptic gapdue to synchronized burst firing.Tonic transmission occurs with slow and irregular firing in neurons without presynaptic action potentials and is regulated bythe activity of other neurons and neurotransmitter reuptake or degradation (Klein et al., 2019). This pacemaker feature mediates the constant preservation of the basal activities of dopaminergic neurons. The determining factor here is thevariety of stimuli consisting of internal and external factors.For example, tonic neurotransmission is observed if there are fast phasic stimuli.However, if there is a reward-related event, there is phasic firing (Jabir et al., 2021).

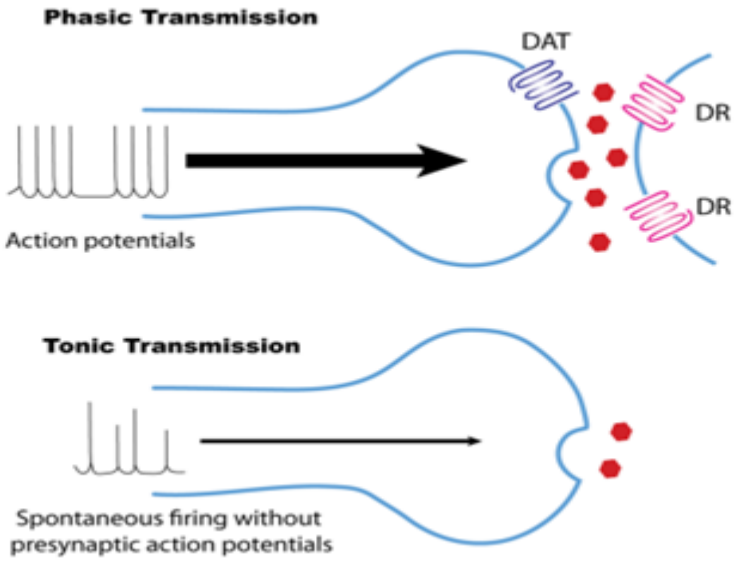

Figure 3. Dopamine phasic/tonic transmissions (Kelin et al., 2019).

Findings from animal studies suggest that in healthy animals, dopaminergic neurons are maintained in a hyperpolarized state by GABAmediated inhibitory inputs from the ventral pallidum, the vSub of the hippocampus regulates that tonic firing via excitatory projections to the NAc, and that this inhibits the ventral pallidum, releasing dopaminergic neurons from inhibition and resulting in tonic neurotransmission in dopaminergic neurons that is effective in generating spike activity. Glutamatergic inputs originating from various areas are held responsible for phasic neurotransmission (Grace et al., 2007).

When dopamine is mentioned, it is the neuromodulatory function that comes to mind due to its relationship with Parkinson's, schizophrenia, attention-deficit/hyperactivity disorder, Huntington's disease, addiction, and cognitive disorders (Klein et al., 2019, Lauretani et al., 2022; Lauretani et al. 2024). Dopamine is produced in other regions of the brain,such as the substantia nigral, ventral tegmental area, and hypothalamus (Latif et al., 2021). The dopaminergic cell group shown in Figure 4, excluding the retina, was determined by tyrosine hydroxylase immunohistochemistry. However,it should be noted that more cells than shown in the tyrosine hydroxylase stained area are located in regions adjacent to the hypothalamus and basal forebrain (Björklund and Dunnett, 2007).

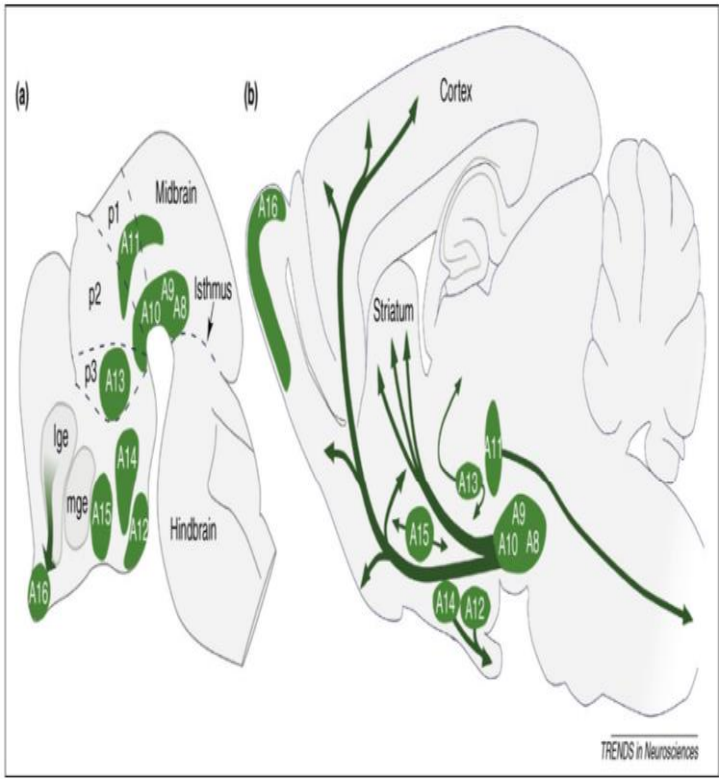

Figure 4. Arrows showing the distribution of dopamine neuron cell groups in the developing (a) and adult (b) rodent brains (Björklund and Dunnett, 2007). lge, lateral ganglionic eminence; mge, medial ganglio-nic eminence; p1–p3, prosomeres 1–3.

Dopamine receptors are divided into two main groups: the D1-like receptor class and the D2-like receptor class. These are then divided into subtypes. While the D1 and D5 subtypes represent D1, the D2,D3,and D4 receptor subtypes resemble D2 (Neve, K., 2009). Dopamine binding to the D1 receptor has three signaling mechanisms: G protein-dependent cell signaling, receptor desensitization, and receptor phosphorylation. In receptor desensitization, phosphorylation of the C-terminal tail and intracellular loops occurs by G protein-coupled receptor kinases(GRK) and protein kinase A(PKA) (Figure 5).β-arrestin binding to the phosphorylated receptor mediates desensitization, internalization, and potentially additional intracellular signaling events produced by activating ERK1/2 and Src protein kinases (Jones-Tabah et al., 2021). The 5q31-q34 gene encodes the D1 receptor, while the genes encoding the D2 and D4 receptors are located on chromosome 11. The gene encoding the D3 receptor is on chromosome 3, and the D5 receptor is on chromosome 4 (Meyers et al., 2022).

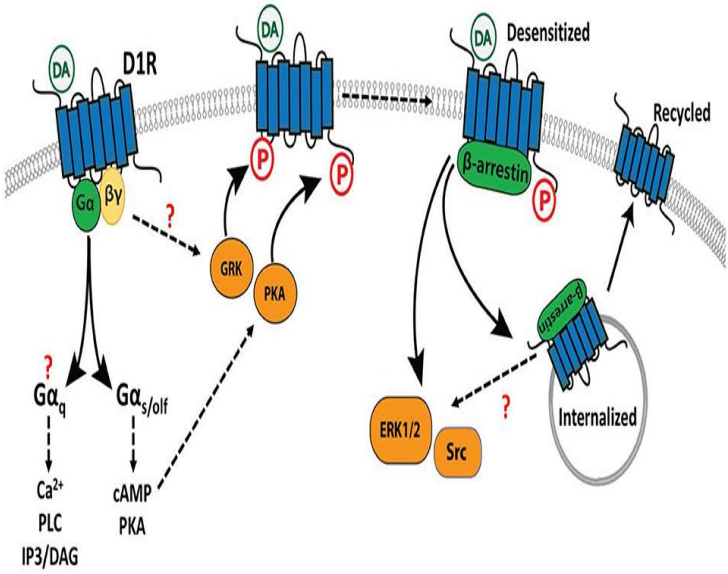

Figure 5. D1 receptor cell signaling scheme (Jones-Tabah et al., 2021).

Dopamine binds to D2, D3, and D4 receptors via G i / G o, activating potassium channels and inhibiting calcium channels and adenylyl cyclase. In addition, it can affect other cellular second messenger cascades. In general, activation of D2 receptors counteracts the effects of D1 receptor activation (James et al., 2024).

Recent studies have revealed the functions of dopamine receptors. To name a few, D3, D4, and D5 are shown to be related to cognition, while D2, D3, and D4 are related to sleep, D1 and D2 are linked to memory, and all dopamine receptors are functional with attention (Mishra et al., 2018; Meyers et al., 2022). While the distribution of D1, D2, and D4 receptors is high in the cortex regarding arousal and mood, D2 and D3 receptors come to the fore in prolactin secretion in the ventral hypothalamus and anterior pituitary (James et al., 2024).

Dopamine receptors are all G protein-coupled receptors(GPCRs). Dopaminergic neurons are mainly located in the ventraltegmental area(VTA), the substantia nigra pars compacta(SNc) of the midbrain,and the arcuate nucleus of the hypothalamus. Therefore, dopaminergic neurons project to many regions in the diencephalon and telencephalon. Dopaminergic pathways have been described as the mesocortical pathway from dopaminergic neurons in the VTA to the cortex,the mesolimbic pathway from the VTA to the nucleus accumbens, the nigrostriatal pathway from the substantia nigra to the striatum,and the

tuberoinfundibular pathway from the hypothalamic nuclei(arcuate nucleus and periventricular nucleus) to the pituitary gland (Figure 6) (Latif et al., 2021, Xu and Yang et al, 2022). While the nigrostrial system is active in motor control, the mesolimbic and mesocortical systems play a role in behavioral and cognitive effects. Endocrine control is related to the tubero-pituitary system. D1 receptors, which are abundant in the limbic system, thalamus, hypothalamus, and striatum, are actually in areas that receive dopaminergic innervation. In addition to these, D2 receptors are also located in glutamatergic, GABAergic, and cholinergic nerve terminals. D3 receptors are not found in the striatum but in the limbic system (De Mei et al., 2009; James et al., 2024).

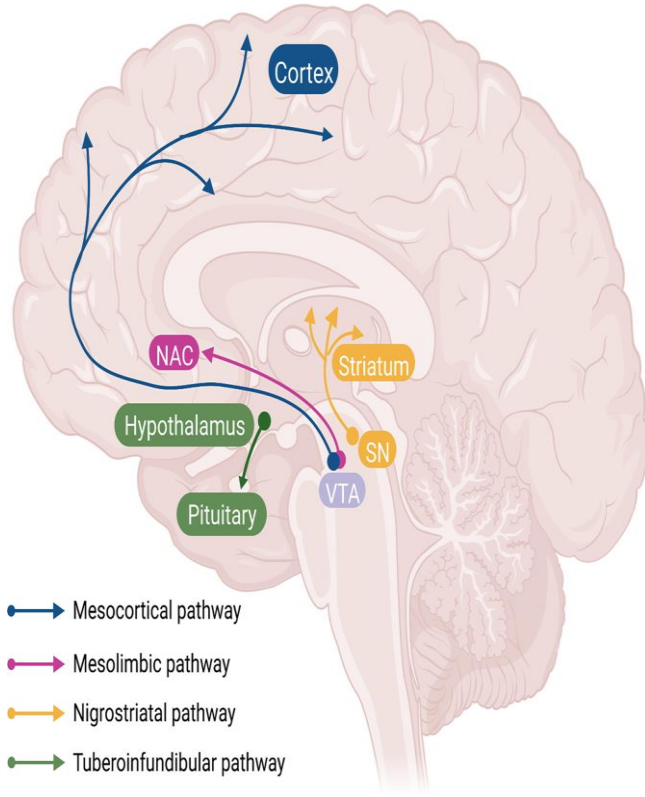

Figure 6. Pathways involved in the dopaminergic system (Xu and Yang et al., 2022).

There is a need for more detailed analysis and investigation of dysfunctions in dopaminergic pathways or receptor levels and related pathophysiological processes to contribute to the early diagnosis and treatment of diseases such as schizophrenia, Alzheimer's, and Parkinson's.

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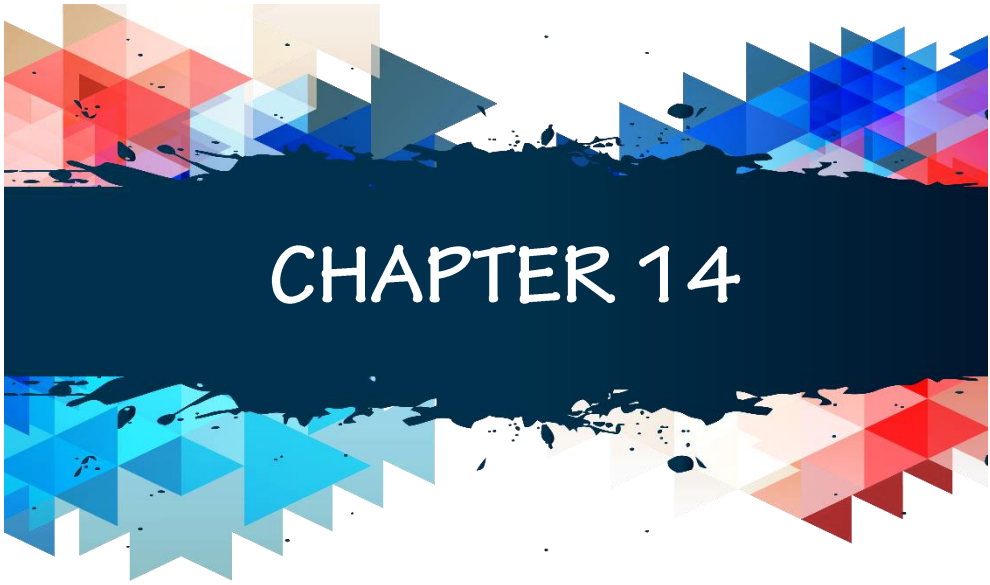

The Elemental Changes in Anxiety Disorder and Depression

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Introduction

The physiopathology of depression involves biological, ecological, psychological and genetic factors. In recent times, many studies have been conducted aiming to explore roles of trace elements in neuropsychiatric disorders. The deficiencies or excessive amounts of trace elements may be associated with the onset and progression of symptoms of anxiety and depression.

The minerals play a multifaceted role in the biological system, from regulating metabolic reactions to acting as nutrients, cofactors and antioxidants. Several studies have shown that changes in mineral levels in the body are linked to the aetiology and pathophysiology of many neuropsychiatric disorders, including depression (Şahin et al. 2020).

Several studies have shown that changes in mineral levels in the body are linked to the aetiology and the pathophysiology of many psychiatric disorders, including depression (Şahin et al. 2020). The aim of this section is to summarize the relationship between trace elements and depression/anxiety, the possible physiopathological mechanisms underlying the relationship between trace elements and the progression of anxiety/depression and the concentrations of determined trace elements. For this purpose, 8 elements including aluminium (Al), calcium (Ca), copper (Cu), manganese (Mn), magnesium (Mg), iron (Fe), selenium (Se), zinc (Zn) were evaluated (Figure 1).

DepressionandAnxiety Disorder

Depression and anxiety disorders are recognised as one of the dıseases that contribute most to non-fatal health losses worldwide. Approximately 24.6 million people are reported to be living with this disorder (World Health Organisation, 2017). In addition to pharmacological agents, cognitive and behavioural therapies are known to be effectivetreatmentmethod in treatment anxietydisorders (Luong et al. 2020).

Trace elements are essential for proper human body function. They are necessary for basic processes such as cell division, differentiation and synthesis of proteins. Therefore, a lack of these trace elements can lead to serious health problems. Recently, studies have shown that trace elements are especially involved in depression and anxiety (Młyniec et al. 2014).

Living organisations require essential elements at appropriate levels. These essential elements are crucial in various physiological processes. Excess or lack of them leads to several pathological conditions, including psychiatric diseases. Researchers state that trace elements play an essential role in anxiety and may be

prognostic indicator of this disease similarly a tool for successful treatment (Islam et al. 2013). Evidence exists that dietary deficiencies in certain elements lead to development of anxiety-like behaviours. Młyniec et al. 2017).

Trace elements are substances found in small amounts in the organism. They are mainly required for vital functions. The main trace elements in humans are fluorine, cobalt, Cu, Mn, molybdenum, Fe, iodine, Zn and suspiciously Se, boron and vanadium. Disruption ofcertain neurophysiological processes dueto deficiency oftraceelements can negatively affect psychological and mental processes. Scientific studies have shown that trace element deficiency can cause diseases such as depression (Zn, Cr, Se, Fe, Co, I), dementia (B, Zn, Fe, Mn, Co, V) and attention deficit hyperactivity disorder (Fe). Chronic exposure to certaintraceelements (Mn, Cr, V, Co, Fe, Cu) may cause depression, anxiety and cognitive dysfunction (Janka 2019).

Different facets of the association between the elements and either depression or anxiety, e.g. the amount of an item in the diet or the serum level of an item and depression or anxiety-like symptoms, are important. The relationship between the amount of an element in the diet or the level of an element in serum and depression or anxiety-like symptomatology is important. In addition, many elements such as selenium, manganese, copper iodine and vanadium influence the physiopathology of depression or anxiety. These elements may affect enzymes as co-factors in the underlying mechanism (Młyniec et al. 2015).

Strong evidence exists to suggest that a deficiency of trace element may lead to development of depressive and anxiogenic behaviour. Supplement drugs may enhance the therapeutic effect of antidepressants and anxiolytics. The elements Zn, Mg, lithium, Fe, Ca and chromium play a role in depression and anxiety disorder. It has been demonstrated that various types of anxiety and depression may respond to treatment at several receptors and the underlying underlying mechanisms may be different. It has been observed that low-dose antidepressant administration supplemented with elemental supplements is effective in the treatment of anxiety and depression. Side effects observed in the treatments of different types of depression and anxiety can be reduced by elemental supplementation (Młyniec et al. 2014).

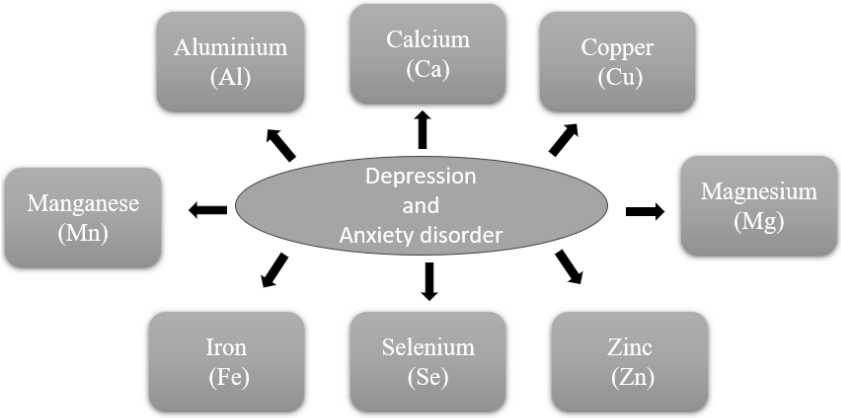

Figure 1. Effect of elements in depression and anxiety disorder

Aluminium (Al)

Patients who are exposed to high Al levels can accumulate it both in their plasma and in their brain. Al entry into CNS is facilitated by transferrin. Neurotoxic effects occur at high plasma Al levels. Al accumulation in the brain has been linked to various cognitive impairments. In additional, this metal may disrupt hippocampal Ca signalling pathways. Neurotoxic effects of Al affects cholinergic neurones and may impair the functions of the cells (Baj et al. 2023).

Oxidative stress caused by aluminium has been found to be associated with various mental illnesses. This association is attributed to certain brain regions that are sensitive to oxidative stress (Tsaluchidu et al. 2008). Reduction of aluminium-induced neurotoxicity can be achieved by the use of lithium potent antioxidants (Haridevamuthu et al. 2023).

Calcium (Ca)

Ca is the mineral most present in the human body. Most calcium is found in bones and a small amount in serum. This mineral has an important role in several physiological processes such as nerve conduction, muscle contraction, intracellular signalling, neurotransmission and synaptic plasticity. Ca overload can result in various neurodegenerative diseases. Conversely, Ca deficiency can cause calcification in the cerebellum, cerebral cortex as well as basal ganglia, leading to extrapyramidal symptoms (Baj et al. 2023).

Ca plays an important role in the control of the mechanisms underlying emotional disorders. Ca is involved in the synthesis and release of neurotransmitters and receptor sensitisation. It is important in neuronal memory of neural stimulation (Paul 2001). Disruption of Ca and signalling pathways may be a factor in psychiatric disorders such as depression or bipolar (Berridge 2014).

Copper (Cu)

Copper (Cu) is third most prevalent transition element in the human body. Its greatest concentrations are found in the liver and brain. Cu play important roles in various physiological processes. One important function of copper is to maintain the balance of Fe levels in body by participation in proper Fe homeostasis. Cu is crucial for effective neural signal transmission. Cu is also essential for brain function and mood regulation. Cu has a role in the synthesis of norepinephrine and dopamine (Baj et al. 2023).

The role of copper in depression is complex. studies suggest that copper plays an important role in the normal development of the nervous system (Scheiber et al. 2014). Some studies have not observed a significant difference in serum copper concentrations between depressed and control groups (Styczeń et al. 2017). In contrast, depressed patients in different studies showed elevated copper levels (ni et al. 2018). Discordant results may be due to different characteristics of patients or differences in measurement methods.

Zinc (Zn)

Zn is one of the most important essential elements in living organisms. Zn plays a regulatory role in transcriptional and structural functions similarly enzymatic proteins. Daily requirement depends on gender and age. Men require 11 mg and women require 8-9 mg. Major sources of Zn element include red meat and whole grains, as well as oysters. Zn is essential for development and function of many tissues, including brain regions. Deficiency of Zn element is linked to many diseases, including psychiatric diseases such as depression-anxiety disorder. Zn has been found to produce both anxiolytic-like and antidepressant effects. Acute administration of Znhydroaspartate at doses of 32.5 and 16.25 mg/kg increased the percentage of entry into open arms of the elevated plus maze in rats. In different studies, 7-day administration of Zn at doses of 15 and 20mg/kg significantly increased the number of open-arm entries and time spent in open arms of elevated plus maze test. Another Zn salt, namely Zn chloride, at doses of 20 and 30 mg/kg showed an anxiolytic-like effect (Młyniec et al. 2017).

Copper (Cu)

Cu is a crucial component of several enzymes involved in the generation of neurotransmitters, such as tyrosine hydroxylase and dopamine hydroxylase. Around 80-95% of the copper in plasma is bound to ceruloplasmin, a Cu binding protein that helps transport copper in the blood. Cu can disrupt synaptic transmission by binding to GABA, NMDA receptors and voltage-gated Ca^{2+} channels. It has been shown that Cu concentrations and metabolic imbalances play a role in various neurodegenerative diseases such as different encephalopathies, Alzheimer's and Wilson's disease (WD) (Baj et al. 2023).

Increased disinhibition and irritability, cognitive impairment, anxiety disorder and depression are common psychiatric characteristics in the patients with WD, an autosomal recessive disorder of Cu metabolism. Cu homeostasis is disrupted due to mutations in the ATP7B gene, which encodes the Cu-transporting P-type ATPase. Reduced biliary excretion and impaired ceruloplasmin formation increase copper levels and trigger oxidative stress, free radical production and mitochondrial dysfunction. WD are common and well-documented psychiatric symptoms such as depression and anxiety. As Dening and Berrios showed, 51% of patients with copper overload were considered to exhibit pathological features, while 20% had seen psychiatrist before a formal diagnosis of WD was made. Psychiatric and behavioral abnormalities are the first symptoms in two-thirds of WD cases and occur in 30-100% of patients. Therefore, the role of Cu in anxiety-related disorders is remarkable (Młyniec et al. 2017).

Iron (Fe)

Fe is a essential element for organisms and is involved in various physiological processes. In human body, it is a major component of haemoglobin, which is particularly important for the storage and delivery of O_2. Fe is also necessary for myoglobin, which acts as a protein in muscles. It also plays an important role in the synthesis of catalase, peroxidase and cytochromes in cellular features. Fe is a constituent of many proteins involved in DNA synthesis. It contributes to cell proliferation, development and growth throughout body. Fe homeostasis is essential for mitochondrial functioning and ATP production in the regulation of energy needs of cells. Fe is a trace element most abundant in central nervous system. Fe is implicated in the synthesis of neurotransmitters such as serotonin and dopamine, synaptic plasticity and myelin sheath for better nerve conduction. Fe homeostasis is important for maintaining cognitive functions and supporting neurophysiological processes. In older individuals, Fe accumulation in brain may lead to cognitive and motor dysfunctions. Fe deficiency or excessive

intake related to damage in monoamine levels or potential for neuronal damage due to cellular toxicity (Baj et al. 2023).

Manganese (Mn)

Mn is an essential element that plays an significant role in regulation of the lipid and glucose metabolism, similarly the synthesis and activation of various enzymes. Mn plays a majör role in the antioxidant defence, energy production, immunity and neuronal activity. In the CNS, areas such as caudate nucleus, putamen and globus pallidus have high manganese concentrations. Mn toxicity can lead to oxidative stress, mitochondrial dysfunction and apoptosis. Imbalances in Mn levels can cause neurodegenerative disorders. High Mn element levels can result in symptoms similar to Parkinson disease, including motor, cognitive and emotional impairments. Excess Mn in the body has been associated with neurotoxicity (Baj et al. 2023).

Mn is an important element found usually in liver, kidneys, bones, pyruvate carboxylase, arginase and mitochondrial SOD. Mn deficiency seldom develops in human population, but its toxic effects are known to come into being in certain occupational settings. Miners and welders are exposed to high Mn through inhalation of manganese-containing dust. The oral route is less efficient in delivering Mn due to weak absorption from the gastrointestinal system. Being exposed to over amounts of Mn can lead to poisoning and is associated with a several of symptoms related to psychiatric and motor functions. The clinical picture of manganese neurotoxicity in adults is known as manganism and consists of a set of symptoms that resemble Parkinson disease. Tremors, rigidity, masked face, bradykinesia, anormal posture and altered behavior. Mn exposure has been shown to lead to accumulation of compound in some brain areas, components of basal ganglia (Młyniec et al. 2017).

Magnesium (Mg)

Most total body Mg is contained in muscles and bones. Mg acts as a cofactor for different enzymes. Mg is involved in various physiological functions in the body. These functions include muscle contraction and neuromuscular conduction. Mg is also necessary for glycolysis and oxidative phosphorylation. It also helps prevent overstimulation of neurons by controlling their N-methyl-D-aspartate receptors. It is also required for the maintenance of protein structures, DNA and RNA, mitochondria, immunological functions and bone mineralisation. Mg is involved in the proper transport of ions through cell membranes. Neurologically, Mg deficiency leads to an increased risk of migraine, stroke and seizures. In severe cases of hypermagnesaemia, it can cause neuromuscular

dysfunction, bradycardia and atrial fibrillation (Baj et al. 2023). Mg+2 may help antidepressants inhibit arteriolar vasoconstriction (Fidan et al. 2022).

Mg is the fourth most abundant trace element in the body and the second most abundant intracellular cation. The adult body contains approximately 24 g of Mg, of which about 50% is found in bones. The rest is found in the intracellular space of soft tissues. Only 1% of total body Mg is found in blood. Intracellular Mg is needed too much biochemical reactions, many of which require energy production. It plays a major role in protein and nucleic acid synthesis. It is essential in maintaining K, Ca and Na homeostasis. Mg is necessary for proper nerve and muscle function, normal heart rhythm and bone formation. Dietary Mg is essentially absorbed in small intestine and excreted via kidneys. The recommended daily allowance is 300 mg (Erikson 2003). Plasma Mg levels in healthy adults range from 0.7 to 1 mmol/L (1.5–2 mEq/L; 17–24 mg/L) (Młyniec et al. 2017).

There is also ample evidence for the role of Mg in the pathophysiology and treatment of neurological diseases (migraine, pain, traumatic brain injury, cerebral ischemia, stroke) and psychiatric diseases (depression, psychoses, hyperactivity, and autism in children). addiction as well as anxiety) (Vink and Nechifor, 2011). Magnesium (Mg) plays an important role in the etiology, progression and treatment of depression. Magnesium acts as a cofactor in many enzymes involved in brain function and mood regulation (Serefko et al. 2013). Studies have emphasized the relevance of magnesium supplementation to the limbic system, suggesting a role in the development and course of depression (Wang et al.2018). Impaired Mg levels can lead to disorders such as depression, schizophrenia, anxiety and eating disorders (Botturi et al.2020).

Likewise, the relationship between Mg intake and anxiety in humans is uncertain. A recent large, cross-sectional, population-based sample of women found no association between Mg intake and anxiety. However, rodent studies indicate an association between dietary magnesium reduction and the emergence of anxiety-related behaviors and suggest that a Mg-deficient diet is useful at a preclinical level as a model of anxiety (Młyniec et al. 2017).

In studies on Mg and anxiety, magnesium lactate, oxide or sulphate have generally been used. However, more studies are needed to compare the anti-anxiety effects of different types of Mg. Because it is still unclear which type of Mg is best for anxiety or depression. In order to benefit from the natural anxiolytic functions of Mg, it is recommended to consume Mg-rich foods such as green leafy vegetables, avocado, dark chocolate (Fidan et al. 2022).

Selenium (Se)

Se is an essential trace mineral that plays a majorrole in physiological processes. Maintaining adequate Se levels is important for general physiological mechanisms. Low levels of Se have been associated with several health problems, cognitive decline and immune function. Se is an important constituent of selenoproteins, which are essential to physiological functions such as antioxidant defence, immune system, DNA synthesis and thyroid metabolism. Sufficient Se prevents oxidative damage and supports cognitive functions. Excessive Se ingestion may cause some neurological problems (Baj et al. 2023).

Low serum selenium concentrations have been observed in depressed patients compared to healthy people. Deficient selenium levels may be a risk factor for anxiety and decreased cognitive function due to antioxidant pathways and decreased enzyme activity (Islam et al. 2018). It has also been observed that pregnant and postpartum women with low selenium intake have a higher risk of experiencing postnatal depression (Jin et al. 2020).

Zinc (Zn)

Zn is the world's second most plentiful essential element in the human body. It plays crucial roles in several physiological processes, among which protein composition and regulation of gene expression, RNA and DNA synthesis. Zn is required for cell metabolism and development. Zn serves as a cofactor for numerous enzymes. In the central nervous system, Zn is most abundant in the hippocampus and the olfactory bulb and is found mainly in the synaptic vesicles of glutaminergic neurones. Zn-containing neurones are highly concentrated in the forebrain. Zinc can inhibit glutamate oscillation and effect GABA receptors involved in neurotransmission. Zn levels are very important for neurogenesis in adults. Zinc serves as a cocofactor in the central nervous system, which can cause neuronal damage, neurodegenerative diseases such as Alzheimer's disease, cognitive dysfunctions, anxiety and depression (Baj et al. 2023).

Zinc deficits can lead to a variety of conditions, including neuropsychiatric disorders such as anxiety and depression. Preclinical studies have shown that zinc deficiency has a significant effect on anxiogenic behavior. In studies, two weeks of zinc deprivation caused anxiety-like behavior as measured by open field testing in rats. It was observed that the frequency of crossing the line decreased similarly the time spent on grooming decreased. Moreover, the time spent in open arms of the plus maze test was observed to be reduced in zinc-deficient mice. According to the authors, a possible explanation for the behavior observed after 2 weeks of a low zinc diet is based on hyperactivity of HPA axis. Their study

found significantly higher levels of corticosterone in zinc-deficient rats (Młyniec et al. 2017).

Conclusion

Depression and anxiety disorders are related to essential elements in the body. The combination of antidepressant treatment and elemental supplementation has shown positive effects in patients with depression and anxiety. Deficiencies of some elements can lead progression symptoms of depression and anxiety, while excessive accumulation of others has been shown to adversely affect the prognosis of the disease. Further studiesareneeded to understand the role otrace elements pathophysiology of depression and anxiety.

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CHAPTER 15

Diabetes and Probiotics

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INTRODUCTION

Diabetes is a chronic metabolic disorder marked by elevated blood glucose levels, leading to severe complications such as neuropathy, nephropathy, and cardiovascular diseases, significantly affecting the quality of life. According to the International Diabetes Federation (IDF) 2021 data, there are 537 million people living with diabetes globally, with 6.7 million deaths attributed to diabetes in 2021. The prevalence of diabetes is also on the rise in our country. Numerous studies have established links between the microbiota and various diseases, including cardiovascular diseases, certain cancers, respiratory illnesses, diabetes. (Hou et al., 2022). Research has shown that patients with diabetes or insulin resistance have different gut microbiota compositions compared to healthy individuals (Wu et al., 2020; Chen et al., 2021). Understanding the connection between microbiota diversity and insulin resistance, along with the mechanisms involved, is crucial for comprehending the development of diabetes and insulin resistance. Based on these findings, intentional modifications to the gut microbiota may offer a potential therapeutic approach for preventing diabetes or regulating blood sugar levels (Tao et al., 2020; Wang et al., 2020).

TYPES of DIABETES

Diabetes is classified into four main types: Type 1, Type 2, gestational diabetes (GDM), and other specific types. Type 1 diabetes, also known as juvenile diabetes, primarily arises from genetic factors that trigger the autoimmune destruction of pancreatic β-cells, leading to insulin deficiency and elevated blood glucose levels. Type 1 diabetes accounts for about 5-10% of all diabetes cases. Its etiology involves autoimmunity, genetic predisposition, and environmental factors. This form of diabetes is progressive, characterized by recurring ketosis episodes and hyperglycemia (Tanaka et al., 2000).

Type 2 Diabetes Mellitus is the most prevalent form of diabetes, typically occurring in adults. It is characterized by insulin resistance due to a decrease in insulin receptor numbers or reduced insulin effectiveness at the post-receptor level in target cells. Insulin resistance and insulin secretion dysfunction are prominent features of Type 2 diabetes, which constitutes about 90-95% of all diabetes cases. Key mechanisms in the pathogenesis of Type 2 diabetes include decreased insulin transcription, β-cell mass reduction leading to insulin deficiency, impaired pulsatile insulin secretion, glucose toxicity, lipotoxicity from prolonged elevated free fatty acids, and receptor defects in peripheral tissues (ADA, 2013b).

Gestational diabetes is a form of diabetes that first appears during pregnancy. It results from increased insulin resistance and insulin deficiency or a combination of both. High-risk groups for gestational diabetes include obese women, those with a history of glucose intolerance, older women, women with a family history of diabetes, women from ethnic groups at high risk for Type 2 diabetes, and pregnant women with high fasting or random blood glucose levels (Almind et al., 2001).

MICROBIOTA

Gut bacteria perform various essential functions, including food fermentation, protection against pathogens, stimulation of immune responses, and vitamin production. Human microbial colonization begins at birth and progresses over approximately three years until the microbiota reaches its adult composition. An average 70 kg human colon contains about 10^{13} bacteria (Sender et al., 2016). The number and diversity of these bacteria change in disease states, and the disruption of microbiota balance is linked to various diseases. The gut microbiota generally consists of six phyla: Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria, Fusobacteria, and Verrucomicrobia. Fungi such as Candida, Saccharomyces, Malassezia, and Cladosporium are also present in the gut microbiota. Besides bacteria and fungi, the gut microbiota includes viruses, phages, and archaea, primarily M. smithii (Hou et al., 2022). Factors such as birth mode, antibiotic and drug use, aging, dietary habits, and consumption of prebiotics and probiotics influence the diversity and composition of the gut microbiome. Maintaining the diversity and balance of the gut microbiota is crucial for enhancing human health.

RELATIONSHIP BETWEEN DIABETES, PROBIOTICS and MICROBIOTA

Alterations in the diversity or structure of the gut microbiota can impact metabolic activities, leading to metabolic disorders such as obesity and diabetes. Large-scale metagenomic studies in China and Europe have documented gut microbial dysbiosis in obese and Type 2 diabetic patients (10,11). These studies commonly found a decrease in butyrate-producing bacteria along with an increase in opportunistic pathogens (10,11,12). Dietary influences on bacterial flora show that excessive intake of nutrients like saturated and polyunsaturated fatty acids or insufficient consumption of oligosaccharides and phytochemicals can alter bacterial metabolic activity. Consequently, the number of Bifidobacterium, beneficial for the host microbiota, decreases while Firmicutes increase. In diabetic individuals, there is a reduction in butyrate-producing bacteria (Roseburia intestinalis and F. Prausnitzii) and an increase in Lactobacillus

gasseri, Streptococcus mutans, Escherichia coli (E. rectale), and some Clostridium species. Roseburia, Eubacterium hallii, and Faecalibacterium prausnitzii reduce insulin resistance, whereas L. gasseri, S. mutans, and E. coli increase insulin resistance, thus raising the risk of diabetes (6). One of the most significant contributions of bacteria in the gut microbiota to the human body is the production of short-chain fatty acids (SCFAs). SCFAs are produced by the fermentation of non-digestible carbohydrates by the gut microbiota. These acids include butyrate, acetate, and propionate (Morisson and Preston, 2016). SCFAs regulate insulin sensitivity, systemic inflammation, glucose, and lipid homeostasis.

Probiotics are extremely important microorganisms for our health. Prebiotics are defined as substrates selectively utilized by host microorganisms that confer health benefits. Additionally, the use of probiotics has been shown to reduce oxidative stress and increase antioxidant capacity (Kayacan et al., 2022).

DISCUSSION and CONCLUSION

Studies in the literature have shown that diabetes is related to the gut microbiota. Alterations in the gut microbiota have also been observed in individuals with impaired glucose tolerance and combined glucose intolerance, linked to insulin resistance (Wu et al., 2020). The abundance of butyrate-producing bacteria, such as SCFAs in the gut microbiota, is reduced in the presence of insulin resistance and diabetes (Zhao et al., 2018; Wu et al., 2020; Chen et al., 2021). Diets rich in probiotics and fiber have shown beneficial effects in T2DM patients by reducing HbA1c, fasting blood glucose, and insulin resistance (Zhao et al., 2018; Tao et al., 2020). As seen in these studies, further research on diabetes and probiotics should be conducted, and advancements in this area should be incorporated into future diabetes treatments.

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CHAPTER 15

Colon Cancer and Gut Microbiota

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Gut Microbiota

The significance of the microbiota for human health is increasingly recognized with new research. Numerous diseases, including cardiovascular diseases, cancer, respiratory illnesses, diabetes, inflammatory bowel diseases, neurological disorders, chronic kidney diseases, and liver diseases, have been linked to the microbiota (Hou et al., 2022). The gut microbiota plays a vital role in nutrition, physiology, metabolism, and immunology. Interest in studies examining the effects of gut microbiota on health is growing. Microbial dysbiosis, characterized by a reduction in beneficial bacteria and an increase in harmful bacteria, has been linked to obesity, diabetes, metabolic syndrome, and gastrointestinal diseases in both animal models and human studies (McCabe et al., 2015; Altuntaş and Batman, 2017). The gut microbiota, which contains the highest number of microorganisms and is considered the most crucial for human health, serves functions such as food fermentation, pathogen protection, immune response stimulation, and vitamin production. An average 70 kg human colon harbors approximately 10^{13} bacteria (Sender et al., 2016). The gut microbiota primarily comprises six phyla: Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria, Fusobacteria, and Verrucomicrobia. Some fungi found in the gut microbiota include Candida, Saccharomyces, Malassezia, and Cladosporium. In addition to bacteria and fungi, the gut microbiota also includes viruses, phages, and archaea, notably M. smithii (Hou et al., 2022). One of the most significant contributions of gut bacteria to human health is the production of short-chain fatty acids (SCFAs). SCFAs, such as butyrate, acetate, and propionate, are produced through the fermentation of indigestible carbohydrates by the gut microbiota (Morisson and Preston, 2016). Besides being a vital nutrient for enterocytes, SCFAs regulate insulin sensitivity, systemic inflammation, and glucose and lipid homeostasis.

Colon Cancer

Cancer, a leading cause of death, results from the uncontrolled proliferation of cells mutated by environmental and genetic factors. It manifests in various forms depending on the affected tissue and organ, one of which is colorectal cancer. Cancer cells proliferate without the need for external signals and are less susceptible to apoptosis signals compared to normal cells. These highly mutative and unstable cells can divide indefinitely and induce angiogenesis to facilitate their nourishment and dissemination, leading to metastasis and proliferation in other tissues (Alberts et al., 2007). Colon cancer (CC) develops from the transformation of normal colon epithelium into adenomatous polyps. CC typically begins as a polyp in the intestinal mucosa, but it can also start as a benign

lesion known as an adenoma, which has the potential to become malignant (Granados-Romero et al., 2017). Over 1 million new colorectal cancer (CRC) cases are diagnosed worldwide each year, making it the third most common cancer and the fourth leading cause of cancer deaths globally (Terzic et al., 2010). The majority of CRC cases are linked to environmental factors rather than inherited genetic changes. Risk factors include environmental and dietary mutagens, specific gut commensals and pathogens, and chronic intestinal inflammation preceding tumor development (Das et al., 2007; Terzic et al., 2010; Jasperson et al., 2010). Genetic abnormalities, involving the activation of several oncogenes and the loss of two or more tumor suppressor genes, are sufficient for cancer development (Langdon, 2004). The incidence of colorectal cancer increases sharply with age, with approximately 90% of new cases occurring in adults over 50. Furthermore, individuals over 65 have a threefold higher risk of developing CRC, with incidence and mortality rates higher in women than in men in this age group (White et al., 2018). It is estimated that about 35% of the risk factors for colorectal cancer are hereditary (Granados-Romero et al., 2017).

Microbiota and Colon Cancer

The role of individual variations in gut flora and genetic traits in the pathogenesis of colon diseases is increasingly recognized. It is understood that the colonization of the colon by beneficial bacteria can prevent potential diseases. Various studies support the notion that the gut microbiota plays a role in cancer etiology. Several hypotheses have been proposed to explain the role of microbial imbalance in carcinogenesis. Some researchers suggest that gut microbiota dysbiosis induces chronic pro-inflammatory responses and epithelial cell changes, ultimately leading to cancer. The Driver-Passenger hypothesis is one such model. According to this hypothesis, native gut bacteria (Drivers) cause DNA damage in epithelial cells, contributing to the initiation of cancer. In later stages, ongoing carcinogenesis affects the surrounding microenvironment, promoting the growth of opportunistic microorganisms (Passengers). This model posits that disease progression induces changes in the microenvironment, replacing the microbiota with agents that have a competitive advantage in the tumor microenvironment and support tumor expansion. In this paradigm, both drivers and passengers have distinct temporal correlations with CRC and can represent specific roles in etiology (Saus et al., 2019).

Recent studies have shown alterations in the gut microbiota in colon cancer, suggesting that the gut microbiota may play a significant role in the initiation and progression of this malignancy (Saus et al., 2019). The gut microbiota helps maintain mucosal homeostasis and epithelial barrier function. In a healthy state,

the gut barrier effectively compartmentalizes bacteria in the lumen. However, impairments in gut barrier function can lead to "leaky gut," associated with various gastrointestinal disorders and diseases, including irritable bowel syndrome (IBS), inflammatory bowel disease (IBD), celiac disease, and colon cancer (Altveş et al., 2020).

Microbiota and Cancer Tumor progression and invasion are influenced by inflammation and anti-tumor immune responses. Approximately 15% of cancer cases are associated with infectious agents or infection-related inflammation (Hou et al., 2022). The disruption of the intestinal barrier and subsequent direct contact between the microbiota and hematopoietic cells can initiate an inflammatory process in carcinogenesis (Altveş et al., 2020). IL-18 has a protective effect on the mucosa, and dysbiosis resulting from the absence of IL-18 production in mice can lead to colon cancer development upon chemical induction (Altveş et al., 2020). It is suggested that probiotics may enhance the effectiveness of immunotherapy in cancer patients, with the effect varying depending on the bacteria (Lee et al., 2021).

Discussion and Conclusion

Colorectal cancer (CRC) is the third most common cancer globally and the third leading cause of cancer-related deaths. CRC is a multifactorial disease resulting from the transformation of normal colon epithelium into adenomatous polyps. Traditional treatments for colon cancer include chemotherapy, radiation, and surgical procedures. Studies have identified various microbiome alterations in CRC patients. The microbiome is often enriched with pro-inflammatory opportunistic pathogens and microorganisms associated with metabolic issues, while butyrate-producing bacteria are deficient. Streptococcus gallolyticus, E. faecalis, Fusobacterium nucleatum, Escherichia coli, and B. fragilis are more prevalent, whereas genera such as Roseburia, Clostridium, Faecalibacterium, and Bifidobacterium are typically reduced in CRC patients compared to healthy individuals (Saus et al., 2019). Recent research has shown that the microbiota can influence the response to chemotherapy and immunotherapy through myeloid-derived cells in the tumor microenvironment. The microbiota is now being considered a new organ, and its effects on cancer are being increasingly studied. Understanding the role of the microbiota in carcinogenesis may enhance its integration into cancer treatment in the future.

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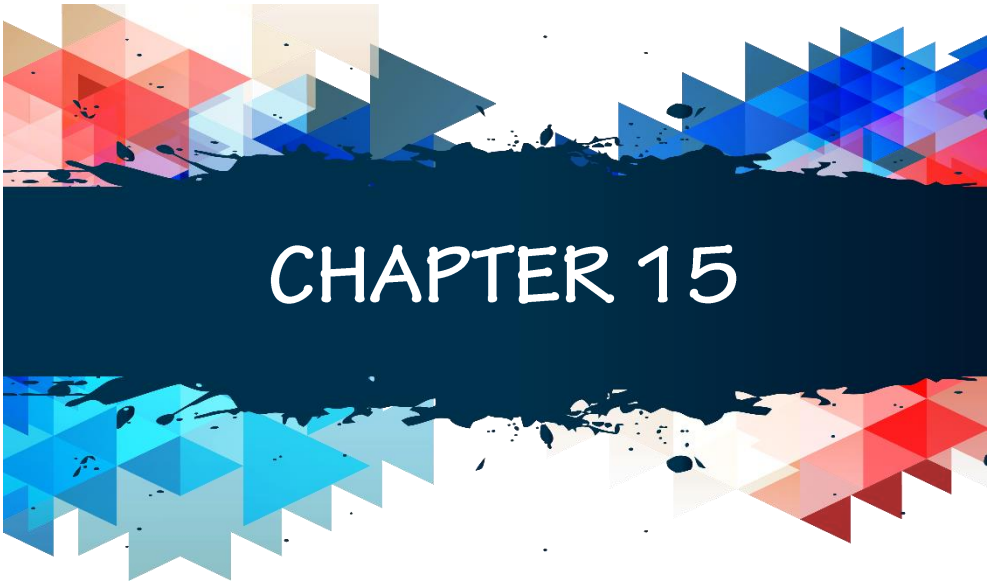

Histopathological Evaluation of Some Heavy Metals Impact on Rat Cerebrum and Cerebellum Tissues

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Introduction

Most of the animal and neurotoxic effects of heavy metals and peripheral nervous system indicated major involvement of blood vessels. However, little is known of its mechanism of neurotoxic action as well as the comparative evaluation of the morphological lesions after identical exposure in developing and adult brain (Murthy et al. 1987). Iron is an essential mineral in humans and plays a crucial role in vital biochemical activities, such as oxygen sensing and transport, electron transfer and catalysis (Kozan et al. 2008).

Heavy metals are naturally occurring metal that is present in food, soil and water. It is relased in to the enviroment from both natural and man-made sources. Chronic exposure to heavy metal-contaminated water and food causes cancer of skin, liver, lung and bladder. Heavy metal is also said to exert its toxicity through oxidative stress by generating reactive oxygen species. Free radicals have been detected in some cell treated with heavy metal. Treament with heavy metal has been shown to induce of hydroxyl radical formation in brain (Zalups and Ahmad 2003; Bridges and Zalups 2005; Bashir et al. 2006).

Heavy metals are the most important source of anorganic pollution in freshwater. Heavy metals are transported by rock pieces transported with erosion, dust transported by wing, volcanic activities, forest fires and plant cover to the water. Chemical pollutants involved to aquatic environment via atmosphere. Because these elements which are in atmosphere get to the water with the rain and wind and has an impact on the aquatic systems (Lee et al. 2007; Sönmez and Akkuş 2009).

Heavy metals left intensively to the environment cause different healthy problems on people by entering the food chain heavy metals. The food chain far reaching people through heavy metals in humans also causes a number of diseases and even death. Our aim is to provide determinig effect of in tissues especially in central nervous system as a result of consuming cause foods included heavy metals and water. Because of this study, the male rat specific ppm-level heavy metals (Fe and Zn) effects on brain tissue damages created recursively, and to reduce the effects of antioxidants (Juglone (5-hydroxy-1,4-naphthoquinone) were determined as the histological and immunohistochemical applications.

Materials and Methods

The damage of heavy metals, iron and zinc formed in the rats brain tissue and juglone (5-hydroxy-1,4-naphthoquinone) antioxidant activities in preventing these damages were explored with histological and immunohistochemical

methods in this study. Five groups were constituded by using 35 adult male Wistar-Albino sexual rats. First group was control group (1 ml. water), second was given iron (0.3 ml. stock solution from Fe/600 ppm. + 0.7 ml.water), third was given Zinc (0.2 ml. stock solution of Zn/ 400 ppm+ 0.8 ml. water), fourth was given Fe (0.3 ml. stock solution from Fe/600 ppm. + 0.7 ml.water) +Antioxidant Juglone (5-hydroxy-1,4-naphthoquinone), fifth was given Zn (0.2 ml. stock solution of Zn/ 400 ppm) + Antioxidant Juglone (5-hydroxy-1,4-naphthoquinone) which was given to the method of rat Gavage. Hematoxylin-eosin (H&E) staining was applied to determine the histological sides of the damages of heavy metals in cerebrum and cerebellum tissues and effects of given juglone (5-hydroxy-1,4-naphthoquinone) for reducing these damages. Tissues were observed with light microscopic (Leica DM 500) examinations. Besides, immunohistochemical TUNNEL method was applied to determine DNA damages in cell (Bancroft et al. 2008) . All methods were performed in accordance with the relevant guidelines and regulations. The manuscript follows the recommendations in the ARRIVE guidelines (Kilkenny et al. 2010).

Results

It was observed that the number of ischemic neurons and vascular dilatation were more density in Fe group. Density of damage in brain tissue of Fe group was higher than in the control group (Figure1-4) but in the other groups were no significant difference with control group ($p<0.05$). Vacuolization in neurons were identificated at Fe group. Number of apoptotic cells were observed in each group. Density of damage in cerebrum of Fe and Fe+Juglone groups were higher than in the control group. Apoptotic cells were increased Fe groups (Figure 4). Apoptotic cells number was decreased in the group consisting of juglone. There was little damage and less apoptotic cells in treated Zn group.

Number of apoptotic cells and purkinje cells were observed in each group. Density of damage in cerebellum of Fe and Fe+Juglone groups were higher than in the control group but in the other groups were no significant difference with control group ($p<0.05$) (Figure 5-10). Degeneration and reduction in the number of purkinje cells were determined in Fe and Fe+Juglone groups. Apoptotic cells were increased Fe groups.Degeneration in Fe+Juglone group was decreased according to the Fe group because of protective effect of the juglone.

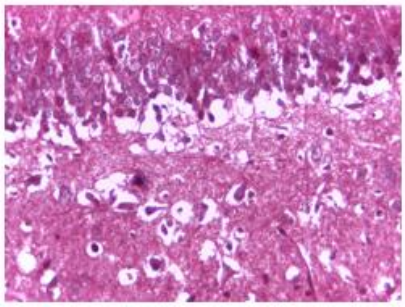

Fig. 1: Fe Group, Masson trichrome, X 40

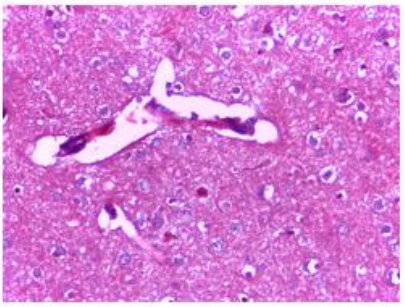

Fig. 2: Fe Group, Masson trichrome, X 40

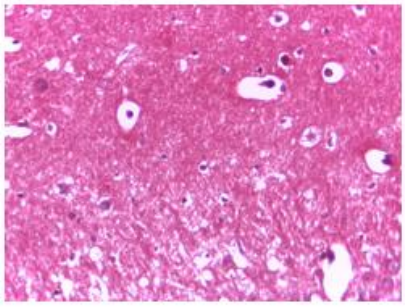

Fig. 3: Fe Group, Masson trichrome, X 40

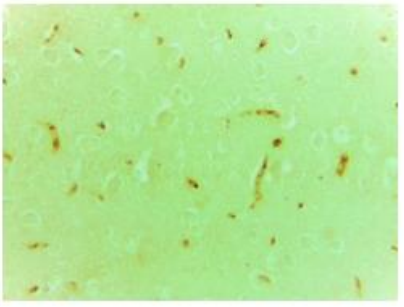

Fig. 4: Fe Group, TUNEL, X 40

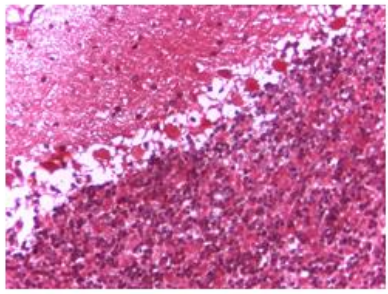

Fig. 5: Fe Group, Masson trichrome, X 40

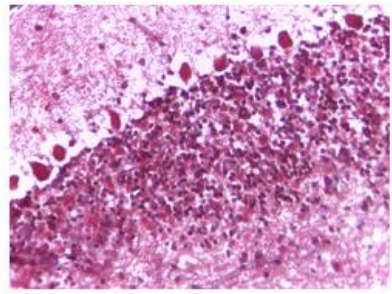

Fig. 6: Fe+Juglone Group, Masson trichrome, X 40

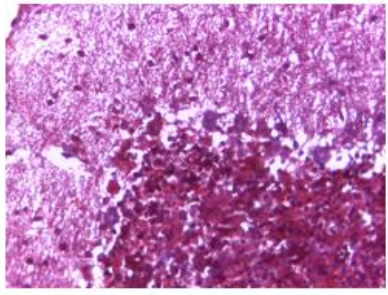

Fig. 7: Zn Group, Masson trichrome, X 40

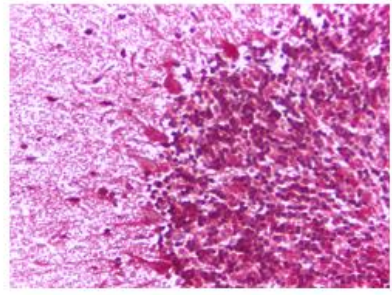

Fig. 8: Zn+Juglone Group, Masson trichrome, X 40

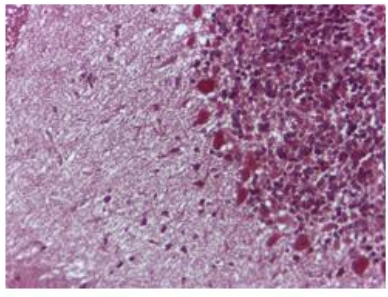

Fig. 9: Control Group, Masson trichrome, X 40

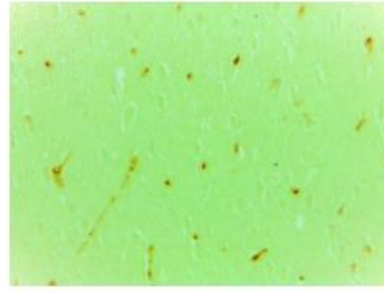

Fig. 10: Fe Group, TUNEL, X 40

Discussion

Various heavy metals such as cadmium, copper, mercury, iron and zinc are well documented to stimulate, tissue metallothionein. Metallothionein (MT) is found in various animal tissues, including the brain, and functions as a scavenger of heavy metals as well as a survey for essential metals (Yasutake et al. 2004).

In spite of exposure to mercury vapor for 3 months, no pathological change was determined in the brains of either MT-null or wild type mice, suggesting a low mercury toxicity of the present dose schedule. The presence of mercury could be detected histochemically in the brains of grains were observed in the cytoplasm of nerve cells and occasional glial cells throughout the brain of both strains, though non-exposed control tissues indicated no mercury grains. It was not observed differences in the distribution and intensity of mercury deposits between the brains of MT-null and wild type mice exposed to mercury (Yasutake et al. 2004). It was reported that mercury deposition at cerebrum and cerebellum cells were observed in rats which exposed to mercury 10 weeks (Warfvinge 1995).

Nehru et al. (1997) were investigated when the weight of the cerebrum and cerebellum was taken separately, a significant rise in the cerebellar weight was seen in the Pb-treated group, and it resulted in a important increase in its rate to brain weight. Following combined application of Pb and Se a substantial improvement in the cerebrum weight and brain weight was declared. Histologically, the transverse section of cortex of group I (control) animals indicated a well-organized cortical layer. The cells were uniform and showed no vacuoles. In the Pb-treated animals, however, the layers were almost absent, and the neurons were diffuse in the cross-section. There were spaces and autophagic vacuoles with debris seen in many places. Pb and Se treated animals, the autophagic vacuoles were present. The pyramidal cells were reduces in size. In this study, it was observed that the number of ischemic neurons and vascular dilatation were more density in Fe group. Density of damage in brain tissue of Fe group was higher than in the control group but in the other groups were no significant difference with control group.

In the central nervous system signs of silver were identicated after higher doses of silver lactate. The sediment was confined to lysosomes of motor neurons in the pontine nuclei and in the spinal cord, but glial cells were also found to contain silver. Neuropathic changes were seen in the myelin sheaths of cauda equina axons (Danscher 1991).

Histochemical changes in several neurons in the central nervous system and spinal ganglia could be detected after about 14 days of exposure to mercury chloride. By investigation light microscopy, the reaction products were seen as black grains in the neuronal somata. In many cases the grains were localized to special regioni juxtanuclearly, but more even distributions were also found (Danscher and Schroder 1979).

Total number of Purkinje cells in iron and iron+nicardipine groups were significantly lower than control animals ($p<0.005$). However, when the iron and iron+nicardipine groups were compared, purkinje cell loss was higher in the iron group ($p<0.05$) (Kozan et al. 2008). Similar results were obtained in this study.

The administration of cadmium (100 ppm) in drinking water to growing rats from 21 days of life for 120 days consistantly induced lesions in the cerebellar cortex. Purkinje cells at places were found to be disintegrated with pyknotic nuclei, eosinophilic cytoplasm and resolution of cellular membranes. The blood vessels, however, appeared normal. The leptomeninges and deep cerebellar nuclei were splitted up (Murthy et al. 1987).

Similar results were observed cerebellum tissues. Density of damage in cerebellum of Fe and Fe+Juglone groups were higher than in the control group but in the other groups were no significant difference with control group ($p<0.05$). Degeneration and reduction in the number of purkinje cells were determined in Fe and Fe+Juglone groups.

Arsenic dose-dependent histopathological changes observed in brain. The section from brain show more frequent nuclear pyknosis. A significant increase in caspase-3 activity was determined at 10.5 and 12.6 mg/kg sodium arsenite in brain (Bashir et al. 2006). In this study, apoptotic cells number was higher Fe groups than other groups.

Fe/600 ppm.doses heavy metals have created a toxic effect on the cerebrum and cerebellum tissues. The damage associated with the Zn and Zn+Juglone was determined to be less significant than the damage by Fe and Fe+Juglone groups. Degeneration in cerebrum and cerebellum tissues were decreased with antioxidant effects of juglone.

Authors' contribution

NŞ and MŞ conceived and planned the study. NŞ drafted and revised the manuscript. NŞ collected the data. NŞ and MŞ analyzed the data. All authors approved the final version of the manuscript for submission.

Data availability statement

All the research data related to this manuscript will be available upon reasonable request to the corresponding authors.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethical approval

The ethical approval of the study was obtained from the Süleyman Demirel University Animal Experiments Local Ethics Committee Chairman Turkey (21438139-338).

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Supplemental material

Supplemental material for this article is available online.

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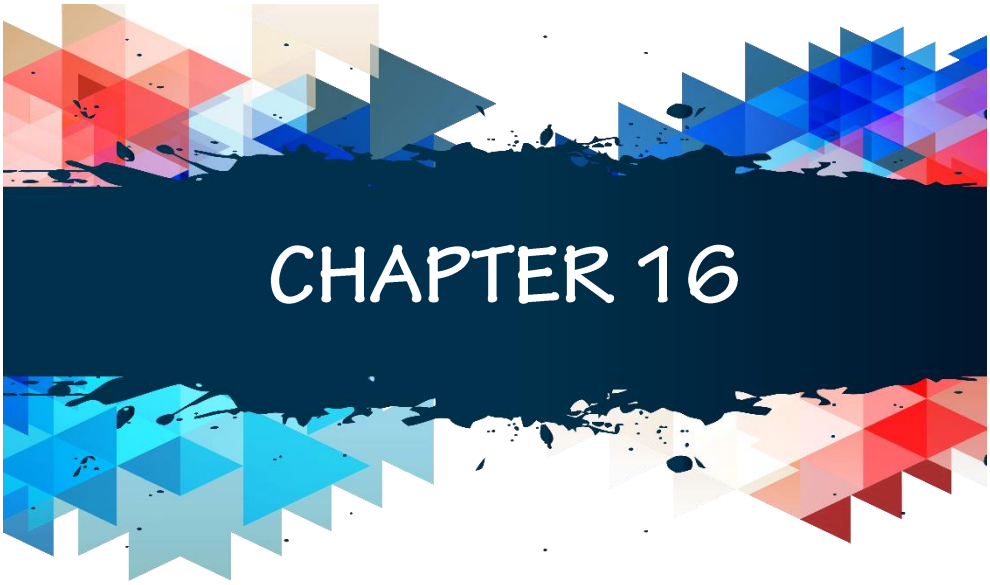

Hypothalamic Regulation of Food Intake and The Impact of Asprosin on Appetite and Consumption

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INTRODUCTION

Adipose tissue not only serves as an energy reservoir but also functions as an endocrine organ. The substances synthesized and secreted by adipose tissue as intercellular signaling proteins are referred to as adipokines. Adipokines exert autocrine, paracrine, and endocrine effects on various organs (Kajimura et al. 2017). To date, approximately 20 members of the adipokine family have been identified. A recent addition to the adipokine group is asprosin (Romere et al. 2016). Asprosin is released in nanomolar levels into circulation from white adipose tissue cells during periods of fasting. This 140-amino acid polypeptide has been shown to perform two major functions. The first is that asprosin, upon being released into the bloodstream, reaches the liver, where it stimulates hepatic cells, promoting the rapid release of glucose into the bloodstream (Romere et al. 2016; Greenhill 2016). The second significant role of asprosin is its ability to cross the blood-brain barrier and exert direct effects on the hypothalamus (Duerrschmid et al. 2017). Asprosin has been found to stimulate AgRP/NPY neurons in the hypothalamus, which are responsible for regulating the hunger center, thereby increasing appetite and food intake (Duerrschmid et al. 2017; Lisa et al. 2018).

Hypothalamus

The hypothalamus is a structure weighing approximately 4 grams, located inferior to the thalamus in the brain and forming the base of the third ventricle. It is composed of a network of nuclei, each of which plays a distinct physiological role within the central nervous system (CNS) (Arıncı and Elhan 2001). As one of the most crucial components of the CNS, the hypothalamus is involved in regulating a wide variety of functions. Due to its central location in the brain, it is connected to several other brain regions, including the brainstem, limbic system, amygdala, pituitary gland, and cerebral cortex, facilitating its integration within the broader neural network of the body (Kandel et al. 2000).

Functions of the Hypothalamus and Its Relation to Food Intake

The hypothalamus is the primary center responsible for regulating homeostasis, which refers to the stability of the internal environment of the body. Several of the hypothalamic nuclei are involved in regulating long-term energy balance, glucose utilization, sodium and water balance, body temperature, sleep/wake cycles, and blood pressure. In this context, the hypothalamus serves as a bridge connecting the central nervous system (CNS), the endocrine system, and the autonomic nervous system (ANS) (Hall 2017).

The hypothalamus also plays a critical role in controlling feeding behavior. The lateral hypothalamic nuclei function to stimulate hunger, while the ventromedial hypothalamic nuclei serve as a center for satiety. Various neurons and peptides in these regions of the hypothalamus contribute to the generation of feelings of hunger and fullness (Shahid and Singh 2019). The arcuate nucleus of the hypothalamus is responsible for regulating food intake and energy expenditure in response to signals from peripheral hormones such as leptin and ghrelin.

Within the arcuate nucleus, two types of neurons are involved in controlling food intake. These include pro-opiomelanocortin (POMC) neurons and neurons that produce Agouti-related protein (AGRP) along with Neuropeptide Y (NPY). POMC neurons are involved in generating the sensation of satiety and inhibiting food intake by producing α-melanocyte-stimulating hormone (α-MSH) and cocaine- and amphetamine-regulated transcript (CART), which also increase energy expenditure. On the other hand, AGRP/NPY neurons contribute to the sensation of hunger and the reduction of energy expenditure through the secretion of their respective peptides. These effects are mediated by the activation and inhibition of melanocortin receptors (MCR-4) located in the paraventricular nucleus (PVN) (Figure 1).

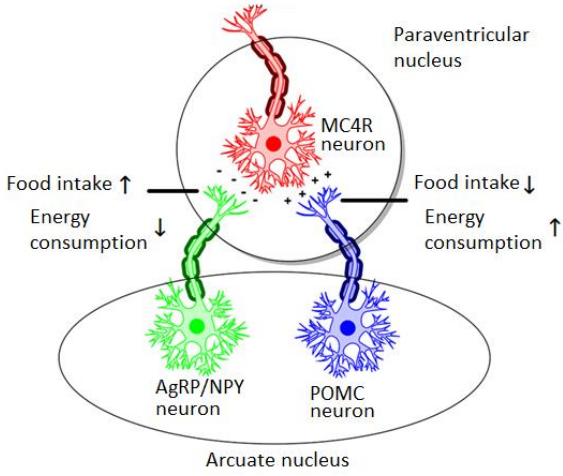

Figure 1. Control of food intake and energy expenditure in the arcuate nucleus (https://www.vanderbilt.edu/vicb/discovery_archives/controlling_energy_balance.html).

Asprosin and Its Effects on Food Intake

Asprosin was serendipitously discovered in 2016 by American scientists led by Romero et al., during their investigation of two distinct patients diagnosed with Neonatal Progeroid Syndrome (NPS) (Romere et al. 2016). NPS is a rare genetic disorder that is primarily characterized by the presence of abnormally low levels of adipose tissue throughout the body, which distinguishes it from other conditions related to premature aging (O'Neill et al. 2007). Patients suffering from NPS often present with insulin resistance and exhibit a tendency to consume excessive amounts of food, conditions that are thought to be associated with the underlying metabolic abnormalities characteristic of the syndrome (Shagun et al. 2015). The discovery of asprosin occurred when Romero and his colleagues identified an anomaly that was distinctly different from other known presentations of NPS in these two patients. Upon further investigation, it was found that this anomaly was linked to a previously unidentified peptide hormone. Since asprosin is released specifically from white adipose tissue, it was named after the Greek word "aspros," meaning white, in reference to its tissue origin.

Asprosin is a peptide hormone consisting of 140 amino acids and is encoded by the last two exons of the FBN1 gene. Exon 65 codes for 11 amino acids, while exon 66 encodes 129 amino acids, together forming the full-length hormone. Research has shown that white adipose tissue exhibits the highest expression of the FBN1 gene, supporting the conclusion that asprosin is secreted predominantly by this type of adipose tissue (Romere et al. 2016; Greenhill 2016). This discovery has contributed significantly to the understanding of how white adipose tissue can influence metabolic processes, especially in the context of conditions like NPS, where both abnormal fat distribution and metabolic dysregulation are central features. Furthermore, the identification of asprosin has opened new avenues of research into the role of adipose tissue-derived hormones in regulating glucose metabolism and overall energy homeostasis.

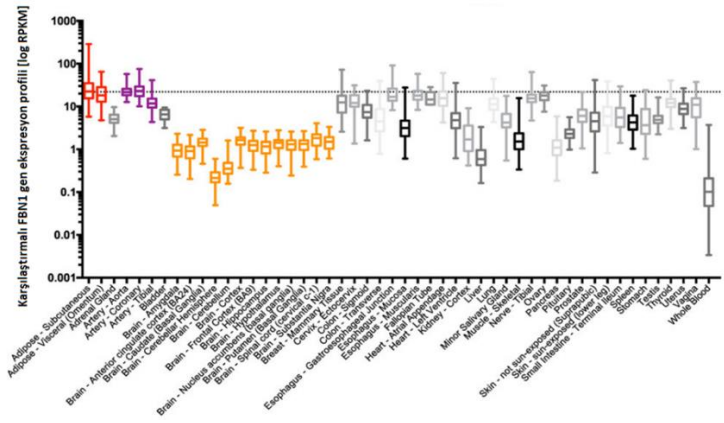

Figure 2. Human FBN1 gene expression profile (Romere et al. 2016).

After being released from white adipose tissue into the bloodstream, asprosin travels to its target organs, including the liver and hypothalamus. As a peptide hormone, asprosin exerts its effects by binding to a receptor on the surface of target cells, which is coupled to a heterotrimeric guanosine triphosphate (GTP)-binding protein (G protein). This binding event triggers a signaling cascade, primarily through the activation of the cyclic adenosine monophosphate (cAMP) - protein kinase A (PKA) pathway (Romere et al. 2016; Duerrschmid et al. 2017).

Upon binding to the G protein-coupled receptor (GPCR) on the cell membrane, asprosin activates the enzyme adenylate cyclase. This enzyme catalyzes the conversion of a small amount of cytoplasmic adenosine triphosphate (ATP) into cAMP. The subsequent increase in cAMP levels activates protein kinase A (PKA), which then phosphorylates specific target proteins within the cell. This phosphorylation event sets off a series of biochemical reactions that culminate in the cellular response associated with asprosin's hormonal effects. These processes are integral to the regulation of key physiological functions, such as glucose metabolism and energy homeostasis, as illustrated in Figure 3.

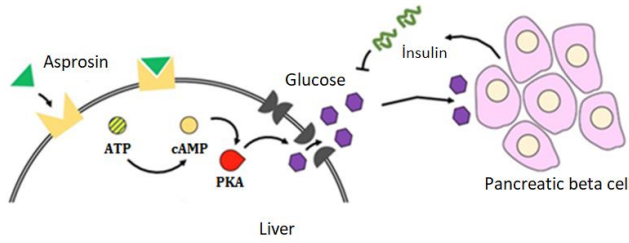

Figure 3. Mechanism of action of asprosin (2).

It has been well-established that, after being released into the bloodstream, asprosin is capable of crossing the blood-brain barrier and exerting direct effects on the hypothalamus. Research has demonstrated that asprosin activates orexigenic neurons, including those that produce Neuropeptide Y (NPY) and Agouti-related protein (AGRP), located in the arcuate nucleus of the hypothalamus, via the cAMP signaling pathway (Duerrschmid et al. 2017). The activation of these neurons leads to the release of substances that promote an increase in appetite and food consumption.

In addition to stimulating orexigenic neurons, asprosin has been found to inhibit pro-opiomelanocortin (POMC) neurons, which play a crucial role in inducing satiety. This inhibition appears to occur indirectly through the activation of AGRP neurons. Upon activation, AGRP neurons inhibit POMC neuron activity through GABA receptors, thereby preventing the generation of the satiety signal (Duerrschmid et al. 2017).

The activation of NPY-AGRP neurons is known to increase food intake while simultaneously reducing energy expenditure (Ilnytska and Argyropoulos 2008). In an effort to understand the role of asprosin in regulating energy consumption, Clemens Duerrschmid and colleagues conducted a study in which they infused asprosin and monitored oxygen consumption as a measure of energy expenditure. However, no statistically significant correlation was found between asprosin administration and oxygen consumption (Duerrschmid et al. 2017). It is worth noting that oxygen consumption alone may not provide a definitive indication of energy expenditure, and thus, the precise interaction between asprosin and overall energy balance remains inconclusive.

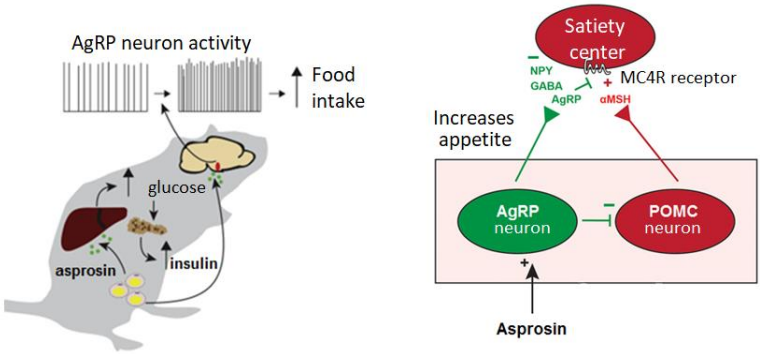

Figure 4. Effects of Asprosin on Food Intake (Duerrschmid et al. 2017). (https://journals.plos.org/plosbiology/article/figure?id=10.1371/journal.pbio.2006188.g001)

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CHAPTER 17

Comparative Analysis of Antinuclear Antibody Detection: Advantages and Limitations of ELISA and IFA Methods

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Introduction

As long as the immune system functions properly, it defends the body against a wide range of foreign antigens without mounting a response against the individual's own antigens (1). While a healthy organism has developed various mechanisms to protect its antigenic structures from its immune system, tolerance to self-antigens may sometimes be lost (2,3). Some immune cells fail to recognize certain structures of their own tissues and mistakenly perceive them as foreign antigens, leading the immune system to attack the individual's own cells and tissues. These molecules that develop against self-antigens are referred to as autoantibodies. There are several subclasses of autoantibodies, and they are named according to the regions they affect (4,5).

What is Antinuclear Antibody

Antinuclear antibodies (ANA) target nuclear components, including DNA, RNA, histones, and centromeres, resulting in cellular damage and functional disruption. ANA is among the most frequently detected autoantibodies, with positivity observed in approximately 55-95% of autoimmune diseases, including Sjögren's syndrome and systemic lupus erythematosus (6). Distinct from other autoantibodies, ANA's ability to infiltrate cells and interact with genetic material, such as DNA within the cell nucleus, thereby inducing genetic damage, renders it particularly hazardous. Moreover, the cellular attack provokes an inflammatory response, which may culminate in irreversible damage to vital organs and tissues, particularly the kidneys, heart, and joints.

The term "antinuclear antibodies" (ANA) was initially introduced to describe autoantibodies targeting nuclear components. However, it has since been broadened to encompass autoantibodies directed against cellular structures beyond the nucleus, including those within the cytoplasm, due to the advent of the indirect immunofluorescence (IIF) technique, which detects all autoantibodies present in the cell (7). While ANA is found at low levels (5-15%) in healthy individuals, its prevalence has been progressively increasing (8). Additionally, its occurrence is significantly higher in individuals with autoimmune diseases (6). These autoantibodies are not only pivotal in the pathogenesis of autoimmune conditions but also serve a critical role in their diagnosis and management. Therefore, the detection of ANA with high sensitivity and specificity is essential. Common methodologies for ANA detection include techniques such as Enzyme-Linked Immunosorbent Assay (ELISA) and Immunofluorescence (IIF).

ANA ELISA Test

Enzyme-Linked Immunosorbent Assay (ELISA) is a widely used and versatile analytical technique in medicine, known for its ability to measure multiple samples simultaneously within a short timeframe. This assay is both highly specific and sensitive. ELISA operates based on the antigen-antibody interaction and can yield either qualitative or quantitative results (9). The technique can be employed to detect antibodies against an antigen or vice versa. To identify the presence of an antibody, the corresponding antigen is immobilized on a 96-well ELISA plate, and the sample to be tested is applied. If the sample contains specific antibodies against the antigen, they will bind to the antigen. Following this, an enzyme-conjugated anti-IgG antibody is introduced to bind to the antigen-bound antibodies. In the final step, a substrate is added, allowing the enzyme to catalyze a color-producing reaction, converting the results into a detectable signal (Figure 1). The results are then determined by measuring the intensity of the color change.

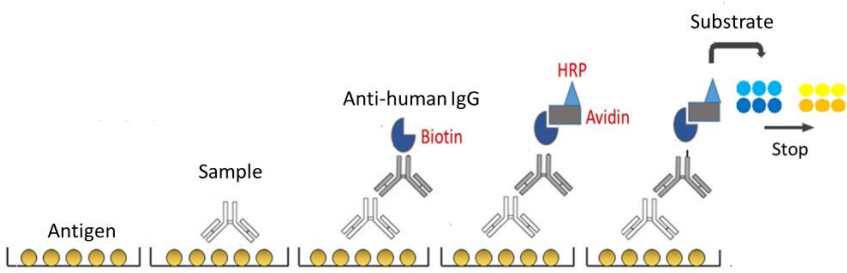

Figure 1. Steps and schematic representation of the ANA ELISA method

Several ELISA assays for the detection of antinuclear antibodies (ANA) have been developed and validated (10,11). Consequently, ELISA has become the most widely used method, not only for routine screening but also for the specific detection of ANA. Furthermore, due to the strong correlation between ANA levels and disease activity, it is commonly employed to monitor disease progression.

ELISA can be utilized to detect total ANA, extractable nuclear antigens (ENA), and specific ANA antibodies, such as anti-dsDNA and anti-La antibodies. Commercially available kits typically use either tissue extracts containing various nuclear components or molecules synthesized via recombinant technology. For total ANA detection, cell nuclei are often used (12), while for ENA, antigens such as calf or rabbit thymus extracts are preferred (13). In the measurement of anti-dsDNA, purified calf thymus DNA is commonly employed (14).

Studies comparing the performance of the ELISA test with the indirect immunofluorescence (IF-ANA) test have shown that the agreement on ANA positivity ranges from 87% to 95% (15). Sensitivity across different commercial ELISA kits has been reported to vary between 69% and 98%, while specificity ranges from 81% to 98% (16).

IF-ANA Technique

The indirect immunofluorescence antinuclear antibody (IF-ANA) test was developed by George Friou in 1957 (186) and has since become widely used for the diagnosis of autoimmune diseases, including BDH (17). IF-ANA detects the presence of ANA in the patient's blood, where it binds to reactive test cells (substrates), forming distinct fluorescence patterns that are associated with specific autoimmune conditions. Initially, various cell types, such as chicken erythrocytes, rat liver, rat kidney, and HeLa cells, were used as substrates (16). However, in 1975, the introduction of HEp-2 cells significantly enhanced the sensitivity of the test (18). HEp-2 cells, derived from a laryngeal squamous cell carcinoma, are now commercially available pre-coated on glass slides. Today, HEp-2 cell substrates are predominantly used worldwide for the IF-ANA test (18).

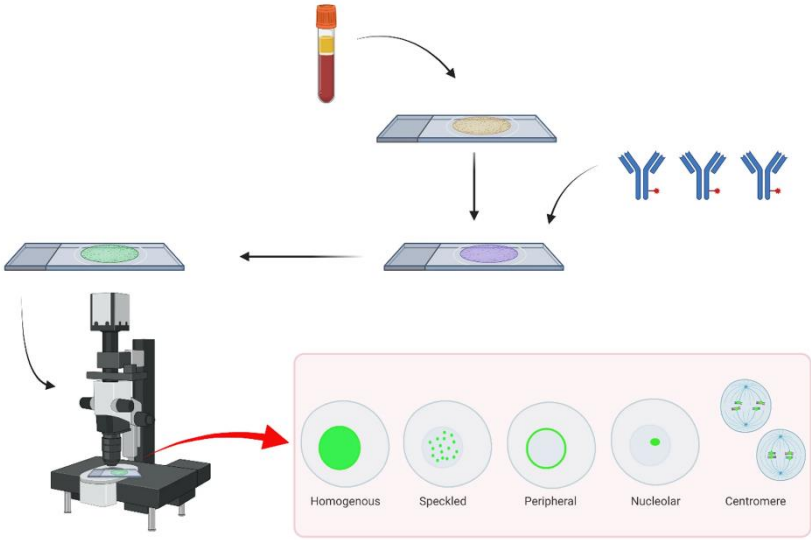

Figure 2. Visual explanation of the IFA measurement method (19)

The fluorescence patterns observed in the indirect immunofluorescence antinuclear antibody (IFA-ANA) test can provide valuable insights into the specificity of ANA, as these patterns reflect the characteristic nuclear localization of the target antigens. Common fluorescence patterns detected by IFA include homogeneous, granular, peripheral, and nucleolar patterns (Figure 2). For example, peripheral ANA is associated with the presence of anti-dsDNA antibodies and is typically indicative of systemic lupus erythematosus (SLE), while granular ANA suggests the presence of antibodies against extractable nuclear antigens (ENA), such as Ro, La, and Sm (17). However, due to the complex topological features within these patterns, technical expertise is required for their accurate interpretation (20, 21). Additionally, since the entire cell is utilized in IFA-ANA, antibodies targeting cytoplasmic, mitotic, and nuclear molecules can also be detected. This broad reactivity may cause confusion in terminology and create challenges when using ANA positivity as a diagnostic and classificatory criterion for autoimmune diseases (22).

ANA positivity is a critical factor in the diagnosis of systemic lupus erythematosus (SLE), with indirect immunofluorescence (IFA) generally regarded as the 'gold standard' for serological testing. However, while IFA-ANA is often considered the gold standard, it may not always yield the expected intensity. The performance of IFA can vary depending on several factors, including the specific test kit used, the conditions of cell fixation, the cellular concentration of antigens, and the specificity of anti-IgG reagents (22). Another issue with IFA is the initial dilution of the serum used for testing. Since even normal sera can result in ANA positivity at low dilutions, the standard starting dilution for routine testing is typically 1:40 or 1:80 (23, 24). Higher dilutions may also be used for initial screening, although this may reduce the frequency of positive results in patient samples. Consequently, the definition of positive and negative results in IFA tests is influenced by the dilution factor of the serum used for the initial dilution (25). The choice of dilution should aim to minimize the number of positive results in the control population while maximizing positivity in individuals with the disease.

IFA is inherently a visual test and is observer-dependent. Specifically, for sera with low antibody titers, determining positivity may vary between observers, leading to reduced sensitivity. Digital imaging and computer-based methods have been proposed to address this limitation; however, inter-analyzer variability in results persists (26).

Beyond observer-dependence, the IFA-ANA test presents other challenges. One such challenge is the difficulty in establishing a clear threshold for positivity.

Additionally, the frequency of positive results in healthy individuals can be problematic. Depending on the test kit used, ANA positivity can be observed in 20-30% of individuals without autoimmune disease (22). The underlying reasons for this high rate of ANA positivity in healthy individuals remain unclear. However, it is hypothesized that false positive results may arise from the cross-linking of antibodies to fixed (and denatured) nuclear molecules (27).

Depending on the test kit used and the demographic characteristics of the population being studied, IFA-ANA results can range from 5% to 20%. This variability can result in elevated ANA positivity rates in healthy individuals and unexpectedly low positivity rates in patients with systemic lupus erythematosus (SLE). Seronegativity in these cases may be due to the insufficient sensitivity of certain test kits or the inherent limitations of IFA in detecting specific antibodies, such as anti-Ro60 antibodies (28). These issues have led to the development of alternative assays, such as ELISA, which offer more reliable detection of ANAs, greater sensitivity, require less specialized personnel, and provide improved cost-efficiency.

Conclusion

In conclusion, both IFA and ELISA are widely used methods for ANA measurement, each with its own advantages and disadvantages. IFA is considered the gold standard due to its ability to provide detailed fluorescence patterns that can offer insights into the specificity of ANA, but it is observer-dependent, subject to variability in interpretation, and has a relatively lower sensitivity, especially in cases with low antibody titers. On the other hand, ELISA is highly specific and sensitive, capable of providing quantitative results and is less dependent on observer skill. However, ELISA may lack the ability to capture the full range of fluorescence patterns seen in IFA and may require careful calibration for accurate results. Overall, ELISA is more suitable for routine screening and quantification, while IFA remains a valuable tool for detecting specific patterns and in-depth analysis of ANA. Both methods complement each other, with their use depending on the clinical context and diagnostic needs.

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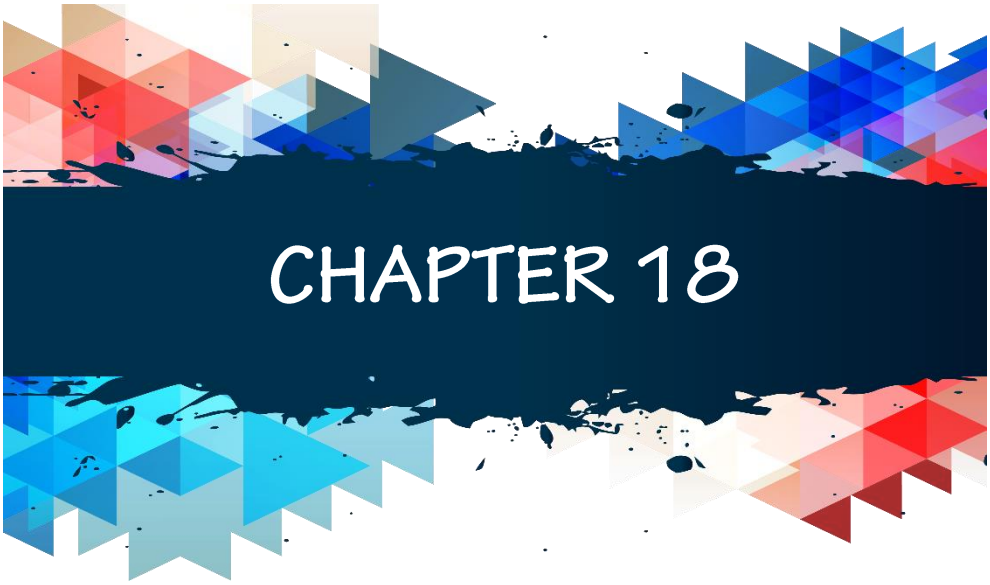

Exercise During Pregnancy

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INTRODUCTION

In recent years, the increasing interest of women in various exercise programs has led to the continuation of these activities during pregnancy, and even an increase in the desire to exercise especially for aesthetic reasons during this period. Evidence that physically active women have easier births dates back to ancient times. Aristotle stated that difficult births were due to a sedentary (passive) lifestyle. It is written in the holy book that Hebrew slaves had easier births than other women. While exercise recommendations at the beginning of the twentieth century were limited to walking in the fresh air, the first foundations of more active exercise programs were laid in the 1930s. Vaugh's squatting exercises to strengthen the perineal muscles (around the vagina), Read's breathing techniques, and Lamaze's psychoprophylactic birth methods are examples from this period. Between 1940 and 1960, exercise programs were limited to the above recommendations and walks in the open air. However, today, more and more women want to participate in various exercise programs such as running, aerobic running, yoga, pilates during pregnancy or continue the sports they do.

The American College of Obstetricians and Gynecologists states that physical activity is generally low-risk during pregnancy and is beneficial for many women. However, exercise programs should be designed considering the specific needs of each pregnant woman and the condition of the fetus. Therefore, it is important for women to have a thorough clinical evaluation for possible contraindications before starting any exercise program. Women with uncomplicated pregnancies should be encouraged to continue aerobic and endurance exercise before, during, and after pregnancy. Healthcare professionals should carefully consider these conditions before making physical activity recommendations for women with medical or obstetric conditions. Although bed rest is often recommended, this is often unnecessary and it may be more appropriate to remain active. These approaches are essential for the health of both mother and baby. Regular physical activity provides a number of important benefits during pregnancy. These activities contribute to maintaining physical fitness, helping with weight management, reducing the risk of gestational diabetes, especially in obese women, and improving overall psychological well-being. Physical activity includes all types of bodily movements resulting from the movement of skeletal muscles and supports heart and lung health at every stage of life, reducing the risk of obesity and related health problems. Women who have adopted a healthy lifestyle should be encouraged to continue exercising, eating well and not smoking during pregnancy. For women who do not have healthy habits, the pre-pregnancy period and the period should be seen as the perfect time to adopt such

healthy behaviors. Exercise consists of planned, structured and repetitive physical movements with the aim of improving certain physical fitness components and is considered an essential element of a healthy lifestyle. "In 2018, the United States Department of Health and Human Services published physical activity guidelines recommending that healthy pregnant and postpartum women engage in at least 150 minutes of moderate-intensity aerobic activity per week . These activities include brisk walking. Additionally, the World Health Organization and the American College of Sports Medicine have made similar recommendations, citing evidence that exercise provides indisputable benefits for adults and that these benefits outweigh any potential risks. According to ACOG (American College of Obstetricians and Gynecologists), exercise should be encouraged throughout pregnancy.

The Effects of Exercise on Pregnancy and Labor

It has been emphasized that maternal, fetal and neonatal adverse outcomes are reduced in women who engage in physical activity during pregnancy and that maternal and child health are positively affected. Although it has been reported that physical activity during pregnancy does not provide weight control, it has been found to reduce the risk of gestational diabetes and hypertension, alleviate symptoms of low back pain and depression, improve women's mental health and improve quality of life. Barakat et al. found that exercise during pregnancy decreased cesarean section rates. Although there are studies indicating that exercise during pregnancy shortens the duration of trauma and reduces obstetric interventions, there are also studies indicating that exercise does not affect the duration of trauma. According to the study conducted by Ünver et al. in Malatya, it was observed that quality of life scores increased as exercise habits increased during pregnancy.

Maternal Effects of Exercise

Many women are restricted in their mobility and participation in routine activities during pregnancy, but studies have shown that a daily exercise program can reduce the risk of miscarriage by 40%. American researchers, James Clapp and colleagues have observed that moderate exercise, such as cycling and walking, protects against gestational hypertension when performed throughout pregnancy. Exercise has also been shown to prevent the onset of early labor and premature rupture of membranes and to help shorten the duration of labor. Reduced risk of pre-eclampsia has been reported with physical activity before or during pregnancy. Another study found that vigorous activity before pregnancy and light to moderate or vigorous exercise during pregnancy reduced the risk of

gestational diabetes and abnormal glucose tolerance. Exercise during pregnancy was found to reduce the likelihood of cesarean delivery.

In their randomized controlled study, Garshasbi and Zadeh found a statistically significant decrease in low back pain in the pregnant group who were exercised compared to the other group. It is emphasized that exercise programs initiated before pregnancy may reduce the likelihood of gestational diabetes mellitus (GDM) and may also delay the need for insulin or reduce the amount of insulin in GDM that occurs during pregnancy. In addition, pelvic floor muscle exercises from conservative treatment methods are recommended as the most appropriate treatment method for women with urinary incontinence during pregnancy. Bø and Haakstad reported that there was no significant difference in the incidence of urinary and fecal incontinence in the exercise group and the control group.

Effects of Exercise on the Fetus

Epidemiological studies have shown that there is a very strong relationship between intrauterine growth and developmental delay in the babies of women who do strenuous physical activity and who are undernourished, that mothers who constantly stand and work in strenuous jobs may give birth earlier and that these babies are small for gestational age. According to Artal and O'Toole, in one study, there was no difference between the birth weights of babies in pregnant women who do vigorous exercise and those who live a sedentary life, while in other studies, there was an increase in birth weight in the group that exercised.

Ghodsi and Asltoghiri found in their study that there was no significant difference in the birth weights of babies in the groups that exercised and did not exercise. Problems that may occur in the fetus due to exercise are hypoxemia, fetal heart rate changes and hypothermia. During exercise, the amount of blood flowing to working muscles and organs increases, unlike other organs. Fetal heart rate may vary depending on the intensity, duration and type of exercise. There may be an increase of 10-30 beats/minute in fetal heart rate during exercise. Fetal heart rate returns to normal within 5-20 minutes after exercise. This increase does not harm the fetus. Fetal heart rate deceleration or bradycardia has been reported at a rate of 8.9% in pregnant women who exercise, and it has been stated that it does not have a prolonged effect on the fetus. Szymanski and Satin stated in their study that there is no relationship between the maximum heart rate of the pregnant woman and fetal bradycardia. Uterine blood flow may decrease by 20-60% during exercise. However, it returns to normal within 20 minutes after exercise. The increase in hematocrit and the oxygen carrying capacity of the blood in the

pregnant woman compensate for fetal blood flow. While some studies in the literature indicate that exercise has very little effect on fetal blood flow, some studies indicate that it does not affect it at all. Another possible risk is that exercise may cause fetal distress. Some studies in the literature indicate that moderate-intensity exercise does not have harmful effects on fetal heart rate. In a meta-analysis study, no significant difference was found in terms of fetal respiratory distress syndrome in the exercise group compared to the control groups.

Scope of Exercise in Pregnancy

The scope of the exercise program to be prepared by taking into account the changes that occur during pregnancy should generally be as follows.

- Proper posture (posture) training,
- Teaching appropriate body mechanics,
- Strengthening arms for postpartum child care,
- Strengthening the legs to carry the increased body weight,
- Exercise and training to prevent edema, varicose veins and cramps,
- Exercise and training for pelvic floor muscle control,
- Strengthening of the abdominal muscles,
- Aerobic exercise program for the maintenance of cardiovascular (cardiovascular) endurance,
- Strengthening the muscles to be used during labor,

The type, intensity and duration of exercise during pregnancy should be well regulated to prevent potential hazards to the mother and fetus. In addition, the gestational week is also used as a criterion for adjusting the type and dose of exercise. Swimming and walking are among the safe exercises recommended during pregnancy.

Women who exercised before becoming pregnant can continue their exercise program. However, the ideal time for non-exercising pregnant women to start exercising is the second trimester. Many women think that the best time to exercise is the second trimester. This is because discomforts such as nausea, vomiting and fatigue, which are common in the first trimester, disappear at the end of the first trimester. Women who were previously sedentary should start an aerobic exercise program 3 times a week for 15 minutes. Gradually, the frequency

and duration of exercise should be increased to 4 days a week for 30 minutes. Duration and intensity of exercise are important in pregnancy. Because both intensity and duration decrease the blood flow to the uterus and increase both maternal temperature and fetal temperature. The duration of exercise should be 15 minutes in each session for beginners. After the second trimester, it should be increased by 1-2 minutes per week. The maximum duration of exercise should be 30 minutes.

Strength exercises during pregnancy should be of moderate intensity and should be repeated several times to create muscle fatigue. To prevent hypotension, the Valsalva maneuver (holding breath) should be avoided and exercises should not be performed in the supine position. If diastasis recti develops, abdominal exercises should not be performed.

Contraindications to Exercise in Pregnancy

- Cervical insufficiency

- 2nd and 3rd trimester vaginal bleeding that does not go away

- Placenta previa after 26 weeks

- Premature rupture of membranes

- Severe pregnancy-induced hypertension

- Cardiovascular disease

- History of recurrent spontaneous abortion or risk or history of preterm birth

- Thrombophlebitis or pulmonary embolism

- Multiple pregnancies at risk for premature birth

- Restrictive lung disease

Pregnant women should seek medical attention if they experience the following signs and symptoms during exercise

- Bloody discharge from the vagina,

-Rupture of membranes

- Sudden swelling of the face or hands or ankles

- Persistent, severe headache or changes in vision or both, unexplained faintness or dizziness.

- Swelling, redness and pain in the leg

- Increased heart rate and blood pressure, extreme fatigue, tachycardia and chest pain after exercise

- Contractions lasting more than 6-8 hours may indicate preterm labor

- Unexpected abdominal pain

- Insufficient weight gain (less than one kilogram per month in the last two trimesters)

- Decrease or disappearance of fetal movements

- Shortness of breath

CONCLUSION

Although pregnancy is not a disease, healthy pregnant women should be encouraged to participate in and maintain physical activities. Exercise performed at the appropriate gestational week, at the appropriate exercise type, intensity and duration positively affects the health and development of the baby as well as maternal health. In a normal pregnancy, a woman can continue to exercise if she has previously participated in exercise programs. Pregnant women who exercise should have regular pregnancy follow-ups. In addition, according to the results of many studies, it is pleasing that although most of the exercise programs applied exceeded the level recommended by ACOG, no adverse effects on fetal and maternal findings were found. Pregnant women should receive counseling from health professionals about exercises. Pregnant women should participate in exercises within a program prepared jointly by health professionals, dieticians and sports specialists. The level of knowledge and behavior of pregnant women should be increased by organizing educational seminars and exercise programs on pregnancy exercises.

In summary, it should be kept in mind that every pregnancy is different and every woman's health condition is special. Therefore, women who want to exercise regularly during pregnancy should create an exercise plan within a program under the supervision of physicians and midwives. Therefore, women who want to exercise regularly during pregnancy should develop an exercise plan under the supervision of physicians and midwives. Midwives and nurses should explain to pregnant women who will apply the exercise program that they should apply to the nearest health institution in case of a health problem and warn them to control their heart rate during exercise. Midwives and nurses should be well aware of the benefits of regular exercise during pregnancy for the mother and fetus and should be able to use their knowledge well to include pregnant women in the exercise program.

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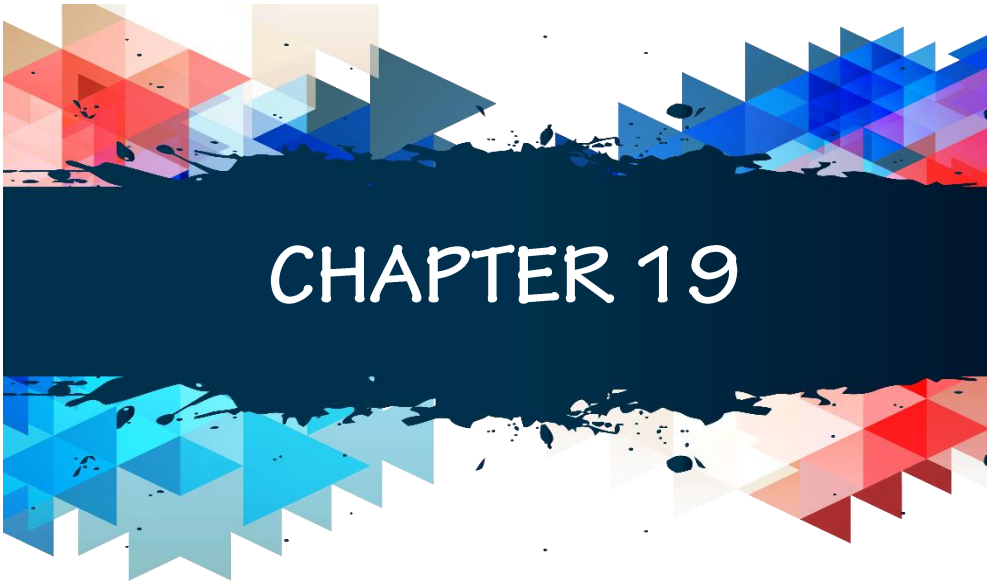

Alzheimer's Disease and Diabetes Mellitus: The Secret of Twin Siblings and Potential Treatment Avenues

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1. Introduction

Alzheimer's disease (AD) and diabetes mellitus (DM) are two of the most common diseases in the elderly, and their prevalence is expected to continue to rise worldwide in response to an aging population. AD is a leading cause of dementia, affecting approximately 10% of individuals aged 65 years and older. Currently, there are approximately 50 million people worldwide with Alzheimer's disease or other forms of dementia, and it is estimated that this number will reach approximately 152 million by 2050. Diabetes is a metabolic disorder that affects over 400 million patients, with more than 90% of them having type 2 diabetes mellitus (T2D). Studies have shown that AD and DM are not independent diseases; instead, they are often closely interconnected at the clinical and pathophysiological levels (1). DM is a chronic condition characterized by absolute or relative insulin deficiency. This condition leads to chronic hyperglycemia (high blood sugar), resulting in insufficient pancreatic beta-cell function and, therefore, inadequate insulin production (type 1 diabetes, T1D) or the development of insulin resistance and subsequent loss of beta-cell function (type 2 diabetes, T2D) (2, 3). Examination of diagnoses shows that AD is the most common cause of dementia in individuals with T2DM, accounting for approximately 91% of all cases (4). Although the exact mechanisms of insulin resistance in this metabolic disorder are not fully understood, obesity and age are recognized as major risk factors (5, 6). Recent epidemiological studies suggest a higher risk of developing AD in individuals with diabetes than in healthy individuals (7). AD is a chronic neurodegenerative disease characterized by the loss of cholinergic neurons, cerebrovascular inflammation, and accumulation of amyloid-beta (Aβ) plaques in cerebral blood vessels and brain parenchyma. The most prominent neuropathological changes in AD are dense senile amyloid plaques in the hippocampus and the cortex. The Aβ peptide is the main component of senile plaques and has numerous toxic effects on neurons, astrocytes, glial cells, and the brain endothelium. Recent research has shed light on oxidative stress and neuroinflammatory pathways (8). In line with this, the studies indicate a close association between Alzheimer's disease and diabetes mellitus through common factors such as chronic inflammation, oxidative stress, mitochondrial dysfunction, vascular damage, impaired insulin signaling, and shared pathogenic mechanisms (9-18). The convergence of Alzheimer's Disease and Diabetes Mellitus has sparked immense interest among researchers and clinicians, leading to a wealth of studies aimed at unraveling the underlying connection between the two conditions. Furthermore, studies have shown that the presence of Diabetes Mellitus can exacerbate the progression of Alzheimer's

Disease, resulting in more severe cognitive impairment and accelerated neurodegeneration. These findings have underscored the need to explore the shared pathophysiological mechanisms that underpin the relationship between these two seemingly distinct diseases. Given the interaction of insulin signaling with Alzheimer's disease (AD) or cognitive impairments and the increasing evidence of shared pathophysiological findings between AD and type 2 diabetes mellitus (T2DM), there is significant interest in exploring the potential benefits of approved antidiabetic drugs for the treatment of AD (19). The research data have shown improvements in the cognitive functions of patients administered antidiabetic drugs such as intranasal insulin, metformin, thiazolidinediones, and incretins (20). Numerous clinical studies have evaluated the effects of antidiabetic drugs on the pathological markers of AD have been conducted, and animal studies have demonstrated positive effects in various areas, including tau protein pathology, β-amyloid accumulation, neurogenesis, oxidative stress, synaptic function, cognitive function, and neuroinflammation (21-35). Findings from clinical trials conducted specifically on AD patients, taking advantage of the understanding of the relationship between AD and insulin resistance, support the development of AD therapeutics and suggest that antidiabetic drugs may be a neuroprotective treatment approach for AD (20). In this comprehensive review, we will explore the shared pathophysiological mechanisms, the impact of insulin resistance on Alzheimer's Disease, the role of amyloid β and oxidative stress, potential treatment avenues including antidiabetic drugs and lifestyle interventions, current research and clinical trials, as well as future perspectives and challenges. By the end of this article, you will have a deeper understanding of the intricate relationship between these two conditions and the potential treatment avenues that could revolutionize the way we approach Alzheimer's Disease and Diabetes Mellitus.

2. The Link Between Alzheimer's Disease and Diabetes Mellitus

The link between Alzheimer's Disease and Diabetes Mellitus has been a subject of intense scientific investigation in recent years. This association has sparked curiosity and led researchers to explore the underlying biological mechanisms that may connect these two seemingly unrelated conditions. As a result of this direction, there are some common risk factors for AD and T2D, including high cholesterol levels, cellular degeneration, beta-amyloid accumulation, oxidative stress, inflammation, cardiovascular problems, metabolic syndrome, glycogen synthase kinase 3, τ protein phosphorylation, apolipoprotein E4, and apoptosis (36-39). Furthermore, reports have shown that insulin plays a significant role in neurological functions, and that a deficiency in

insulin or reduced sensitivity of insulin receptors may lead to decreased acetylcholine levels. This suggests a possible biochemical connection between AD and T2D (36, 39, 40). These relationships may be due to uncontrolled elevation of blood sugar directly impacting neurodegenerative changes in the brain or may have originated from other health issues associated with diabetes or the effects of excessive insulin (41-43). Hyperinsulinemia accompanying type 2 diabetes may serve as a link between AD and DM because it can influence the accumulation of amyloid-beta peptide in the brain by competing with the enzyme that breaks down insulin. Receptors for advanced glycation end products have also been implicated in the pathogenesis of diabetes. These receptors can be found in cells associated with neurofibrillary tangles and may function as cell surface receptors for amyloid-beta peptide. Another significant factor is the presence of excess adipose tissue in the body, which can increase inflammation through cytokines that lead to the production of adipocytes critical for metabolism and insulin resistance. Adiponectin, leptin, resistin, tumor necrosis factor alpha (TNF-α), and interleukin 6 (IL-6) are associated with insulin resistance and hyperinsulinemia and may directly or indirectly influence AD risk (44). This is because oxidative stress in AD, caused by the accumulation of Aβ plaques, activates microglia and astrocytes to create proinflammatory cytokines, such as tumor necrosis factor alpha (TNF-α) and interleukins (ILs), which can have proapoptotic and synaptotoxic effects on neurons (45). Insulin resistance and AD lead to activation of molecular markers such as nuclear factor kappa B (NF-κB) and tumor necrosis factor alpha (TNF-α), increased cytokine release, and elevated levels of reactive oxygen species (ROS). This leads to an increase in amyloid-beta (Aβ) accumulation and hyperphosphorylation of the tau protein. Additionally, insulin resistance reduces the levels of insulin-degrading enzyme (IDE), an enzyme that breaks down insulin, leading to problems in the clearance of amyloid β. Higher levels of amyloid β can lead to a decrease in insulin receptor expression, further increasing insulin resistance and creating a vicious cycle (46). In light of this information, AD can be considered as a metabolic disease in the brain caused by insulin resistance, leading to the proposed term "type 3 DM." Type 3 diabetes primarily refers to a condition in which brain cells do not respond to insulin, resulting in disruption of synaptic function, metabolism, and immune response, among other areas (47).

3. Shared Pathophysiological Mechanisms

Several pathophysiological mechanisms are common between Alzheimer's Disease and Diabetes Mellitus, which might explain the increased risk of developing AD among individuals with DM. These mechanisms span from

insulin resistance, glucose metabolism dysregulation, chronic inflammation, oxidative stress, mitochondrial dysfunction. Understanding these connections sheds light on the intricate relationship between metabolic and neurodegenerative disorders.

3.1. Insulin Resistance and Glucose Metabolism

The brain, like other organs in the body, is insulin-sensitive, and insulin signaling regulates energy metabolism, cell survival, and neuroplasticity. Insulin's neuroprotective effects not only support neuronal growth and survival but are also vital for cognitive processes such as learning and memory. Recent evidence suggests that insulin regulates the concentration of neurotransmitters such as acetylcholine, norepinephrine, and epinephrine, which play crucial roles in memory formation. Additionally, insulin supports neuronal plasticity and cholinergic functions essential for learning, memory, and myelin maintenance. However, disruptions in insulin signaling have emerged as a significant factor linking Alzheimer's disease (AD) and diabetes mellitus (DM) (48). Impaired insulin signaling is a common denominator between AD and DM. In AD, insulin resistance is not confined to systemic levels but also occurs in the brain, impairing neuronal function, synaptic plasticity, and memory formation, thereby exacerbating cognitive decline (48-52). Alterations in molecules involved in the insulin signaling pathway are thought to contribute to the pathogenesis of AD (52). Insulin receptors (IRs), with their tyrosine kinase activity, regulate biological processes such as cell growth, protein synthesis, and glucose transport (52). In addition to peripheral tissues, IRs are present in the hippocampus and cerebral cortex, playing crucial roles in learning and memory. Insulin can cross into the brain from circulation or be endogenously produced (52). By binding to IRs, insulin triggers autophosphorylation and activates insulin receptor substrates (IRS-1 and IRS-2), leading to the activation of signaling pathways such as phosphoinositide 3-kinase/protein kinase B (PI3K/AKT) (53). The PI3K/AKT pathway regulates critical processes, including protein synthesis, cell survival, energy metabolism, and amyloid-beta (Aβ) clearance. It also activates signaling cascades like the extracellular signal-regulated kinase (ERK), associated with synaptic plasticity and memory formation (54). The PI3K-AKT pathway supports mechanisms that promote neuronal growth and survival while suppressing apoptosis (52). This pathway also regulates the activity of glycogen synthase kinase-3β (GSK-3β), which can influence tau expression. Hyperactivation of GSK-3β may lead to tau hyperphosphorylation, a key process in the formation of neurofibrillary tangles (NFTs) in AD pathology (48). Inhibition of GSK-3β prevents tau hyperphosphorylation. Dysregulation of this pathway can exacerbate

AD pathology by promoting abnormal tau phosphorylation (52). Furthermore, the PI3K-AKT cascade modulates GABAergic transmission, which plays a critical role in learning and memory processes (55). Alterations in the mTOR signaling pathway can downregulate insulin receptors, exacerbating insulin resistance, and contributing to AD pathogenesis under hyperinsulinemic conditions (56). Data from animal and cell culture models reveal insulin's effects on both the production and degradation of Aβ (52). Insulin can elevate extracellular Aβ levels by modulating γ-secretase activity or increasing Aβ release from neurons. Insulin-degrading enzyme (IDE), which plays a vital role in Aβ degradation, may be competitively inhibited by insulin, reducing Aβ breakdown (57-59). Moreover, reduced IDE levels and activity have been reported in hyperinsulinemic animals. Aβ has also been shown to interfere with insulin signaling. Aβ peptides may inhibit insulin binding and receptor autophosphorylation, directly impairing insulin's effects (52). Recent studies suggest that Aβ can induce insulin resistance by downregulating insulin receptors (60). The impact of insulin resistance on AD pathophysiology has been studied in various animal models. Diet-induced insulin resistance has been shown to increase Aβ production, enhance γ-secretase activity, and reduce IDE activity in AD transgenic mice (61). Factors like excessive caloric intake can lead to metabolic changes such as obesity, hyperglycemia, and hyperlipidemia, which independently contribute to AD pathology (52). Postmortem analyses of AD brains reveal significant reductions in the expression of insulin and insulin signaling molecules (e.g., IR, IRS-1, IRS-2) (62, 63). These findings suggest impaired insulin signaling in AD brains, though it remains unclear whether this is a cause or consequence of neurodegeneration. Experimental models with intentionally disrupted insulin functions have demonstrated that AD neuropathologies, such as amyloid accumulation and hyperphosphorylated tau protein, can be induced. Furthermore, diabetes mellitus (DM) is thought to contribute to the relationship between AD and DM by altering the structure and function of cerebral blood vessels. DM has been shown to impair cerebral blood flow regulation, disrupt the blood-brain barrier (BBB), and reduce the brain's repair potential. Glycation, a process in which free glucose molecules non-enzymatically bind to proteins, lipids, and nucleic acids, accelerates under hyperglycemic conditions, leading to the formation of advanced glycation end products (AGEs). AGE accumulation causes tissue damage and disrupts cellular functions, while its receptor, RAGE (Receptor for Advanced Glycation End Products), has been identified as a key pathway triggering inflammation and oxidative stress (48-52). Overactivation of RAGE plays a central role in the pathogenesis of chronic inflammatory and neurodegenerative diseases such as

DM and AD. AGE-RAGE interactions can initiate vascular dysfunction and oxidative stress processes in AD. This increases BBB permeability and disrupts cerebral circulation, thereby accelerating amyloid-beta (Aβ) accumulation and neurodegeneration. Aβ, a major component of senile plaques (SP)—one of the histopathological markers of AD—induces damage to both neuronal and vascular structures. Vascular inflammation and oxidative stress mechanisms triggered via RAGE are also implicated in this process. RAGE is suggested to play a significant role in the vascular dysfunction effects of Aβ and may serve as an alternative therapeutic target in AD (52). The interaction of RAGE with Aβ can further impair cerebral circulation by increasing the expression of inflammatory cytokines and endothelin-1. This process lies at the intersection of DM and AD pathophysiology, providing critical insights for identifying therapeutic targets for these diseases. In addition, one of DM's contributions to AD pathogenesis involves disturbances in energy metabolism. AGE accumulation, mitochondrial dysfunction, and the generation of reactive oxygen species (ROS) impair ATP production, leading to neuronal dysfunction. Studies in diabetic animal models have demonstrated a decline in glycolytic capacity, which, together with reduced ATP production, negatively impacts synaptic activity. Dysregulation of glucose metabolism is another common feature. Chronic hyperglycemia in DM impairs glucose transport to the brain, reducing the availability of energy substrates for neurons. This metabolic dysfunction increases the risk of AD by causing brain energy deficits and neuronal damage (48, 49). Glucose transporter proteins (GLUT), particularly GLUT4 and GLUT8, are insulin-sensitive, and insulin signaling regulates their phosphorylation. However, in diabetic conditions, disruptions in insulin signaling inhibit glucose transport and utilization in the brain, accelerating cognitive decline. Apolipoprotein E (APOE) genotype is another significant factor in the relationship between DM and AD. In individuals carrying the APOE4 allele, reduced glucose metabolism has been observed in the posterior cingulate, precuneus, and lateral parietal regions. These metabolic abnormalities exacerbate neuronal damage in both AD and DM, triggering neurodegenerative processes (49). Considering the significant role of RAGE-induced vascular inflammation and oxidative stress in these pathways, the AGE-RAGE axis is proposed as a potential therapeutic target for both DM and AD. The reciprocal interactions between AGE accumulation and insulin signaling—affecting energy metabolism, synaptic activity, and mitochondrial function—are crucial elements to consider in treatment strategies for DM and AD (52). The accumulation of Aβ plaques, a hallmark feature of AD, is exacerbated in individuals with DM. Mechanisms such as increased AGE production and oxidative stress contribute to enhanced Aβ deposition (48, 51, 64). Tau

pathology, characterized by the formation of neurofibrillary tangles, is also associated with metabolic disruptions commonly observed in DM. Factors such as oxidative stress and mitochondrial dysfunction further promote tau phosphorylation and aggregation (48, 65).

In this context, approaches aimed at optimizing glycemic control may not only slow the progression of AD but also alleviate the cognitive complications of DM. Therapeutic strategies targeting AGE accumulation and RAGE activation may play a critical role in reducing vascular inflammation and improving cerebral energy metabolism.

3.2. The Impact of Inflammation on Neural and Metabolic Processes

Both diseases are characterized by systemic and neuroinflammation, with increased levels of pro-inflammatory cytokines and immune cell activation, promoting the pathological changes in both brain and peripheral tissues (65, 66). In DM, chronic hyperglycemia and insulin resistance lead to the persistent activation of inflammatory responses. Hyperglycemia increases the accumulation of advanced glycation end products (AGEs) and their receptors (RAGE), which in turn amplify inflammation and oxidative stress. The AGE-RAGE interaction enhances the release of proinflammatory cytokines and promotes the generation of reactive oxygen species (ROS), exacerbating both vascular and neuronal damage. Activation of the AGE-RAGE axis further reinforces the peripheral inflammation reflected in the central nervous system (51, 52). This process increases the release of cytokines such as IL-1β, IL-6, and TNF-α, supporting systemic inflammation. These cytokines disrupt insulin signaling pathways by enhancing serine phosphorylation of insulin receptor substrate-1 (IRS-1), thereby inhibiting glucose uptake. IL-1β triggers β-cell apoptosis, while IL-6 strengthens inflammatory signaling pathways, further increasing systemic insulin resistance. TNF-α reduces insulin's effectiveness and increases free fatty acids, perpetuating the inflammatory cycle. These processes not only contribute to the chronic complications of DM but also serve as critical triggers for the pathogenesis of AD. Consequently, elevated proinflammatory cytokine levels in DM patients hyperactivate immune cells, causing damage in both peripheral tissues and the brain (65).

In AD, neuroinflammation is characterized by the activation of microglia and astrocytes. Overactivation of microglia promotes chronic neuroinflammation and neurodegeneration in AD brains, while in DM, metabolic insult due to hyperglycemia primes microglia toward an exaggerated inflammatory response

(51). Activated microglia evoke significant alterations in both their morphology and functionality, and once activated, they react by taking on an amoeboid or bushy shape and show an enhanced capacity for chemotaxis, phagocytosis, and the release of a large number of bioactive compounds, including cytokines, chemokines, reactive oxygen species (ROS), and nitric oxide bioactive compounds. In neurodegenerative diseases, activated microglia play a major role in the modulation of chronic local inflammation, which becomes an important factor in modifying neuronal function leading to cell damage and death. Activation of the multifunctional protein complex nuclear factor-kappa B (NF-kB), a major proinflammatory signaling system. (67). The brain is particularly vulnerable to oxidative stress and inflammation, exhibiting significant increases in the response to different initiators. Although previously primarily associated with cell damage and cell death, these altered states of neurons and glia also contribute to the damage observed during neurodegenerative diseases associated with inflammatory and oxidative stress processes. The occurrence and progression of neuroinflammation involve a complex interaction between various types of cells, including astrocytes, microglia, and neurons. While astrocytes and microglia are the major immune cells of the brain, neurons have been shown to generate local inflammatory mediators, such as the chemokine fractalkine (FKN or CX3CL1), essential for crosstalk between neurons and microglia. Microglia, in particular, are highly dynamic cells designed to react to cellular stress conditions. Hence, even in their resting or surveillance state, microglia continuously monitor the tissue and, in response to tissue disturbances, can adopt various morphological states to fulfill their typical functions. (68)

The activation of glial cells in response to Aβ plaques and hyperphosphorylated tau protein triggers the release of IL-1β, IL-6, and TNF-α, leading to the chronicization of the inflammatory response. IL-1β and TNF-α enhance β-secretase (BACE1) activity, accelerating Aβ production and suppressing microglial phagocytosis, thereby promoting Aβ accumulation. This process results in impairments in synaptic plasticity and accelerates cognitive decline. When released by microglia, IL-1β strengthens inflammatory cycles and disrupts synaptic function, inducing long-term depression (LTD) in hippocampal neurons (65). IL-6, primarily produced by activated astrocytes, sustains both central and peripheral inflammation. IL-6 has been shown to accumulate around amyloid plaques and stabilize their formation. Additionally, IL-6 promotes the activation of kinases such as GSK-3β and CDK5, which support tau protein hyperphosphorylation, thereby disrupting microtubule stability. TNF-α and IL-1β also increase the permeability of the blood-brain barrier (BBB), facilitating

the entry of peripheral inflammatory mediators into the central nervous system. This BBB disruption further exacerbates Aβ accumulation, intensifying the neuroinflammatory cycle and contributing to the progression of AD (48, 65).

The activation of macrophages and microglial cells provides a common foundation for the inflammatory processes in both DM and AD. The increase in inflammatory cytokines in DM serves as a trigger that accelerates the neurodegenerative processes of AD. For instance, elevated peripheral levels of cytokines such as IL-6 and TNF-α enhance microglial activation and support Aβ pathology. Additionally, hyperglycemia and insulin resistance disrupt energy metabolism, further aggravating the neuroinflammatory processes characteristic of AD. Excessive production of TNF-α and IL-1β facilitates the transition of astrocytes into a reactive phenotype. These reactive astrocytes cause polarization loss in AQP4 (aquaporin 4) channels, disrupting the brain's glymphatic system and impairing Aβ clearance. Reduced efficiency of the glymphatic pathway plays a critical role in AD progression. Chemokines released by microglial cells can also exacerbate neuronal damage; for example, chemokines like CCL2 trigger the clustering of inflammatory cells around neurons, further worsening synaptic dysfunction (65).

The combination of neuroinflammation and systemic inflammation forms the shared mechanisms of AD and DM. In AD, IL-6 and TNF-α enhance glutamate-mediated excitotoxicity in neurons, triggering oxidative stress and mitochondrial dysfunction. In DM, these cytokines impair insulin production and signaling pathways, weakening energy metabolism. Additionally, AGE accumulation and RAGE activation create a feedback loop that further amplifies the inflammatory response (51, 52, 65).

The inflammatory link between DM and AD requires a deeper understanding of immune responses and the development of therapeutic targets. Achieving glycemic control in the early stages of DM and suppressing the AGE-RAGE axis can help regulate inflammatory processes in both diseases. Similarly, therapeutic approaches targeting cytokine-mediated pathways may play a critical role in reducing neuroinflammation. Treatments that prevent AGE accumulation and inhibit RAGE activation may not only slow the progression of AD but also alleviate the cognitive complications of DM. In this context, strategies targeting inflammation offer new hope for breaking the mutually

reinforcing effects of AD and DM, providing promising avenues for the treatment of both conditions.

3.3. Oxidative Stress and Mitochondrial Dysfunction

Oxidative stress and mitochondrial dysfunction are interconnected processes that exacerbate cellular damage in both conditions. In these states, excessive reactive oxygen species (ROS) production overwhelms the antioxidant defense system, leading to protein, lipid, and DNA damage. Mitochondrial dysfunction further amplifies this process by reducing ATP production and increasing ROS generation, creating a vicious cycle that promotes cellular and neuronal injury.

Mitochondrial dysfunction and oxidative stress are central mechanisms in the pathogenesis of both Alzheimer's disease (AD) and type 2 diabetes mellitus (T2DM). Mitochondria play a crucial role in cellular processes, including energy metabolism, reactive oxygen species (ROS) production, and calcium homeostasis. However, in AD patients, imbalances in mitochondrial dynamics (fusion and fission), insufficient mitophagy, and increased ROS production accelerate synaptic dysfunction and neurodegeneration (69). Specifically, mitochondrial DNA (mtDNA) damage contributes to reduced energy production and cellular dysfunction, leading to impaired synaptic plasticity and cognitive decline (70). Insulin resistance and hyperglycemia in T2DM are directly linked to the pathogenesis of AD. Impairments in insulin signaling promote glycogen synthase kinase-3β (GSK-3β) activation, leading to tau hyperphosphorylation and amyloid-beta (Aβ) accumulation. Systemic inflammation and glucose metabolism disturbances in T2DM exacerbate neuroinflammation and mitochondrial dysfunction observed in AD (71, 72). The loss of insulin's neuroprotective effects restricts glucose uptake, resulting in energy imbalance and increased oxidative stress. These processes contribute to synaptic plasticity deficits and accelerate neurodegeneration in both diseases (70). Mitochondrial dysfunction, together with increased oxidative stress, activates cell death pathways, which are associated with reduced cerebral glucose metabolism in T2DM and neuronal damage in AD. In T2DM patients, reduced cerebral glucose metabolism exacerbates the cognitive complications of AD. Moreover, increased ROS production leads to damage to proteins, lipids, and DNA, further impairing mitochondrial functions (69). Hyperglycemia, in particular, disrupts oxidative phosphorylation processes, decreases mitochondrial biogenesis, and negatively impacts energy metabolism (72).

These findings highlight the reciprocal interaction of oxidative stress, mitochondrial dysfunction, and insulin resistance in the pathogenesis of AD and

T2DM. Strategies that support mitochondrial biogenesis, reduce oxidative stress through antioxidant therapies, and improve insulin sensitivity are considered potential therapeutic targets to slow disease progression. Lifestyle modifications, regular physical exercise, and multi-targeted pharmacological interventions are promising approaches for managing these processes (69, 70, 71, 72).

4. Conclusion

The growing body of evidence highlights the intricate relationship between Alzheimer's disease (AD) and type 2 diabetes mellitus (T2DM), emphasizing shared pathophysiological mechanisms such as oxidative stress, mitochondrial dysfunction, insulin resistance, and chronic inflammation. These interconnected processes exacerbate neuronal injury, synaptic dysfunction, and cognitive decline, providing a foundation for understanding the overlap between metabolic and neurodegenerative disorders. Mitochondrial imbalances, including disrupted dynamics (fusion and fission), impaired mitophagy, and increased reactive oxygen species (ROS) production, emerge as key contributors to the progression of both conditions. Concurrently, insulin resistance and hyperglycemia disrupt energy metabolism, fueling oxidative stress and inflammatory cascades that amplify neurodegeneration in AD.

Given these findings, future research should prioritize integrative approaches targeting the shared molecular pathways of AD and T2DM. Strategies aimed at enhancing mitochondrial biogenesis, reducing oxidative stress, and restoring insulin sensitivity hold significant therapeutic potential. Lifestyle interventions, such as regular physical activity, dietary modifications, and glycemic control, may serve as accessible and cost-effective measures to mitigate the risk of AD in diabetic patients. In addition, pharmacological approaches, including repurposed antidiabetic drugs such as metformin, intranasal insulin, thiazolidinediones, and incretin-based therapies, have demonstrated promising results in both preclinical and clinical settings, improving cognitive functions and reducing pathological markers of AD.

Future perspectives should focus on advancing multi-targeted therapies that simultaneously address oxidative damage, mitochondrial dysfunction, and insulin resistance. Innovative research into personalized medicine approaches—considering genetic, metabolic, and lifestyle factors—could offer tailored treatments for individuals at risk of both conditions. The development of novel biomarkers to detect early-stage mitochondrial and metabolic dysfunction may also aid in timely diagnosis and intervention. Furthermore, long-term clinical

trials are needed to assess the efficacy and safety of combination therapies targeting these shared mechanisms.

In conclusion, understanding the complex interplay between AD and T2DM not only sheds light on the pathogenesis of these diseases but also opens new avenues for prevention and treatment. By bridging the gap between metabolic and neurodegenerative research, it may be possible to develop comprehensive strategies that slow disease progression, preserve cognitive function, and ultimately improve patient outcomes.

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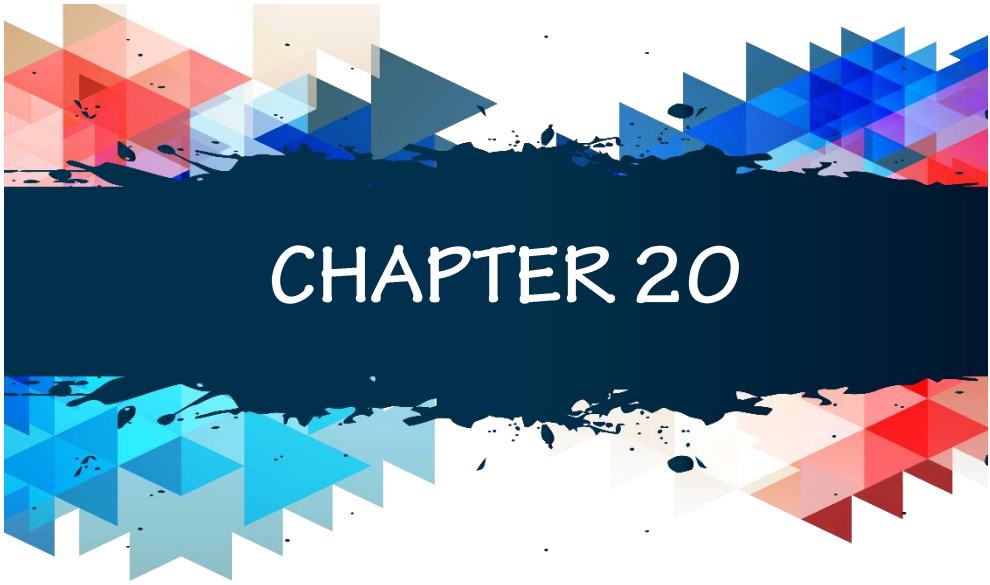

The Role of Telomere Attrition in Neurodegenerative Disorders

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1. INTRODUCTION

Telomeres are repeatedly repeated nucleotide sequences at the ends of linear chromosomes. Because telomere attrition is increased in some conditions, it can be used as a biomarker to help determine the diagnosis and/or prognosis of these conditions (Vaiserman and Krasnienkov, 2020). A class of neurological conditions known as neurodegenerative diseases (NDs) are defined by the progressive loss of neurons or myelin sheaths, which results in cognitive and movement disability (Lamptey et al. 2022). Huntington's disease (HD), Parkinson's disease (PD), Alzheimer's disease (AD), and amyotrophic lateral sclerosis (ALS) are the most prevalent types of these diseases. It is not yet clear how telomere attrition is involved in the pathophysiology of neurodegenerative diseases. In this review, we will review what is now known about the biology of telomeres in AD and PD, two common neurodegenerative diseases.

2. NEURODEGENERATIVE DISEASES

A class of diseases known as neurodegenerative diseases is defined by the progressive loss of neuronal structure and function in either the peripheral or central nervous systems (brain and spinal cord). These illnesses, which are frequently chronic and irreversible, cause mobility, cognitive function, and other neurological processes to gradually deteriorate (Tanaka et al. 2020).

2.1. Alzheimer's disease (AD)

AD is a neurodegenerative disease characterized by progressive memory loss, decreased reasoning ability, language problems, behavioral and personality disorders, and a persistent decline in cognitive functions (Soria Lopez et al. 2019). As the disease advances, individuals with AD may experience difficulty with activities of daily living and require full-time care. There are approximately 50 million people with dementia worldwide, and approximately two-thirds of them have AD (Gustavsson et al. 2023).

Etiology and Risk Factors: Although the precise etiology of AD is unknown, genetic and environmental risk factors are believed to be involved. The biggest risk factor is age. While the probability of having AD is 3% at the age of 65, this rate increases to over 30% at the age of 85 (Alzheimer’s Association, 2019). Other risk factors for AD include family history of the disease, certain genetic mutations (e.g., in the genes encoding amyloid precursor protein and presenilin proteins), cardiovascular risk factors, traumatic brain injury, and certain lifestyle factors (e.g., physical inactivity, poor diet, smoking) (Sehar et al. 2022).

Pathology: AD is characterized by synapse loss and the subsequent atrophy of neurons throughout the cerebral cortex. The buildup of aberrant protein aggregates in the brain is one of the pathophysiological hallmarks of AD:

Beta-amyloid plaques: Beta-amyloid plaques are abnormal clumps or aggregates of beta-amyloid protein fragments that accumulate between neurons in the brain. Beta-amyloid is a naturally occurring protein that is typically broken down and eliminated in healthy brains, but in AD and certain other neurodegenerative disorders, it accumulates and forms plaques (Gustavsson et al. 2023). One of the defining characteristics of AD is the presence of these plaques, which are thought to play a role in the progressive loss of cognitive function that affected people experience. They can interfere with neuronal communication, disrupt cell function, and trigger inflammation, ultimately leading to cell death and the characteristic symptoms of AD, including memory loss, cognitive decline, and changes in behavior.

Tau protein tangles: Tau protein tangles, also known as neurofibrillary tangles (NFTs), are another hallmark feature of AD and several other neurodegenerative disorders, collectively known as tauopathies (Rawat et al. 2022). Tau is a protein normally found in the brain that helps stabilize microtubules, which are essential for the structure and function of neurons. In AD and related tauopathies, tau proteins become abnormally phosphorylated, causing them to detach from microtubules and aggregate into tangles inside neurons. These tangles disrupt the normal functioning of neurons, impairing their ability to transport essential molecules and maintain their structural integrity. As a result, neurons may become dysfunctional and eventually die (Mietelska-Porowska et al. 2014).

Diagnosis: Diagnosis of AD typically involves a comprehensive assessment of cognitive function, medical history, physical examination, and various diagnostic tests, including neuroimaging (e.g., MRI, CT scans) and cognitive assessments (Arvanitakis et al. 2019). A definitive diagnosis often requires a postmortem examination of brain tissue to identify characteristic pathological changes.

2.2. Parkinson's disease (PD)

PD is a progressive neurodegenerative disorder that primarily affects movement (Ramesh and Arachchige, 2023). It is distinguished by the death of neurons in the brain that produce dopamine, especially in an area known as the substantia nigra. Dopamine is a neurotransmitter important in movement

coordination, and its deficiency causes the motor symptoms of PD (Ramesh and Arachchige, 2023).

Etiology: Although the precise origin of PD is unknown, a mix of lifestyle, environmental, and genetic variables are thought to be involved. Although most cases of PD are sporadic, meaning they occur without a clear familial inheritance pattern, genetic factors can play a role in some cases (Inamdar et al. 2007). Mutations in certain genes have been linked to familial forms of PD, which account for a small percentage of cases. Variations in some genes can affect various cellular processes, including protein aggregation, mitochondrial function, and protein degradation pathways, all of which are implicated in PD pathology.

There is evidence linking exposure to specific environmental contaminants and poisons to a higher incidence of PD (De Miranda et al. 2022). These include pesticides and herbicides, such as rotenone and paraquat, as well as industrial chemicals like solvents and heavy metals (e.g., manganese). Chronic exposure to these substances may contribute to the development of PD by damaging neurons and disrupting cellular processes involved in maintaining brain health.

PD is more common in older adults, with the risk increasing with age. While PD can occur at any age, it most commonly develops after the age of 60 (Reeve et al. 2017). Aging is associated with a decline in cellular function and an increased vulnerability to neurodegenerative processes, which may contribute to the development of PD.

Diagnosis: The diagnosis of PD is primarily based on clinical symptoms and neurological examination.

Treatment: Although there isn't a cure for PD, treatment attempts to reduce symptoms, enhance quality of life, and halt the disease's development. Physical therapy, occupational therapy, speech therapy, deep brain stimulation (DBS) surgery, and drugs that raise dopamine levels in the brain (such as levodopa and dopamine agonists) are among the possible treatment options (Church 2021).

3. TELOMERES

Telomeres are specialized structures located at the ends of chromosomes, which are the thread-like structures that contain DNA and associated proteins in the nucleus of a cell. They consist of repetitive nucleotide sequences and associated proteins (O'Sullivan and Karlseder, 2010). One of their primary functions is to protect the integrity of the chromosome by preventing the ends from fraying or sticking to each other, which could lead to genetic instability or the loss of genetic information. During each cell division, the DNA replication machinery

cannot fully replicate the ends of linear chromosomes. This phenomenon, known as the end-replication problem, results in the gradual shortening of telomeres with each cell division (O'Sullivan and Karlseder, 2010). Cells may eventually experience apoptosis (programmed cell death) or replicative senescence when their telomeres get critically short, which would essentially limit their ability to proliferate. This procedure serves as a safeguard against the growth of aging or damaged cells, which would otherwise lead to diseases or malfunctions (Childs et al. 2014).

3.1 Telomere attrition

Telomere attrition refers to the gradual shortening of telomeres over time. Telomeres naturally shorten with each cell division due to the end-replication problem, where the DNA polymerase enzyme cannot fully replicate the ends of linear chromosomes (Bonnell et al. 2021). This process occurs in most somatic cells throughout an individual's lifespan. As telomeres shorten, cells may eventually reach a critical threshold where they undergo cellular senescence or apoptosis. Several factors contribute to telomere attrition.

Oxidative Stress: Reactive oxygen species (ROS) generated during cellular metabolism can damage telomeric DNA and accelerate telomere shortening (Passos et al. 2007).

Chronic Inflammation: Inflammatory processes can trigger the production of cytokines and other molecules that contribute to telomere shortening (Lustig et al. 2017).

Poor Lifestyle Choices: Rapid telomere shortening has been associated with unhealthy lifestyle choices like smoking, binge drinking, eating poorly, and being sedentary (Latifovic et al. 2016).

Psychological Stress: Chronic stress can activate the hypothalamic-pituitary-adrenal (HPA) axis and increase levels of cortisol, which may contribute to telomere shortening (Lin and Epel, 2022).

Genetic Factors: Certain genetic variations can influence telomere length and the rate of telomere shortening.

3.2 Telomere attrition in diseases

Telomeres play a crucial role in cellular aging and are associated with various age-related illnesses, including cancer, heart disease, and neurological conditions. Accelerated aging and a higher risk of age-related disorders have been linked to shorter telomeres (Gruber et al. 2021). Conversely, maintaining telomere length is thought to promote longevity and overall health. Several factors can

influence telomere length and dynamics, including genetics, environmental factors, lifestyle choices, and stress. For example, longer telomeres and less cellular aging have been linked to healthy lifestyle choices like consistent exercise, a balanced diet, enough sleep, and stress management (Galiè et al. 2020). On the other hand, telomere shortening may be accelerated by variables such as smoking, obesity, poor diet, lack of exercise, and chronic stress, which can also increase the risk of disease and cellular aging.

4. TELOMERES AND AD

Research on the relationship between telomeres and AD has garnered significant attention in recent years, as telomere biology is increasingly recognized as a potential contributor to AD pathogenesis and progression (Yu et al. 2023). Several lines of evidence suggest potential associations between telomeres and AD:

1. Cellular senescence: Telomere shortening can lead to cellular senescence, a state of irreversible growth arrest associated with aging. Senescent cells accumulate in the aging brain and are implicated in neurodegenerative diseases, including AD (Martínez-Cué and Rueda N, 2020). Senescent cells secrete pro-inflammatory molecules and contribute to neuroinflammation, oxidative stress, and neuronal dysfunction, all of which are hallmarks of AD pathology.

2. Oxidative stress: Telomeres are susceptible to oxidative damage, and oxidative stress can accelerate telomere shortening. Oxidative stress is implicated in AD pathology, contributing to the accumulation of beta-amyloid plaques and tau tangles, synaptic dysfunction, and neuronal loss (Tönnies and Trushina, 2017). Telomerase has antioxidant properties and may help protect cells from oxidative damage.

3. Neuroinflammation: Telomere dysfunction can trigger an inflammatory response, characterized by the activation of microglia and the release of pro-inflammatory cytokines (Hsiao et al. 2021). Chronic neuroinflammation is a prominent feature of AD and contributes to disease progression. Telomerase has been shown to modulate immune responses and may have anti-inflammatory effects in the brain.

4. Neurogenesis: Telomerase activity is critical for maintaining neural stem cell function and promoting neurogenesis, the process by which

new neurons are generated in the adult brain (Liu et al. 2018). Impaired neurogenesis is observed in AD and may contribute to cognitive decline and neuronal loss. Restoring telomerase activity or preserving telomere length could potentially enhance neurogenesis and mitigate AD-related pathology.

While these findings suggest a potential link between telomeres, telomerase, and AD, more research is needed to fully understand their role in disease pathogenesis and to explore the therapeutic potential of targeting telomere/telomerase pathways in AD treatment and prevention. Clinical studies investigating telomere dynamics and telomerase activity in individuals with AD, as well as preclinical research using animal models, are ongoing to further elucidate these mechanisms and identify novel therapeutic targets.

5. TELOMERES AND PD

Research on the relationship between telomeres and PD is still evolving, but there is growing interest in understanding how telomere biology may intersect with the pathogenesis and progression of PD. While the exact mechanisms linking telomeres to PD remain to be fully elucidated, several lines of evidence suggest potential associations:

1. Oxidative stress and mitochondrial dysfunction: PD is characterized by the progressive degeneration of dopaminergic neurons, often attributed to oxidative stress and mitochondrial dysfunction (Subramaniam and Chesselet, 2013). Telomeres are vulnerable to oxidative damage, and oxidative stress can accelerate telomere shortening. Thus, the oxidative stress observed in PD could potentially impact telomere integrity and contribute to disease progression.

2. Inflammation and immune dysregulation: Chronic inflammation and dysregulated immune responses are implicated in PD pathology (Tansey et al. 2022). Microglia can become activated, and pro-inflammatory cytokines can be released when telomere disruption sets off an inflammatory response. Moreover, telomerase has immunomodulatory effects and may influence the function of immune cells in the brain. Dysregulation of immune responses mediated by telomeres and telomerase could potentially contribute to neuroinflammation and neuronal damage in PD.

3. Cellular senescence: Telomere shortening can lead to cellular senescence, a state of irreversible growth arrest associated with aging. Senescent cells accumulate in the aging brain and secrete pro-inflammatory molecules, contributing to neuroinflammation and neuronal dysfunction. Senescence-

associated markers have been observed in the brains of individuals with PD, suggesting a potential role for cellular senescence in disease pathogenesis.

4. Genetic associations: Some studies have identified genetic variants associated with telomere length that may also influence susceptibility to PD. For example, certain single nucleotide polymorphisms (SNPs) in genes involved in telomere maintenance pathways have been linked to both telomere length and PD risk.

While these findings suggest potential links between telomeres and PD, further research is needed to fully elucidate the underlying mechanisms and determine the clinical relevance of telomere biology in PD. Longitudinal studies investigating telomere dynamics in individuals with PD, as well as preclinical research using animal models, are ongoing to better understand the role of telomeres in PD pathogenesis and identify potential therapeutic targets.

6. CONCLUSIONS

Overall, telomere shortening appears to be a common feature of neurodegenerative diseases and may contribute to disease pathogenesis and progression by exacerbating neuronal dysfunction, oxidative stress, inflammation, and DNA damage. Further research is needed to elucidate the specific mechanisms linking telomere shortening to each neurodegenerative disease and to explore the potential therapeutic implications of targeting telomere biology in these conditions.

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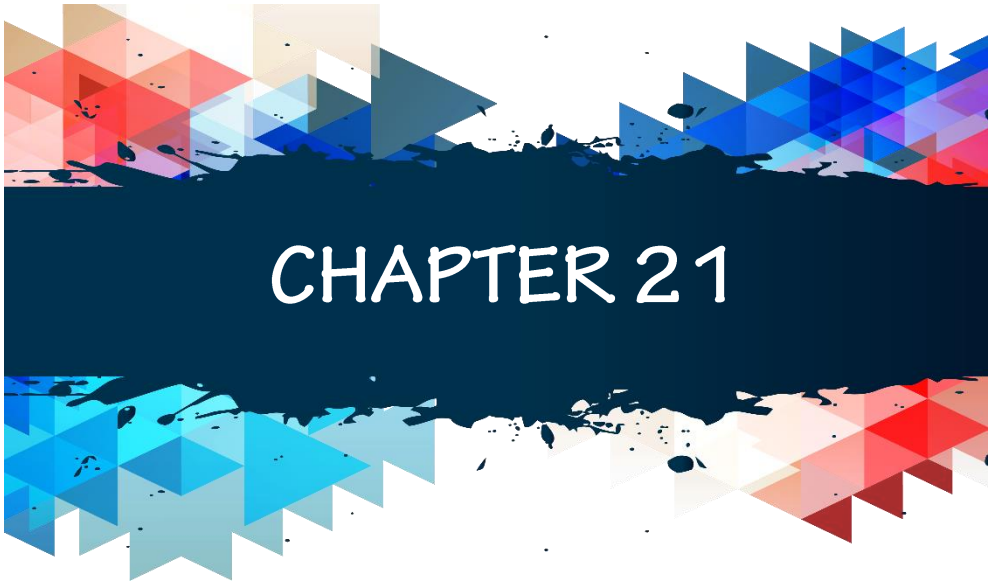

Birth Preparation Training and Its Importance

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Introduction

The historical development of childbirth preparation trainings started in the 1930s and it is seen that it has been developed in terms of method and philosophy over the years (1). In the 1920s, Dick Read was the person who talked about natural birth, explaining birth as "Birth is a physiological event and is not painful in this respect." According to Dick Read, he argued that it was due to fear due to the unknown (2). One of the important names related to prenatal education is Lamaze. In the 1950s, Lamaze observed the behaviors of women in Russia during childbirth and established a connection with Pavlov's conditioning response in these movements. He developed the "painless birth" method in France. The method he found with this method includes relaxing exercises such as deep breathing, efflorescence, massage and focusing on another object to reduce the feeling of discomfort and pain during birth (3,4). American Doctor Robert Bradley conducted studies on relaxation techniques for labor in the 1950-60s (5). Sheila Kitzinger gave trainings to women in the 1960s about pregnancy and methods of explaining their emotional-physical thoughts about this period. The Kitzinger method is based on the philosophy of control and focus to respond to pain and adapt to contractions (2). The method developed by Robert A. Bradley to prepare the pregnant woman and her partner for normal and natural birth without unnecessary medical intervention or medication is the natural birth model with partner support. The role of the partner in this engine is coaching. In the Bradley method, the body-mind connection that increases pain acceptance and relaxation is emphasized. Hypnobirthing philosophy was initiated by Marie Mongan in 1990. Although she did not teach methods to cope with birth pain, she emphasized deep relaxation, hypnosis, visualization and the idea of no birth (6).

In Turkey, childbirth preparation classes started in private universities and organizations in the 1980s (1). With the circular in 2014, the Ministry of Health published a pregnant information class circular and aimed to standardize information and counseling services for pregnant women and to activate service delivery (7). With the circular, 128,589 pregnant women were trained in the last 3 months in 2014, 163,912 pregnant women in 2015 and 216,982 pregnant women in 2016 (1,8).

Content of Prenatal Education

In 2018, with the circular published by the Ministry of Health, the educational content of programs for prenatal education was standardized (9). In the prenatal education program in our country; organs of the reproductive system, the formation of pregnancy, physiological and psychological changes that occur

during pregnancy, common disorders and solutions during pregnancy, exercise during pregnancy, nutrition during pregnancy, smoking, alcohol and substance use during pregnancy, pregnancy follow-up, routine examinations, danger signs in pregnancy, risky pregnancies, non-pharmacological methods in coping with pain, postpartum process and its characteristics, physiological and changes in the mother at the end of labor, newborn care, postpartum family planning methods and father's education in preparation for birth are explained (9,10)

Methods Used in Childbirth Preparation Training

Various training methods were used in the prenatal period in line with the needs of families, institutional policies and resources (11).

- **One-on-One Education :** It is one of the most frequently used methods in the education of the expectant mother. In order to increase the effectiveness of the one-to-one education method, it is necessary to have educators who support the person in changing behavior and who can use priorities in client education. This method is used when discussing emotional and special needs (12-14).

- **Group Education Method:** In this method, the topics of preterm birth symptoms, family planning methods, management of diabetes process during pregnancy, family concept, preparation for birth and breastfeeding process are discussed in detail with pregnant women and their families and behavioral change is provided by increasing the level of knowledge of the group. Midwives and nurses are preferred as educators in this education to be given to pregnant women (12-14).

- **Education Method with Visual Materials:** Recently, there has been an increase in the use of visual materials in many trainings. The use of visual materials in the learning process and the widespread use of tools such as television, video and computer have been shown as the reasons for this increase. In this method, the presence of a knowledgeable and experienced midwife or nurse was emphasized as a prerequisite for answering the questions of the client and the audience and for the success of the education (12-14).

- **Education Method through Computer and Internet:** It is one of the health education methods. Most of the computer-aided education programs are used by health educators. Many people have information on the computer with special programs prepared on health and disease issues. Most of the computer programs are

prepared by organizing the materials for the needs of individuals and making them educational. It is emphasized that the reason why computer-based education has become widespread is due to the accessibility of information on websites with search engines. Pregnant women access the latest researches from websites and find answers to their questions (12).Apart from these advantages of computer and internet-assisted education in health education, there are also disadvantages due to the fact that the information on the internet is not legally controlled (11).

The Effects of Childbirth Preparation Training

Pregnancy is a period in which physiologic, psychological and social changes occur in women's life periods (15). With these changes, women have difficulty in adapting to pregnancy and experience fear due to the unknowns about birth. As labor approaches, the fear of birth increases (16). Prenatal education is an effective health education that facilitates adaptation to pregnancy, reduces the fear of childbirth in pregnant women and develops positive thoughts related to childbirth. Prenatal education reduces anxiety and stress that develops with fear during pregnancy, strengthens mother-fetus bonding during pregnancy, and provides guidance to help meet the need for care and support in education (17,18). When we look at the main objectives of childbirth preparation trainings; preparing the expectant mother and/or father cognitively, sensory and socially, providing them with information, basic knowledge and skills for the care of the pregnant woman and the baby, gaining self-confidence in care, supporting this development process of becoming a family and adapting to new roles by meeting these processes with a love-oriented approach with family integrity, eliminating the fear of childbirth that develops due to the influence of modern culture, and enabling the woman to cope with the birth process regardless of its form. Many studies have demonstrated the positive effects of pregnancy education/birth preparation education. It is known that in some countries, it is integrated into the health system and it is made compulsory to receive pregnant education. In Turkey, pregnant education is provided both in public hospitals within the scope of the goal of mother-friendly hospitals and in the private sector (19,20).

Conclusion

Professional health personnel (midwives and nurses) should prepare women with trainings including pregnancy, birth and postpartum processes. Midwives/nurses who take part in childbirth preparation trainings contribute to maintaining the health of mother and baby and increasing social support. It has been reported that women who are prepared for pregnancy, birth and postpartum processes with awareness increase their pregnancy satisfaction levels and self-confidence, manage labor and actively participate in birth. Comprehensive care, education and counseling provided during pregnancy will facilitate women's adaptation to the physiological and psychological changes that occur during pregnancy.

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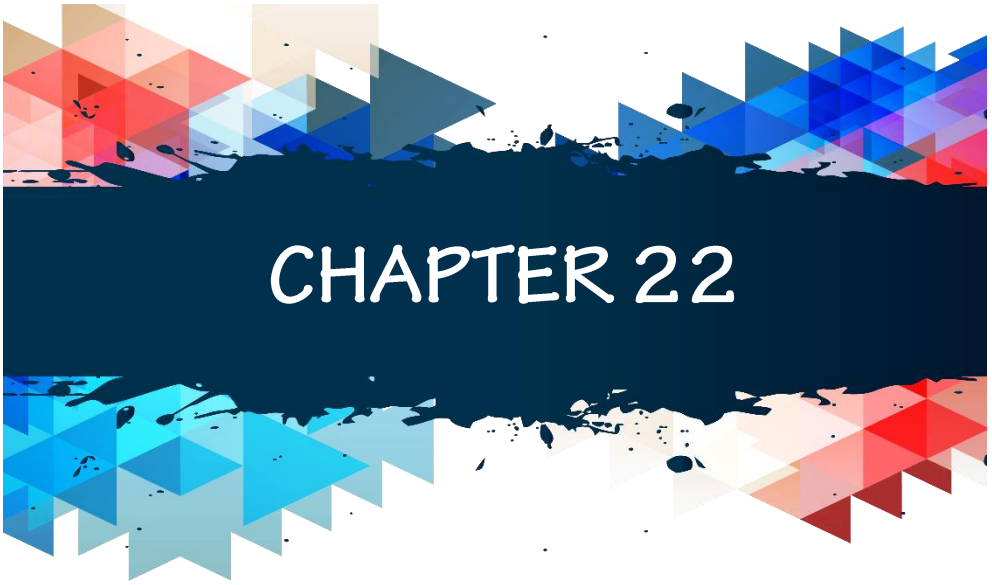

Aristotle on the Anatomical Sciences

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The long history of anatomical sciences can be divided into three distinct phases. The first phase, spanning approximately 800 years, is marked by Greek scientific achievements, which laid the foundation for anatomical knowledge. The Romans did not carry this tradition forward in a progressive manner. The second phase encompasses the medieval period, a time often seen as one of intellectual stagnation, it lasted more than 1000 years. True progress in learning was largely halted during those times, though it may not have been as bleak as traditionally portrayed. Finally, the third phase is the resurgence of original research, beginning in the sixteenth century and continuing through to the present day, marking the era of modern science with an uninterrupted legacy of discovery. Aristotle and Galen are two eminent figures and unquestionably the most important ones of the golden Greek era when it comes to the anatomical sciences. They two not only pioneered the line of scientific development during their time but also dominated the following centuries almost until the 17th century (Conti ve Paternostro, 2019; Colle 1944, Zagris, 2022) .

Aristotle's approach, like Galen's, to nature was not driven by a pure love or fascination with it, nor did he observe it for its own intrinsic value. Instead, he engaged with it primarily as an intellectual pursuit. Consequently, his catalog of animal species is relatively limited, yet his keen interest lies in the structure and behavior of living organisms, which he explores with deep human relevance. His ability to shed light on these aspects is, in fact, quite remarkable. It is noteworthy that while Greek art captures the human form's superficial anatomy with extraordinary accuracy, the environment in which humans exist is given only a cursory or symbolic treatment (Aristotle, 2021).

One could encounter the central philosophical motives in his works as a passionate effort to understand the world and a strong desire to comprehend human beings. He posed fundamental questions dealing the nature of knowledge and its eventual foundations, inquiring into the very layers of ideas and the fundamental elements of the universe. Aristotle also explored the relationship between language, thought, and the items of them, delved into the connection between mind and body, and examined the individual's role in the population. Furthermore, he questioned the ultimate purpose of human life and to what ends it should be directed. These questions remain vibrant and relevant today, and Aristotle's answers continue to hold significant value and influence (Zagris, 2022, Peck 1983).

Though his contributions spanned various fields such as biology including the anatomical sciences, physics, chemistry, political theory, metaphysics, logic, history, ethics, rhetoric, psychology, aesthetics, and poetics, Aristotle pushed

ahaed with his overarching aim: to unify knowledge and understand the world as a coherent whole (Humphreys 2024, Marroquín-Arroyave & Vespa 2023).

Aristotle authored several key works on anatomy, including History of Animals, Parts of Animals, and Generation of Animals, that would later lead him to be called the father of anatomical sciences. Even he also mentions two other biological texts in his writings, these have not survived to the present day. Forming part of a theoretical analysis of nature he investigated the fundamental questions of natural sciences to evaluate the detailed descriptions of hundreds of mammals in these texts (Blits 1999, Peck 1983).

Life

The most considerable reference to the life of Aristotle, is that of Diogenes Laertius in his breakthrough work Lives and Opinions of Eminent Philosophers, estimated to have been written around the second quarter of the 3rd century AD. The book is a good exception to his time, mostly citing the sources to particular details about Aristotle. Regarding his biographical information on Aristotle, his work is in several manners the major source of information. Aristotle was born in Stagira, a small coastal town in Chalcidice, a Greek colony on north of the Aegean sea, in 384 BC after 15 years of Socrates' death. His father Nicomachus served as the court physician to King Amyntas II of Macedonia and came from a long line of medical practitioners. Aristotle likely spent part of his childhood in the Macedonian palace in Pella, thereby forming lasting connections with the Macedonian monarchy that would influence his entire life. His mother Phaestis passed away at a young age of him and Aristotle migrated to Athens, nearly 500 kilometers from Stagira, at around 17 to pursue his studies. There, he became a disciple of Plato, remaining at the Academy for approximately two decades, before he left the city around the time Plato died. He may be forced to flee due to anti-Macedonian sentiment in Athens. The relationship between the Macedonians and the Athenians was marked by a mix of occasional peace and frequent conflict, yet the underlying tension was constant. Afterward, Aristotle lived in Assos, a city in Today's Canakkale of Turkiye, and later in Macedonia, where he tutored Alexander the Great, the son of King Philip. Aristotle's extensive references to the region around Mytilene suggest that he conducted many of his marine biology observations during this period. Eventually, at the age of 49, returned to Athens, where he founded his own school at the Lyceum, a public space outside the city's walls. It is said that Aristotle and his students were called "Peripatetic" derived from the Greek peripatos, meaning "walking" because they would engage in discussions while walking along a covered walkway. Aristotle's second stay in Athens, from 338 to 322 B.C., is regarded as the most productive period of his

life, during which he composed or completed many of his most important philosophical works. After the death of Alexander in 323 B.C., Aristotle faced charges of impiety in Athens, likely due to his perceived threat to the city's political and social stability. As a resident alien rather than a citizen, he chose to leave Athens to avoid standing trial. Aristotle spent his final year in Chalcis, at his mother's estate in the homeland of his mother, where he died of a stomach illness or a chronic intestinal condition (Blits 1999, Dunn 2016, Connell 2021, Düring 1957, Humphreys 2024) .

During his lifetime, most of Aristotle's writings were not published. The works that were made public earned him some respect, but not the deep admiration he would later receive. It took several centuries before Aristotle came to be recognized as more than just a skilled disciple of Plato (Humphreys 2024).

Before Aristotle in Ancient Greece

Alcmaeon of Croton, who was born at least a hundred years before Aristotle, is thought to have been the first person to perform dissection, and perhaps even vivisection, of the human body for research purposes. He truely defined the optic nerve and sensory nerves, and claimed that brain is the center for intelligence. He made observations on the functional organization of the sensory organs. He also discerned the arteries from the veins. Alcmaeon's treatises have not survived in their original form. However, his findings and ideas are known primarily through citations and references by later authors, such as Aristotle, Hippocrates, and other ancient philosophers and medical writers. Alcmaeon made significant contributions to early anatomy and physiology, particularly regarding the brain and senses, but much of his work is preserved only in fragments or through the writings of later thinkers who built on his ideas (Debernardi 2010, Celesia 2012).

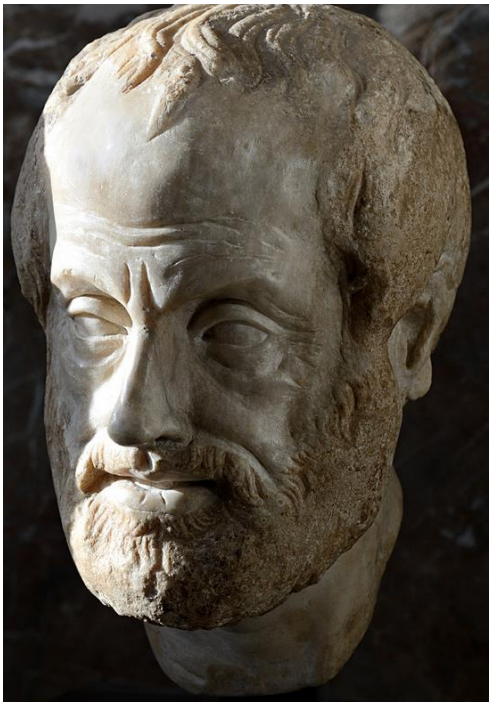
The oldest surviving text directly related to anatomical sciences is the Hippocratic Corpus, a collection of anonymous writings on medicine and surgery, representative of medical literature of the Classical Greek era, primarily composed in the last decades of the 4th century and first decades of 3rd century BC. The Hippocratic writings contain a wealth of anatomical knowledge. Notable works like On Joints and On Fractures, which address the treatment of dislocations and fractures, are particularly significant for presenting what emerges to be the first systematic comparison between the human skeleton and those of other vertebrates. In general, the Hippocratic Corpus demonstrates a comprehensive understanding of the skeleton, revealing the relationships between bones joints, ligaments, and superficial muscles (Blits 1999, King 2017).

Hippocrates of Cos (460–375 B.C.), offered some of the earliest detailed descriptions of the heart and blood vessels. These are found in the Hippocratic Corpus, a collection of numerous medical writings attributed to Hippocrates, his contemporaries, and later physicians. Among the texts included is “the heart," one of the first known works in the history of Western medicine to describe the major blood vessels, the heart valves, and the pericardium. In Hippocratic Corpus it is assumes that the heart and caval veins are responsible for respiration, and that the inspired pneuma travels through the body to reach the heart. Similarly, Aristotle believes that the heart regulates breathing, with pneuma moving upward to the heart, which he identifies as the source of heat (Colle 1944, Dunn 2006, Blits 1999).

Anatomical Works Just after Aristotle in Ancient Greece

Herophilus and Erasistratus, both physicians, in the 3rd and 4th centuries B.C., practised systematic dissections even vivisections on human beings, in Alexandria. Herophilus (335-280 B.C.), in particular, studied the brain, the eyes and nerves, the ovaries, uterine tubes, the liver and pancreas. He also examined the human arterial pulse as a means of diagnosing various pathologies and introduced the use of experimental method to medicine. Also Erasistratus (305-250 B.C.) studied the human pulse, and the blood vessels and the functional anatomy of the human heart as well as its structure. Herophilos differentiated the cerebrum from the cerebellum, evaluated the internal surface of the cranium and its sutures. He described the brainstem and spinal cord as a single anatomical structure, and also he defined the concavity on the internal surface of the occipital bone, housing the posterior confluent of the dura mater sinuses, referred as torcular Herophili. Herophilus described arteries to have thicker walls than the veins through dissections of him on human cadavers. He certainly believed the pulse was associated with the heart beat (Reverón 2015, Bay ve Bay 2010, Conti 2019, Sallam 2010) .

It was widely believed that the body housed a soul, which upon death became trapped within it. Human dissection was allowed for only a brief period of three or four decades during the era of Herophilus and Erasistratus. After that, it was banned and remained forbidden almost for two milleniums, with dissection not being revived until the 16th century (Reveron 2015).



Marble bust of Aristotle in Paris, Louvre Museum, photo: Ilya Shurygin

Anatomical Sights of Aristotle Through his Philosophy

Aristotle believed that the human heart had three ventricles, right, left, and middle, and accepted the right atrium as a venous dilatation, but a chamber of the heart, after his examinations. Aristotle assessed the right cavity connecting with the great vein and the lungs as the largest, the left cavity connecting with the lungs as the smallest, and the middle cavity connecting with the aorta and the lungs as intermediate. One could figure out that the middle cavity should be the left ventricule since it conncets with the aorta; the left cavity should be the left atrium since the left auricle lays laterally the left ventricle, and it connects with the lungs through the pulmonary veins. Aristotle identified the right cavity as the right ventricle, describing it as the largest chamber, located on the right side, and connecting both to the lungs through the pulmonary artery and to the caval veins. His statement that the caval veins adhere to the right ventricle suggests that he did not view the right atrium as a distinct chamber, but rather as a dilatation of the venous system. He saw the heart, as the natural seat of the heat, had the function of forming the blood. The heart has the fuction of being the centre of perceptual activity. Aristotle stated that the channels emanating from the eyes and

ears reach the vessels around the brain and from there the stimulus returns to the heart (Oleksowicz 2018, Praagh ve Praagh 1983).

Aristotle linked the senses to the classical elements: water to sight, air to hearing and smell, fire to taste and touch, which he regarded as analogous, to earth. The rationale behind this association is explained in various works by Aristotle. This doctrine of sensory associations with the elements was then applied to the spatial topography of the body, particularly in understanding the relationships between the sense organs, the brain, and the heart (Connell 2021).

In his treatise on the breath, Aristotle asserts that respiration serves the purpose of drawing air into the body to generate pneuma, the vital breath or spirit, which is the force that enables an animal to move and function. His mechanical explanation of respiration suggests that the air heated within the lungs is expelled by the cooler air from outside the body (internet encyclopedia of philosophy. https://iep.utm.edu/aristotle/#SSH3aii). He mentions his predecessors to misunderstand the functional anatomy of the lungs due to their lack of dissection experiences. Aristotle does not try to understand the reason why humans have lungs. He first classifies lungs within the broader category of features. Then he inquires why all members of that category possess them. He concludes that all creatures with lungs have them because they are land-dwellers that need to cool their blood with air, a rationale that applies to humans as part of this broader group. Aristotle then examines specific variations in lung structure that distinguish different forms within this category. The mammals have large, blood-filled lungs, while reptiles and birds have smaller, drier lungs that are spongy and foam-like. Aristotle asserted the heart to be located above the lungs. This misconception may have arisen due to the collapse of the lungs during dissection. In some animal species, while lying on their back, the lungs could collapse posteriorly and laterally after the thoracic cavity is opened, leading to a mistaken impression of the heart's position relative to the lungs (Blits 1999, Oleksowicz 2018, Marroquín-Arroyave & Vespa 2023, Connell 2021).

Aristotle evaluated through his dissections that the main sensors are connected to the heart by means of the blood vessels. He claims that the channels of the eyes communicate with the veins around the brain. Furthermore, he asserted that the touch and the taste are connected to the heart (Oleksowicz 2018).

His On the Generation of Animals, composed of five books, is the first great compendium of developmental anatomy ever written. It includes the structural analysis of animals in a scientific and systematic fashion, promising the future discipline of comparative anatomy. The study of embryonic development and

fetal nourishment, the characteristics of sex, the variations in the forms of the gonadal organs, as well as the diverse methods of copulation across different species, were all subjects of exploration. Aristotle claims that semen is a genuine excretion and explains why male fertilization is essential. He proposes that semen provides the "form" to the embryo, while the female contributes the matter that is shaped into the developing organism. He brilliantly categorized biological entities into a hierarchical system based on varying degrees of perfection (Zagris 2022, Connell 2021).

Aristotle was a keen reader of Hippocrates, and was highly aware of both earlier and contemporary medical ideas. He openly recognized the significant contributions made by physicians to the study of nature. According to Aristotle, living organisms are composed only of true individuals of the same species, and therefore, their existence can be explained by considering both its metaphysical and biological precedence. He clearly emphasizes this precedence while critiquing his predecessors. The first book of Historia Animalium provides the most crucial information regarding the organs and other anatomical structures of humans. The second book, in contrast, focuses on comparative anatomy, examining both intrinsic and extrinsic structures of various animals. Aristotle's extensive knowledge of human anatomy, particularly internal organs, reflects a systematic effort to outline the variations that discern the anatomical features of whole living creatures. Although the first book primarily discusses human anatomy, Aristotle emphasizes that the study of the internal structures of the human body should be based on comparative anatomy, with a particular focus on observing the morphology of mammals, which are anatomically closest to humans. Nevertheless, Aristotle is keenly aware of the distinct body patterns that differentiate quadrupeds from humans, particularly in terms of their spatial orientation (Oleksowicz 2018, Cole 1944, Connell 2021, Aristotle 2021, Caullery 1966)

According to the sources, although it is understood that Aristotle did not dissect human beings, a personality like him, who dissected hundreds of animals of many different kinds and had questions about human beings, must have dissected human beings in a way that would not be recorded. The information in the literature that his pupil Diocles performed dissections on the human body seems to support this argument.

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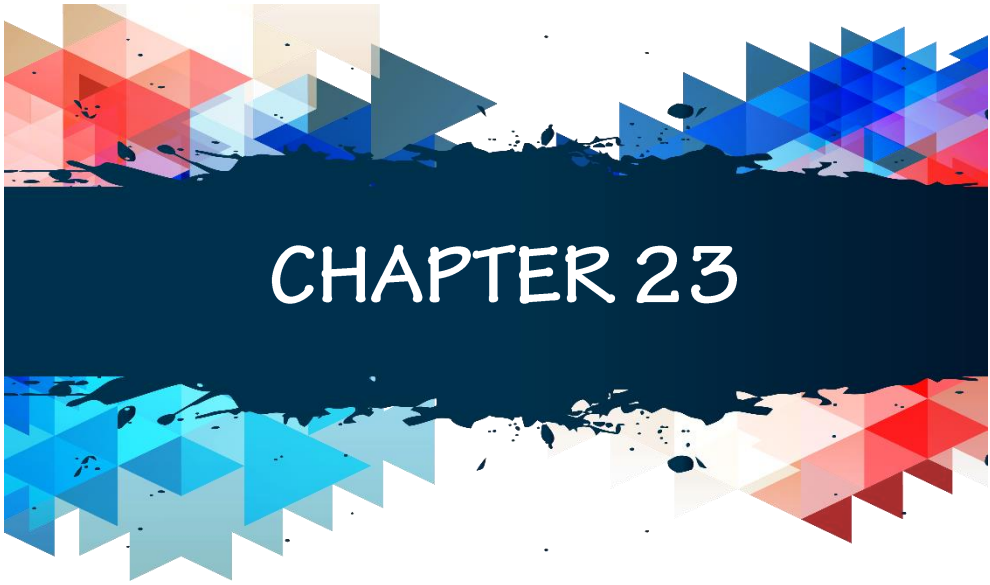

Multiple Sclerosis and Fatigue

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Multiple sclerosis (MS) is a chronic inflammatory autoimmune disease of the central nervous system (CNS) and usually occurs in patients aged 20-40 years. It is characterised by demyelination and subsequent degeneration leading to axonal loss and neuronal damage (Moustafaa et al., 2022). Since MS mostly affects young adults, it causes a decrease in productivity, adversely affects quality of life, and causes patients to withdraw from daily life activities due to the symptoms they experience (Coates et al., 2020).

There are studies indicating that multiple sclerosis is more common in Caucasians and in the temperate zone. It is reported that the prevalence increases in regions far from the equator, and that it is more common in regions such as Northern Europe, North America, Northern Australia than in regions close to the equator (Czerwińska-Mazur et al., 2018). When the epidemiology of MS is analysed worldwide, it is seen that the prevalence is 126 per 100 thousand (median 112) and the incidence is 6.5 per 100 thousand (median 5.2) (Melcon et al., 2014). In Europe, the prevalence has been reported as 17-216 per 100 thousand (median 83) and the incidence as 0.5-12 per 100 thousand (median 4.3) in the last thirty years (Pugliatti et al., 2006). In our country, it is reported that MS is seen in an average of 2000-2500 people, prevalence and incidence rates are lower than in Europe, and may be higher than in Middle Eastern and Asian countries (Evans et al., n.d.; Polat et al., 2017).

In a prevalence study on MS in Turkey, the rate was found to be 101/100,000 in Maltepe, Istanbul and 30/100,000 in Edirne. In a prevalence study conducted in two regions (Karabük-Akçakoca) located on the same latitude but different in terms of air pollution, different figures were found. The prevalence of MS in Turkey was calculated as 96.4 per 100,000 and the female/male ratio was 2.1/1 (Öztürk et al., 2024). The prevalence was found to be 95/100,000 in the region where air pollution is more intense (Karabük), while this rate was found to be 46/100,000 in the cleaner Akçakoca region (Börü et al., 2018). In MS, the disease can also be seen under the age of 15 and over the age of 55. It has a higher incidence rate in individuals aged 20-40 years and in women (Akdemir et al., 2017).

1.1. Etiology

Although the exact cause is not yet known, immunopathogenesis suggests that genetic predispositions and environmental variables interact to cause an immunological dysregulation (Harirchian et al., 2018).

Epstein-Barr virus infection, smoking and vitamin D deficiency are among the environmental parameters closely associated with MS risk (Harirchian et al.,

2018; Hedström et al., 2009). In addition, another data on the role of genetic factors in the etiology of MS is that some histocompatibility antigens (HLA) are more frequently observed in MS patients compared to controls. The strongest known association has been shown in the DR region on chromosome 6. Other HLA haplotypes (HLA- DR2 and to a lesser extent DR3, B7 and A3) that are more frequent in MS patients are believed to be markers of an MS "predisposition gene", possibly an immune response gene. The presence of one of these markers is reported to increase the risk of developing MS by 3 to 5 times. Although these antigens have been shown to be associated with disease frequency, their presence is not essential and their exact role is not clear (Soyder et al, 2004).

1.2. Physiopathology

The heterogeneity of the clinical presentation of multiple sclerosis cannot be explained by a single immune mechanism. The basic pathology results from CNS inflammation and damage to myelin-producing cells (oligodentrocytes). The myelin sheath is a structure consisting of special proteins that surrounds the axon and is necessary for the rapid progression of the conduction. Triggers from an environmental factor activate T lymphocytes and oligodentrocytes are targeted, and the blood-brain barrier becomes permeable (Czerwińska-Mazur et al., 2018). Although it is considered an autoimmune disease predominantly caused by T lymphocytes, there is evidence that B lymphocytes also play an important role (Hemmer and Selter, 2013).

As a result of inflammation in the central nervous system and damage to the myelin sheath, axon and glial cells, nerve conduction is disrupted. While attacks occur in this acute period, nerve damage leading to axon loss in the long term causes permanent disability (Polat Dunya et al., 2020). When the necessary medical intervention is provided after the attacks in the early stage, it is seen that the damage caused by the attacks can be reversed by repair, remyelination and restoration of conduction. The patient may recover from attacks with complete or partial damage. However, the accumulation of attacks over time and the formation of permanent axon damage leads to irreversible disability. However, there is controversy about the pathophysiological process, whether the inflammatory process is the cause or the result of some processes (Cortez et al., 2015).

1.3. Symptoms and Signs

Since disseminated lesions in multiple sclerosis can develop in any region of the CNS from the cerebral cortex to the spinal cord, the symptoms and signs show great variability. The clinical picture may be acute or may start with slowly progressive symptoms. Symptoms are often seen as 65-75% exacerbations and remissions and the duration of symptoms may vary. In multiple sclerosis, there may sometimes be suspicious symptoms such as fatigue, headache, depression, pain in the extremities and may initially suggest a psychoneurosis picture (Dobson & Giovannoni, 2019).

Most patients with multiple sclerosis present to the clinic with recurrent episodes of remission of initially new or recurrent neurological symptoms (Garg and Smith, 2015). The definition of an attack is largely based on the evaluation of clinical findings. After the last revision, according to the 2010 McDonald criteria, it is defined as "one or more neurological deficits consistent with an acute inflammatory demyelinating process described by the patient or detected by the physician and lasting at least 24 hours in the absence of fever and infection" (Yamout and Alroughani, 2018). Symptoms of MS may vary greatly depending on the affected nerve location.

Motor disorders; spasticity is observed in approximately 60-84% of MS patients. Spasticity leads to resistance to passive movements, increased muscle contraction, weakening of manual skills, spasms, difficulty in walking, and coordination disorders

Somatosensory disorders: It constitutes a large part of the initial manifestations of MS. It is seen in 52-70% of patients. There is numbness, burning sensation and pins and needles. There is deterioration in the sense of position and vibration. More rarely, pain, heat and sense of touch may be impaired. Lhermitte's sign may occur in MS. Impaired taste, hearing and vertigo may occur (Christogianni et al., 2018).

Cerebellar dysfunctions are seen in 50% of MS patients. Trunk ataxia is the most common. Intensional tremor, dysmetria, dysdiacokinesia and cerebellar dysarthria are also observed (Berger, 2013).

Visual dysfunctions; One of the first common clinical manifestations of relapsing-remitting MS (RRMS) is unilateral optic neuritis (Ford, 2020). Patients with optic neuritis may experience progressive vision loss, eye pain that increases with movement and changes in colour vision. Optic neuritis is a symptom seen in

25% of MS patients. Diplopia, vision loss, oscillopsia are also common findings in MS patients (Zafeiropoulos et al., 2021).

Bladder and bowel dysfunctions; 80% of patients with multiple sclerosis have bladder dysfunction. Symptoms such as inability to urinate, frequent urination, inability to urinate may be observed. In the neurogenic bladder, which is common in MS, inadequacy in filling or emptying or both are seen. Failure in filling is the most common. Intestinal dysfunction is seen in 60% of MS patients. The most common complaint is constipation. Other bowel dysfunctions include diarrhoea, flatulence, slowing of colonic movements and ileus (Zielińska-Nowak et al., 2020).

Spasticity is an important problem that is frequently encountered in multiple sclerosis patients and leads to walking and transfer difficulties, joint contractures, limitation of movement, poor hygiene and restriction of activities of daily living (ADL). It is characterised by transient or persistent increased muscle tone and sometimes painful cramps. It occurs especially in the lower extremities. PPMS may start with spastic paraparesis (Torres-Pareja et al., 2019).

Cognitive disorders; cognitive problems are very common findings in MS. In studies, the prevalence in MS patients varies between 34-65% (Kalb et al., 2018). Cognitive impairment may start from the early stages of MS and may have negative effects on activities of daily living. The most common cognitive problems are memory-memory loss, impaired information processing, impaired attention and concentration, and impaired executive functioning. Symptoms such as information processing speed problems in 70% of patients, memory difficulties in 40-60%, attention impairment in 20-50%, and difficulty in executing executive functions in 15-20% were found (Oreja-Guevara et al., 2019).

Psychiatric disorders are seen in 72% of patients, 54% of which are depressive disorders. Depression usually develops as a reaction to the restrictive aspects of the disease (Dauwan et al., 2021). While the annual prevalence of depression in MS is 20% , the lifetime prevalence rate approaches 50%. Although there is no strong evidence of a close relationship between lesion location and the development of depression, it is thought that depression is more associated with neuropathologies developing in the left anterior temporal/parietal region (Siegert and Abernethy, 2005). Although more rare than depression, abnormal laughing and crying episodes, euphoria and bipolar disorder can be seen in MS patients (Penner, 2014).

Although *sexual dysfunctions* are seen in 70-73% of men, there are some studies showing that this rate reaches up to 90% in men. In men, erectile

dysfunction and ejaculation problems also trigger mood disorders (Guo et al., 2012; Kajbafvala et al., 2022; Qaderi & Merghati Khoei, 2014). The main sexual problems in women can be counted as decreased libido, orgasm disorder, decreased vaginal lubrication, loss of genital sensation. Constipation and faecal incontinence are bowel problems seen in MS patients (Guo et al., 2012; Kajbafvala et al., 2022; Khemiri et al., 2020).

Fatigue; Fatigue, which can be seen in 83% of patients, greatly affects the quality of life of patients (Kalb et al., 2018). Although the cause of the most common and most ignored fatigue is not fully understood, it is explained by cytokine production, which is related to the central nervous system and autoimmune factors such as demyelination and axon loss. Depression, weakness, severe exercise, ataxia, spasticity, age, stress, infection, infection, sleep problems, and medications aggravate the fatigue picture and negatively affect the individual's social relations, daily life activities, cognitive and physical functions (Dilek et al., 2019).

1.4. MS Classification

Multiple sclerosis presents in four types according to its clinical course. The most common type is RRMS, which is characterised by attacks and remissions; secondary progressive multiple sclerosis (SPMS), which is progressive from the onset of the disease; primary progressive multiple sclerosis (PPMS), which is progressive from the onset of the disease; and benign MS, which is characterised by mild attacks occurring at long intervals. In addition, a single episode of clinical demyelination occurring in a certain region of the brain is defined as clinical isolated syndrome (CSS). CIS does not mean that the person has MS, but it indicates that the patient has a high risk of developing MS. While lesions are detected on brain MRI in 60% of patients initially diagnosed with CIS, in approximately 20% of these patients, the diagnosis of MS becomes definite in the following period (Grup., 2018; Miller et al., 2012).

1.4.1. Clinical Isolated Syndrome (CIS)

A clinically isolated syndrome is the first clinical episode suggestive of MS. It is described in the revised McDonald 2017 criteria as follows;

- Presence of a monophasic clinical episode with symptoms and objective findings reflecting a focal or multifocal inflammatory demyelinating event in the central nervous system

- This process should last at least 24 hours, have an acute or subacute onset, and there should be no fever or infection during the process

- This episode is similar to a typical MS relapse (attack and exacerbation) but occurs in a patient without a diagnosis of MS.

In order to make a diagnosis of CSC, the patient should not have symptoms suggestive of another demyelinating event in the clinical history or radiological imaging. The clinical picture may be one or more of the symptoms mentioned above (Förster et al., 2019). Epidemiologically, the risk of developing MS has been found to be higher in patients diagnosed with WMD. In different studies, the transformation of WMD into clinical definite MS varies between 20-75% depending on the prevalence of MS in the country (Miller et al., 2012). On the other hand, there is also a study showing that 6% of patients with WMD enter a progressive disease phase within approximately 7 years after the first demyelinating attack (Kremenchutzky et al., 2006).

1.4.2. Relapsing-Remitting Multiple Sclerosis (RRMS)

RRMS is the most common type of MS at disease onset, especially in young people. It is characterised by attacks with complete or partial recovery. Disease progression between attacks is low, but the attacks themselves can leave permanent disability, which can sometimes be severe. The diagnosis of RRMS depends on the demonstration of an inflammatory state in different parts of the CNS at two different times. If the above-mentioned disease presenting with an attack fulfils the McDonald 2017 criteria, it can be diagnosed as RRMS with a single attack. RRMS accounts for approximately 85-90% of patients at the time of diagnosis. Past clinical episodes may be temporarily worsened by fever, physical activity, high environmental temperature or metabolic disturbances and may last for hours or a day or more. This worsening, termed "pseudo-attack", is thought to reflect conduction block in demyelinating axons . It can be categorised as active or inactive RRMS according to the Lublin criteria (Group., 2018).

1.4.3. Secondary Progressive Multiple Sclerosis (SPMS)

It has been reported that approximately half of MS cases progressing with attacks and remission turn into secondary progressive form after 10 years. While the patient initially experiences attacks and remissions, the clinical condition worsens and disability progresses as the disease progresses.

1.4.4. Primary Progressive Multiple Sclerosis (PPMS)

It is defined as a poor prognosis from the onset of the disease. PPMS accounts for approximately 10-15% of MS patients and is generally more common in elderly patients. In this form of the disease, spinal involvement is at the forefront

and the disease progresses more progressively although the cortex is preserved. It causes loss of mobility in many patients.

**Active disease* is defined as the development of new lesions with complete clinical recovery or sequelae and/or contrast enhancement on magnetic resonance imaging (MRI) T1 examination and/or hyperintense new lesions on T2 examination.

1.4.5. Benign MS

It is a retrospective diagnosis characterised by infrequent attacks without severe sequelae and low lesion burden on MRI. Patients with "Expanded Disability Status Scale" (EDSS) scores ≤3 15 years after the onset of the disease are considered benign MS (Kuhlmann and Antel, 2023a).

1.5. Diagnosis

It is not possible to diagnose MS with a single diagnostic test or a specific clinical finding. A detailed neurological examination and anamnesis and clinical symptoms specific to MS are sought. Immunoglobulin G (IgG) and oligoclonal bands in cerebrospinal fluid (CSF) examination and demyelinating plaques with specific features of MS plaques formed in the brain and spinal cord are sought by MR imaging. In addition, secondary tests such as optical coherence, evoked potentials, neurocognitive tests and other tests such as urodynamics, angiography, biopsy, electrophysiology and chest radiography are used for differential diagnosis. The main aim is to demonstrate the spread of lesions in the central nervous system and the clinical condition in time and space and to rule out other diseases with clinical findings similar to MS. Poser and McDonald criteria are used in MS, which is a difficult disease to diagnose, and it is reported that the patient should have at least two attacks and MS plaques should be seen in at least two different regions of the brain (Thompson et al., 2018).

1.6.Treatment

Although there is no definite treatment for MS, the treatment approach can be summarised in three ways as preventive treatment, attack treatment and symptomatic treatment. Attack treatment is the intravenous administration of corticosteroids such as methylprednisolone for periods varying according to the state of the attack, such as 3-7 days. The aim of this treatment is that steroids suppress the immune system strongly and rapidly and reduce the severity of symptoms. Steroids accelerate the recovery of MS attacks, but do not change the long-term course of the disease.

Symptomatic treatment is the treatment that does not affect the activity of the disease and is applied to improve the quality of life of the individual and to treat the symptoms of MS. ¸ Another treatment method, preventive therapies, are a group of drugs that have emerged in the last 30 years and have effects on the immune system that works incorrectly . These drugs can be administered intravenously, orally, subcutaneously and intramuscularly in different forms and may cause different side effects depending on the active substance of the drug and the site of administration (Kuhlmann and Antel, 2023). Almost all of the preventive treatments are for the form of MS with attacks, and while they do not completely cure the disease, they prevent its progressive course (Kuhlmann and Antel, 2023; Thompson et al., 2018). MS and Fatigue

Fatigue is defined as an overwhelming feeling of exhaustion and lack of energy. Patients have the impression that the effort required to fulfil a given task is disproportionately high, leading to avoidance of physical activity. Fatigue is a subjective symptom, so it can be confused with a general feeling of malaise or depression (Comi et al., 2001). Patients should be carefully examined physically and psychologically to avoid confusing symptoms of fatigue syndrome with depression or other disorders, as they may require separate treatments. MS-related fatigue differs from physiological fatigue in that it is more intense, does not diminish with sleep or rest, and also lasts longer (Michel and Bogousslavsky, 2006). In addition, fatigue in MS usually increases in the afternoon and may be exacerbated by stress, mild physical or mental exertion (Coates et al., 2020). Physical fatigue refers to physical exhaustion after performing a task that requires physical energy, while cognitive fatigue is associated with mental fatigue (Chen et al., 2020).

Heat sensitivity can cause a feeling of fatigue characterised by the appearance of a conduction block in the nodes of Ranvier (Uhthoff phenomenon) (Flensner et al., 2011). Factors such as depression, certain medications (opioids, long-term benzodiazepines, sedatives, painkillers, muscle relaxants), alcohol or nicotine, sleep disorders, infections and fever also exacerbate fatigue (Charvet et al., 2014; Mollaòlu and Üstün, 2009; Weiland et al., 2015).

Fatigue in multiple sclerosis can occur due to primary and secondary causes. Primary causes are mostly related to myelin sheath inflammation, axonal transection and brain hypometabolism. Secondary causes include factors such as depression, physical activity level and sleep quality. Poor sleep quality is among the most important etiological factors of fatigue in MS patients (Sıvacı et al., 2018). In a study, it was found that 47.5% of MS patients had poor sleep quality (Merlino et al., 2009). There are studies showing that factors such as advanced

age, female gender, number of years since diagnosis, having several children, lower education level, type of MS, number of comorbidities, smoking, low physical activity level are also associated with fatigue in MS patients (Weiland et al., 2015; Kaya & Ergin., 2021).

Severe fatigue is more frequently observed in progressive relapsing MS compared with relapsing-remitting and primary progressive MS (Hadjimichael et al., 2008a). The symptom of fatigue may be a harbinger of a relapse and may occur even a few weeks or months before it, but it may persist for a long time and occur at a time independent of relapse or remission (Comi et al., 2001). Fatigue is the most common cause that negatively affects quality of life in MS patients. For many patients, it affects quality of life more than pain and physical disability. It also has socioeconomic consequences by causing loss of labour force. According to data from the United States of America, the national cost of MS patients is more than 6.8 million dollars and 50-80% of people were deprived of the labour force within 10 years due to fatigue from the onset of the disease (Al-Dughmi et al., 2016; Aldughmi et al., 2016; Caminero and Bartolomé, 2011).

Although many studies have been conducted so far, it is still not possible to clearly establish the underlying causes of the fatigue symptom. Among the many hypotheses, the most common are immune system disorders or sequelae from CNS damage. Specific causes include proinflammatory cytokines (increased TNF-α mRNA expression, TNF-α levels and interferon-γ levels), endocrine effects (decreased DHEA concentration), axonal loss or increased volumes and cerebral activation patterns (Braley and Chervin, 2010).

1.5. Fatigue and Sleep Relationship

Sleep is a reversible state in which the threshold of response to external stimuli increases. On the basis of electrophysiological, behavioural and neuronal activity characteristics; 2 types of sleep stages have been defined as rapid eye movement (rapid eye movements, REM) and slow eye movement (non-rapid eye movements, NREM) (Merlino et al., 2009). REM and NREM periods of sleep last alternately throughout the night. There is a 'reciprocal' relationship between REM sleep and NREM sleep. In other words, while the effect of one of them weakens, the other one strengthens and takes over the sleep. The period from the beginning of sleep until the end of the first REM sleep is a sleep cycle. This cycle varies between 90-120 minutes and is repeated 4-6 times a night. It is reported that even if a person sleeps for a short time, he/she wakes up more rested when woken up at the end of this cycle. The length of night sleep depends on many

factors such as age, genetic factors and habits (Barun, 2013; Merlino et al., 2009; Sparasci et al., 2022).

The International Classification of Sleep Disorders includes insomnia, sleep-related respiratory disorders, central disorders of hypersomnolence, circadian rhythm sleep-wake disorders, parasomnias, sleep-related movement disorders and other sleep disorders. Sleep problems in MS patients who are categorised as other sleep disorders are also at a considerable level. Poor sleep quality was determined by PDQI in 47.5% of these patients (Mogavero et al., 2023). In fact, in studies in which the severity was also evaluated, it was shown that 13.3% had mild, 21.5% had moderate, and 30.0% had severe sleep problems (Qomi et al., 2023).

Sleep disorders are more common in MS than in the general population (Brass et al., n.d.). Sphincter disorders, spasticity and immobility in MS patients may cause sleep disturbance (Cortez et al., 2015). Spasticity, which is seen at a rate of 40-70% in the course of the disease, is an important factor in the occurrence of insomnia. Neurogenic bladder symptoms seen in 50-80% of patients with multiple sclerosis contribute to sleep disturbance (Kajbafvala et al., 2022). Nocturia causes the patient to get out of bed several times each night and interrupts sleep. In one study, 42% of patients with MS had mild insomnia, 53% moderate insomnia and 58% severe insomnia (Veauthier et al., 2011). Although there are no clear studies on this subject, steroids, immunomodulators, chemotherapeutics, antidepressants, anticonvulsants, opioids, antispastic agents used in MS may lead to sleep disorders (Baron et al., 2011). It has been shown that there is a decrease in sleep efficiency in two thirds of the night following interferon beta injection (Nadjar et al., 2011). Conventional MS treatment primarily focuses on the treatment of motor symptoms. However, sleep disturbance in MS patients is closely related to functional status, decreases quality of life and is one of the important causes of fatigue.

1.6.2. Fatigue and Quality of Life Relationship

Quality of life is the ability of the individual to experience psychological, social and physical satisfaction and to perform activities of daily living on their own (Nag et al., 2021). Although the prognosis of multiple sclerosis is variable, it can cause different degrees of limitations in patients. As the limitations associated with the disease increase, quality of life decreases (Aldughmi et al., 2016). Many psychosocial factors, including coping, mood, self-efficacy and perceived support, affect the quality of life of patients with MS more than indicators of disease prognosis (such as MRI lesions). Neuropsychiatric

complications such as cognitive impairment and fatigue are also moderate quality of life determinants even in patients in the early stages of the disease. In addition, psychological disorders such as depression, which have a direct cause-and-effect relationship with fatigue, also significantly affect quality of life (Siegert and Abernethy, 2005).

1.7. MS and Fatigue Management

Uncertainties about the definition, pathogenesis and measurement of fatigue associated with multiple sclerosis clearly hinder the testing of specific therapeutic anti-fatigue strategies. However, there are unproven strategies that could be trialled to help improve MS-related fatigue. As fatigue appears to be related to mood and quality of life, it is important to address these issues at the outset. Similarly, it is important to exclude common medical conditions that may exacerbate fatigue (e.g. hypothyroidism) and optimise doses of medications known to exacerbate fatigue. Non-pharmacological approaches for the specific management of fatigue include behavioural therapy, graded aerobic exercise programmes, energy-saving strategies, dietary advice, environmental cooling and improvement in basic sleep hygiene (Oliva Ramirez et al., 2021a). Despite its widespread use, modafinil has not yet been shown to be effective in MS-related fatigue (Tur, 2016). Antidepressant drugs, particularly serotonin and noradrenaline reuptake inhibitors, are widely used with little or no evidence supporting their efficacy in MS-related fatigue. The use of stimulants such as amphetamines, methylphenidate and pemoline (not manufactured in the UK) in MS-related fatigue cannot be approved due to their unproven efficacy and possible side effects (Ballon et al., 2006).

In order to manage fatigue, it would be useful to focus on the factors associated with fatigue. As a result of the trainings given to MS patients about lifestyle changes such as diet, smoking, sleep hygiene, physical activity, there is evidence that the person can implement and sustain these recommendations even after 1-3 years, and there are studies showing that there is a significant improvement in quality of life (Latimer-Cheung et al., 2013; Marck et al., 2018).

In another study, it was found that an Internet-based rehabilitation programme reduced the level of fatigue in MS patients and improved cognitive activity (Moustafaa et al., 2022). With the advancing technological developments, internet-based training programmes that patients can quickly adapt to their lives, educational and motivational applications have started to take their place in the fields of study. There are project initiatives that patients can access quickly using technological devices, which are considered to be useful in the management of

fatigue and other symptoms (Cortés-Pérez et al., 2021; Giunti et al., 2018; Jongen et al., 2020; Latimer-Cheung et al., 2013; Thomas et al., 2021).

The concepts of e-health technologies or telehealth, including online education and training programmes, have the potential for a better, easily accessible and cheaper infrastructure in healthcare (Campion et al., 2016; Van Gemert-Pijnen et al., 2011). Today, every individual can access the internet in very easy ways. MS patients are not only a patient group with high internet usage, but also easily adapt to online applications (Fischer et al., n.d.; Nielsen et al., n.d.; pöttgen et al., 2018). The integration of telehealth and wearable technology significantly affects the strength and sustainability of the service provided. Smart watches, which can be considered as the beginning of wearable technologies, have been enabling their users to perform their daily activities and work follow-ups in a more organised way thanks to the notifications they receive since the 2010s. The smartwatch, one of the first wearable fashion components introduced to the market, can be defined in the most comprehensive way for consumers as a type of device worn on the wrist, which basically has many functions from e-mail to video recording (Bölen, 2020). A smartwatch is a computationally powerful, wrist-worn device that can be connected to other devices via short-range wireless connectivity and performs alert notifications by collecting and storing personal data through a series of sensors (Wu et al., 2016). Although there are studies in the literature on the use and continuity of these devices in the healthy population, there are no studies supported by smartwatch measurements on MS patients. It is of great importance that the use of these easily accessible devices to improve the quality of life patients is encouraged and supported by scientific results. In a recent Cochrane review on telerehabilitation in MS, five of the studies had interventions via the internet. The authors concluded that there was limited evidence for the effectiveness of telerehabilitation in improving functional activity, quality of life and fatigue (Khan et al., 2015).

In a review, roles such as care coordinator, care provider and expert resource were identified as the roles of nurses specialised in MS (Meehan & Doody, 2020). In a study conducted in 2013, 83% of MS patients stated that they preferred to communicate with the MS specialist nurse rather than other health professionals because they thought that the MS nurse understood their specific conditions better than other health professionals such as family physicians and neurologists (Ward-Abel et al., 2013). There are studies showing that nurses with expertise in chronic disease management have sufficient competence to provide comprehensive care to MS patients and are important in providing cost savings in health care (Buerhaus, 2018; Maloni, 2013; Roman & Menning, 2017).

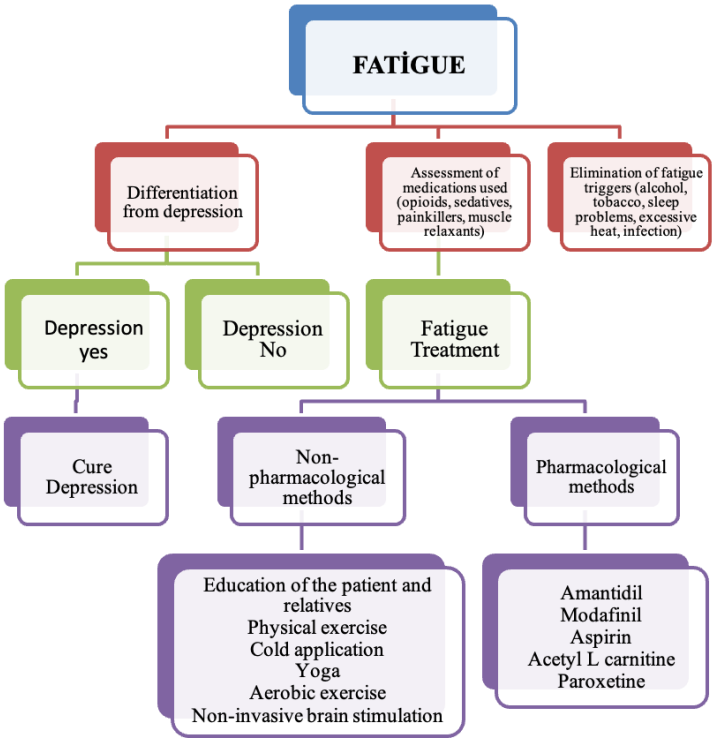

Figure: Fatigue Management (Braley and Chervin, 2010)

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CHAPTER 24

Dopamine and Leptin

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Food intake has significant effects on our emotional behaviors. These behaviors can also be a companion to cope with situations such as food selection, presentation style, nutrition styles, need for socialization, depression, stress, excitement, and sadness (Adolphs et al., 2019; Devonport et al., 2019; Ljubičić et al., 2023). It is thought that the pleasure or happiness felt as a result of food intake is similar to the euphoric feeling after drug use (Krupa et al., 2024). Using strong reinforcers to increase this pleasure even more activates the pleasure regions in the brain after a while and leads to neurophysiological changes. Processed foods are at the forefront of these powerful reinforcers, and the term "food addiction" and, recently, "sugar addiction" is frequently encountered (Meule A, 2019; Lane et al., 2024). The basic mechanism that initiates the formation of this addiction is the sense of taste. This sense begins with the stimulation of taste receptors located on the tongue and then continues by being resolved in the gastrointestinal system, bladder, and fat tissue. This transmission is also encoded in the brain by stimulating the reward system (Laffitte et al., 2014). Just like drugs, amphetamines, or alcohol, foods also realize their addictive properties through the mesolimbic dopaminergic system. With the discovery of Leptin, a hormone produced by fat cells, findings on the effects of leptin in the control of nutrition and energy expenditure are increasing daily in the literature. In addition, experimental and clinical studies have shown that Leptin regulates behavioral patterns associated with the mesolimbic dopaminergic system (Opland et al., 2010, Watts et al., 2022)

It is known that leptin reduces dopamine output by activating local GABA neurons in the mesolimbic dopaminergic system. In contrast, dopamine directly inhibits the NAc in the ventral tegmental area (VTA) (Figure 1). In the lateral hypothalamus, leptin inhibits VTA-projecting LepR neurons that form inhibitory synapses with VTA GABA neurons, thus reducing the inhibitory effect on GABA neurons in the VTA. This does not inhibit VTA GABA neurons but decreases dopamine neuronal activity (Omrani et al., 2021).

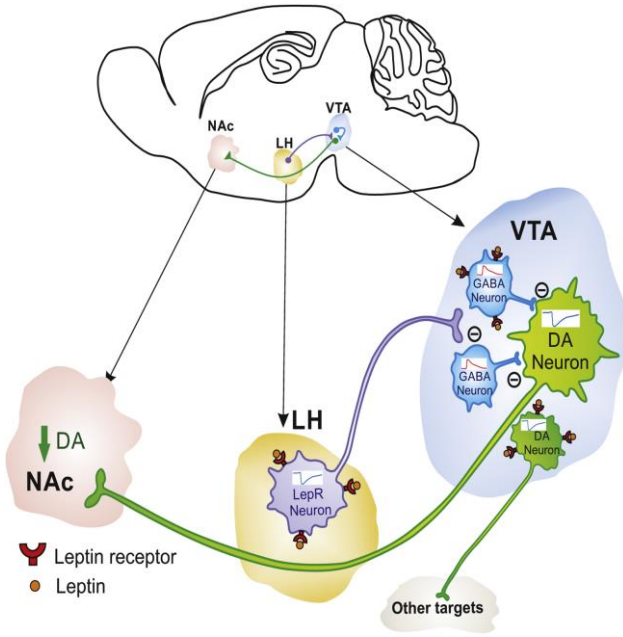

Figure 1. Effects of leptin on the mesolimbic dopaminergic system (Shale et al., 2021).

DA, dopamine; GABA, gamma-aminobutyric acid; LepR, leptin receptor; LH, lateral hypothalamus; NAc, nucleus accumbens; VTA, ventral tegmental area,

The focus of research on leptin is on the homeostatic effects of leptin. There are minimal studies on the interaction of leptin with reward mechanisms. Especially with the demonstration that leptin can regulate drug relapse, its functionality with mesolimbic dopamine circuits becomes remarkable, and it is suggested that changes in leptin levels may affect feeding behavior via VTA dopamine neurons. Therefore, it is suggested that food intake can be changed by modulating leptin transmission in the VTA (Shalev et al., 2001; Hommel et al., 2006, Asgari et al., 2024). However, it has been demonstrated that the rewarding effects of intracranial self-stimulation are suppressed due to intracerebroventricular or intraventral tegmental area leptin applications (Fulton et al., 2000). Dopamine release resulting from action potential bursts in dopaminergic neurons in the VTA is phasic (Watts et al., 2022).

The hedonic value of food, motivation, and reinforcement of food determines the area of action of dopamine in the brain. The hedonic state, i.e. liking delicious food, motivation, i.e. wanting food, and finally, reinforcement of food, i.e. learning the smell and taste of food, is regulated by the brain's reward pathways

(Volkow et al., 2011). As a result, appetite and eating behavior patterns are formed. The effects of leptin on the mesolimbic pathway are two-way, direct, or indirect (Figure 2). However, it should also be noted that an increase in dopamine levels does not necessarily indicate an increase in food intake (Asgari et al., 2024). The mechanism underlying feeding behavior is the inhibition of VTA dopamine neurons by leptin and the modulation of the dopamine VTA nucleus accumbens reward circuit to control feeding behavior. In summary, leptin provides control of feeding by indirectly affecting the dopamine reward system (Baik J.H, 2021).

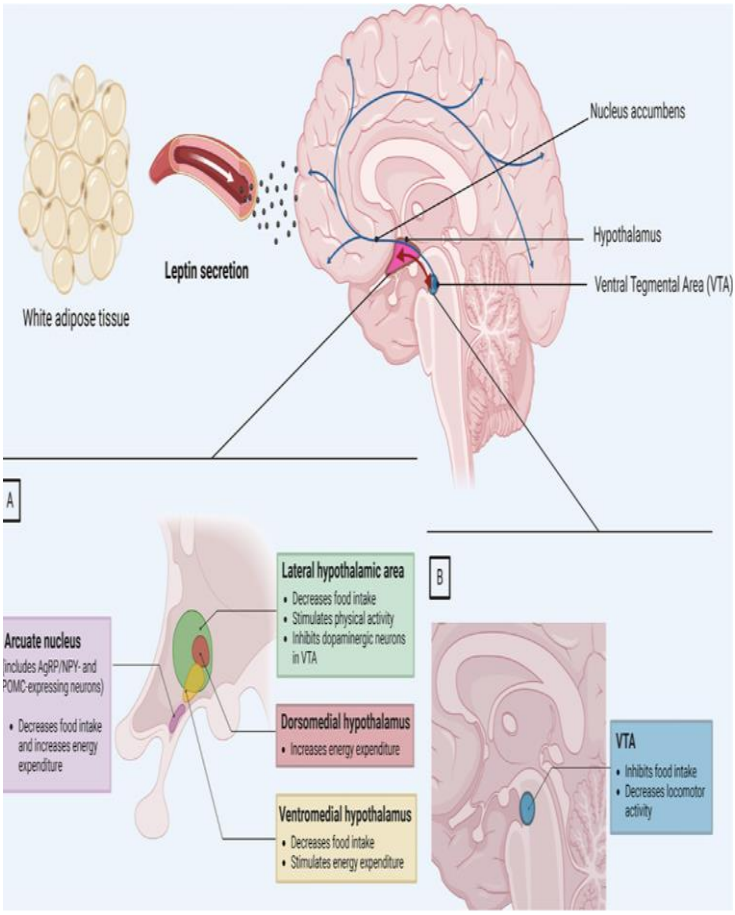

Figure 2. Effects of leptin release on the hypothalamus and ventral tegmental area (Asgari et al., 2024)

Leptin shows its effects in the arcuate nucleus of the hypothalamus through decreased food intake and increased energy expenditure proopiomelanocortin (POMC neurons) as well as agouti-related protein and neuropeptide Y (AgRP/NPY neurons) neurons (Asgari et al., 2024). In the lateral hypothalamic area, it decreases food intake and inhibits dopaminergic neurons in VTA, while in the ventromedial hypothalamus, it stimulates energy expenditure (Figure 2). Leptin also has the feature of decreased locomotor activity in VTA dopaminergic neurons. This effect is also done via the activation of the signal transducer and activator of transcription-3 (STAT3) signaling (Ruegsegger et al., 2017).

There are many studies on leptin in areas such as energy homeostasis, nutrition, and hormonal interactions. On the other hand, the literature has limited information on its effects on cognitive functions. New experimental studies should be designed to provide data on the extent to which energy homeostasis and cognitive status or motivational conditions affect each other. The data obtained will shed light on the neuroanatomical, neuropharmacological, and even neurophysiological valid mechanisms in these conditions.

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CHAPTER 24

Home Care After Oncology Surgery

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Cancer is a major life-threatening global health problem. It has been reported by the World Health Organization that approximately 19.3 million people are currently diagnosed with cancer. This number is expected to increase in the coming years (1). According to the Yearbook of Health Statistics, the most common cancer types in Turkey are breast, lung, prostate, stomach, colorectal, thyroid, uterine, bladder, leukemia and ovarian cancer (2).

Cancer is a disease in which normal cells divide and multiply uncontrollably and spread to other parts of the body. Risk factors such as genetic factors, overweight, tobacco and alcohol use, sedentary lifestyle, unhealthy diet and air pollution are involved in cancer formation. Tobacco use is reported to be the most important risk factor in cancer formation (1,3,4).

Cancer is often a disease that progresses rapidly and sometimes has no symptoms. For this reason, some patients are diagnosed at an advanced stage. To prevent this, it is important to follow the signs and symptoms of cancer in addition to regular health checks. Although the symptoms of cancer vary depending on the type of cancer, common symptoms include fatigue, weight loss, loss of appetite, pain and bleeding. (5,6).

Once patients are diagnosed with life-threatening cancer, they need to be treated adequately and effectively. In general, the primary goal of treatment is to cure the cancer or prolong the patient's life. Improving the patient's quality of life is another important goal. Cancer treatment includes one or more options such as surgery, radiotherapy and chemotherapy depending on the stage of the cancer. In cancer treatment, palliative surgery options are applied as curative or to control symptoms (7,9) .

Surgical interventions, which are an important option in the treatment of oncology patients, affect the daily life of patients and their families.Therefore, it is important to continue cancer treatment with effective care.Patients need physical, emotional, cognitive and spiritual care support in the postoperative period (10,11). Physical signs and symptoms of the disease such as vomiting, nausea, loss of appetite, fatigue, shortness of breath, pain, sleep problems, sexual problems seen in the preoperative period may continue in the postoperative period (12,13). In the emotional domain, fear of death, hopelessness, changes in body image, and a sense of dependence on others can occur (3,4).

Patients receiving oncologic surgical treatment tend to experience negative psychosocial problems.The problems experienced vary according to the stage of the disease and the treatment method, but also vary individually. In this process,

supportive care is recognized as an important service to help meet the physiological, psychosocial and spiritual needs of oncology patients. (14)

Patients after oncologic surgery should be supported by health professionals and their relatives with a multidisciplinary team approach in the management of their symptoms and treatment side effects. Nurses are one of the most important members of the multidisciplinary team, supporting patients and their relatives at every stage of the disease (16). Ensuring patients' well-being and meeting their needs holistically is possible by providing individualized and quality nursing care in a behavioral, cognitive and comprehensive manner. Identifying and meeting the individualized care needs of patients undergoing oncologic surgery and their families at an early stage in order to help them cope with the problems they experience during this process is very important in terms of maintaining the well-being of patients and increasing their quality of life (13,15).

As it is known, cancer patients face many problems due to both the symptoms caused by the disease process and the side effects of treatments such as chemotherapy and radiotherapy. Regardless of the focus of the cancer, the problems experienced by patients in the home environment are generally similar. However, with each passing day, the care of cancer patients is becoming more complex and they need care not only in the hospital setting but also in the home environment (17,18).

Public health nurses aim to promote and improve health and prevent diseases by making home visits and health education to all individuals and families in the society. For this purpose, home care nursing has been accepted as a sub-component of public health nursing for many years and continues to be accepted as such today. However, although public health and home care nursing have common aspects, they are both specialties in their own right (19). Therefore, a home care nurse is neither a public health nurse nor a clinical nurse (20). Home care nurses constitute a common synthesis of public health and clinical fields. It has become important to clearly define the competencies and training standards of home care nurses in order to provide safe, effective and quality health care services to individuals receiving home care services with a different approach than the service provision they receive in the hospital environment (21). The nurse providing home care services should collect information about the patient's condition in the clinic or after hospitalization and the period of hospitalization, create a recording system that provides information flow, and initiate initiatives to get to know the patient and his/her family. The functions to be carried out by the nurse in the home care process should be organized according to a care model, care plan and nursing process (22). Care related to activities of daily living; Pain

control, Nausea and vomiting, Oral mucositis, Fatigue and fatigue, Loss of appetite, Difficulty in adhering to treatment regimen, Psychosocial support services (17, 23, 24).

Oncology patients need the most help with activities of daily living such as bathing, toileting, moving, dressing and undressing, shopping and cleaning. It has been reported that oncology patients have problems in home environments such as shopping, house cleaning, bathing, getting in and out of vehicles, moving around in social areas, dressing and undressing, food preparation, nutrition, going to the toilet, making phone calls, using medications (17).

Pain Management: Since oncology patients have different diagnoses, stages of cancer and types of treatment, an individualized pain plan should be implemented. Pain should be considered and treated before and after treatment. In addition to pharmacologic treatments, non-pharmacologic methods should also be used. Recommendations for pain management and care:

- Non-pharmacological or pharmacological methods should be applied without waiting for the pain to intensify
- Painkillers must be used when necessary and in the correct dose. Information should be obtained from the home care nurse about the method of use and dosage, and this information should be supported by the nurse with brochures.
- Education about the possible side effects of painkillers should be provided by the home care nurse.
- If there is no decrease in the severity of pain, if it affects daily life such as nutrition and movement and if there is an increase in its frequency, the physician should be informed (25).

Nausea and Vomiting: Although not seen in all patients, cancer diagnosis, radiotherapy and chemotherapy can cause nausea and vomiting. The duration, frequency and amount of nausea and vomiting depending on the cause may vary from patient to patient. Suggestions to reduce nausea and vomiting:

- If nausea and vomiting are persistent, antiemetic medication should be used regularly.
- Easily digestible foods such as pretzels, chicken soup, vegetable soup, baked and grilled chicken, boiled potatoes, low-fat rice pilaf, yogurt should be consumed.

- Fluids should not be taken during feeding and should be taken one hour before or after feeding.
- Do not lie on your back for the first 30 minutes after meals.
- -Meals should be eaten little, often and slowly.
- -The room should be ventilated.
- -If the smell of food bothers you, someone else should cook the food. Wait for the food to cool down before eating it.
- If there is no physician-ordered salt restriction, salty crackers, cheese, bread, toast can be consumed to prevent nausea.
- Spicy, very salty and sugary foods should be avoided.
- Mint menthol chewing gum can be chewed as a relaxant in case of a change in taste.
- Deep breathing and coughing exercises before treatments will reduce nausea and vomiting.
- It should be reminded that light meals and snacks two hours before chemotrapy can be relaxing.
- Despite all these recommendations, if vomiting occurs more than three times in one hour and the patient cannot drink more than four glasses of fluid a day, a physician should be consulted (25,26).

Oral Mucositis: Redness and sores in the mouth due to cancer and its treatment. This may lead to malnutrition and infections. The most effective treatment method of oral mucositis is proper oral care (27). Recommendations regarding oral care;

- Regular check-ups with the dentist should be made during treatment.
- The oral mucosa and tongue should be checked every day. It will be an opportunity to diagnose possible problems early.
- Teeth should be cleaned twice a day with a soft toothbrush.
- Before going to bed, one teaspoon of baking soda should be mixed into a tea glass of water and gargle with the mixture.
- If dentures are used, they must be cared for after meals.
- Moisturizer should be used for the lips in every oral care.

- Foods that threaten the integrity of the oral mucosa should be avoided.
- If there is pain during feeding, difficulty in swallowing, redness of the oral mucosa, the physician should be informed (27).

Fatigue and Weakness: It is a symptom that can be observed from mild to severe levels in all patients related to cancer and the treatment process. While many causes lead to fatigue and fatigue, the most common cause is chemotherapy treatment (24,26). Recommendations to reduce fatigue and weakness:

- Activities should be planned in order of priority.
- Rest intervals should be determined at periodic intervals during the day.
- To regulate night sleep, bedtime and wake-up time should be determined. Daily sleep time should be set at 6-8 hours
- You can listen to music or do relaxation exercises to make it easier to fall asleep.
- Regular nutrition will enable active and effective use of energy, so attention should be paid to nutrition. If necessary, help from a dietitian should be sought
- Drink at least 2,000 mL (8 glasses) of water during the day.
- 15-20 minutes of light exercise daily will make it easier to fall asleep.
- Despite all these recommendations, if you feel severe fatigue and weakness, you should consult a physician (28).

Eating disorder: Loss of appetite is common in oncology patients due to nausea, vomiting, oral mucositis, chemotherapy, radiotherapy and other medical treatments. Suggestions to reduce anorexia:

- High-calorie and protein-rich foods should be preferred.
- Nutrition should be planned as 3 main and 3 snacks.
- If there is no problem with the oral mucosa, lemon, vinegar, spices, mint and thyme can be consumed with meals.
- Fruit juices and soups should be consumed according to the patient's condition.

- Some chemotherapy drugs can leave a metal taste in the mouth. To eliminate this problem, a few drops of orange or lemon juice can be added to warm water..
- Foods that are very popular should not be consumed in case of severe anorexia.
- Odorless and cool foods should be preferred.
- Light exercise before meals can reduce anorexia.
- Eat in a comfortable, pleasant and odorless environment.
- If the mouth is not sore, favorite spices can be used to increase the sense of taste.
- -Green leafy foods and unpeeled fruits should be soaked in vinegar water for 15 minutes and consumed after washing.
- -Foods cooked over coal fire should be avoided.
- Avoid grapefruit and its juice, excessively salty and sugary foods, and excessively fatty foods.
- If there is no improvement in anorexia despite all these suggestions, a physician should be consulted (26)

Postoperative Care Applications: Postoperative Care Practices: Patients with cancer and their relatives need care and information on many issues such as dressing, use of assistive devices and drugs (dose, interaction, side effects), exercise and diet after cancer-related surgical procedures (29,30). Recommendations in postoperative care;

- Bad odors should be removed by ventilating the house,
- Pay attention to the cleaning of upholstery and floors as they can create bad odors,
- Hygiene of body, clothing, bed, room and home should be ensured
- Food should be properly prepared and stored
- The temperature of the room where the patient bed is located should be controlled
- Care should be taken against the patient's body temperature dropping
- Patient-oriented medication should be administered

- Complications that may develop due to surgical procedures and poor environmental conditions should be monitored
- Create a hygienic environment suitable for care
- Infection control methods should be followed against postoperative infections (33).

Psychosocial Support Services: Cancer negatively affects individuals physiologically, psychologically and socially. This effect leads to various symptoms such as anxiety, fear and depression in patients, and when the main source of the problems is evaluated, it is determined that cancer is associated with death. In order to prevent or eliminate the occurrence of these problems, the nurse providing home care services should contact the hospital, request appointments from the necessary clinics and direct the patients to these areas (28).

An individual's illness affects all family members individually. Depending on the patient's prognosis, families experience uncertainty about the patient's current and future health status. Roles may change among family members due to the physical limitations and fatigue of the patient (29). The importance of each member of the family making their own decisions should be emphasized and reassured by the health care team. The patient and family should realize that they are being guided by professional individuals. One of the various home care service organizations in Europe and the USA is hospital-based home care services. Home care, which is hospital-based, was born as an alternative for inpatients and outpatients requiring chronic care and has become an expanding type of care. As health technology develops and expectations change, the scope and accessibility of home care services are expanding (32-33).

RESULTS

Patients undergoing oncologic surgery face many psychological, social and physical problems that will negatively affect their lives. Patients need supportive care needs due to these problems. The care needs of patients are met by their relatives or healthcare professionals. The care needs of patients undergoing oncologic surgery should be determined individually in the early period from the diagnosis of the disease. Since all these problems directly affect the treatment process, quality of life, mortality and morbidity rates of patients with cancer and their relatives, the concept of home care continues to be up-to-date. In this direction, it is necessary to evaluate and solve the problems faced by patients with cancer and their caregivers in the home environment within the framework of home care It is seen that the services provided by the home care team and nurses in cancer patients in the home environment have many positive effects such as the possibility of patient-specific care, not the disease, and ensuring the continuity of care without restricting the freedom of patients. The fact that cancer is an increasing chronic disease increases the need for home care services team. For this reason, it is seen that nurses in the home care team have an important place in improving the quality of life of cancer patients. It is recommended that nurses should feel responsible in this field, provide development for the field, and evaluate the opportunities of home care services throughout the country and present policies for their development.

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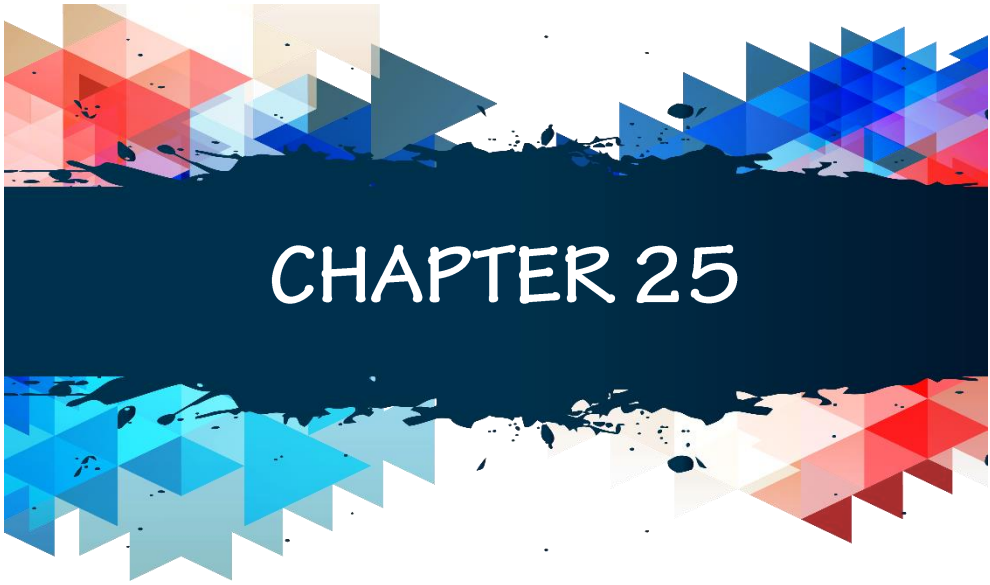

Lithium Toxicity

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1.INTRODUCTION

Lithium is a pharmacological agent with proven efficacy whose medical use dates back to the mid-19th century. Over time, it has been the subject of various controversies. Initially introduced for the treatment of uric acid metabolism disorders, such as gout and rheumatic diseases, its effectiveness in these indications was not adequately substantiated through clinical studies. By the late 19th century, lithium-containing mineral waters and tablets had become widely popular; however, the lithium content in these products was typically negligible from a clinical perspective. In the early 20th century, the introduction of more concentrated lithium preparations led to an increase in toxicity cases, with adverse effects such as tremors, nausea, and vomiting frequently reported. In 1949, the use of lithium chloride as a salt substitute in low-sodium diets caused severe poisonings and fatalities, leading to the rapid abandonment of this practice. During the same period, the efficacy of lithium in treating manic disorders was demonstrated, although it did not gain widespread acceptance initially. Following its approval by the FDA in 1970, lithium entered clinical practice as a safe and effective treatment for bipolar disorder.(1)

Bipolar disorders (BD) are severe psychiatric conditions characterized by cycles of depression and mania. BD type I is associated with manic episodes, while BD type II is linked to hypomania and major depressive episodes. Lithium (Li^+), particularly effective in the treatment of mania, is widely used in the management of both BD type I and BD type II.(2). Alzheimer's disease (AD) is a chronic form of dementia characterized by memory loss, behavioral changes, and impaired concentration. Studies have shown a significantly lower incidence of AD among bipolar disorder patients undergoing chronic lithium (Li^+) therapy. The neuroprotective effects observed in bipolar disorder patients support the potential use of Li^+ in the treatment of AD. Clinical trials are currently ongoing to validate this potential (3,4). Lithium (Li^+) can be used as an adjuvant agent in the treatment of thyroid cancer and hyperthyroidism. Li^+ inhibits the synthesis and release of thyroxine (T4) and triiodothyronine (T3), thereby reducing their serum levels while increasing intrathyroidal iodine accumulation. This mechanism allows for greater uptake of radioactive iodine by both normal and cancerous thyroid cells, enhancing the efficacy of radioactive iodine therapy. Additionally, Li^+ modulates the activity of deiodinase enzymes, regulating the conversion of thyroid hormones between their active and inactive forms. Through these effects, Li^+ is considered an effective supportive treatment option in managing thyroid cancer.(5,6)

The narrow therapeutic window of lithium is a significant clinical factor that increases the risk of toxicity. The therapeutic window defines the effective and safe dosage range of a drug, and for lithium, this range is notably narrow, with minimal difference between therapeutic and toxic doses. The epidemiology of lithium toxicity is categorized into two main forms: acute and chronic toxicity. Acute toxicity is typically associated with the ingestion of high doses of lithium over a short period, often observed in younger individuals and suicide attempts. This form of toxicity is more common in patients who are new to lithium therapy, and its incidence is generally linked to exceeding the therapeutic range. Chronic toxicity, on the other hand, develops gradually due to the accumulation of serum lithium levels during long-term use at therapeutic doses. It is more frequently seen in elderly patients, individuals with impaired renal function, and those exposed to drug interactions. (7). The development of lithium toxicity can be influenced by various factors, including renal function, age, hydration status, concurrent medication use, and overdose. Since lithium elimination occurs almost exclusively through the kidneys, patients with impaired renal function are at a heightened risk for drug accumulation and subsequent toxicity. In elderly individuals, physiological declines in renal function and a reduction in total body water are notable factors that increase the risk of toxicity. Dehydration can further elevate serum lithium levels by reducing renal clearance. Additionally, medications such as diuretics, ACE inhibitors, ARBs, and NSAIDs can alter lithium pharmacokinetics, increasing the likelihood of toxicity. Accidental or intentional overdose can lead to acute lithium poisoning. In this context, regular monitoring of serum lithium levels, careful dose adjustment, and proactive management of coexisting risk factors are critical to preventing toxicity in patients receiving lithium therapy. (8,9)

2. PHARMACOKINETICS AND PHARMACODYNAMICS

Lithium is an agent metabolized in the body in a manner similar to sodium ions and is widely used in the treatment of neuropsychiatric disorders. Pharmacodynamically, although its exact mechanism of action is not fully elucidated, lithium has been shown to inhibit the inositol monophosphate and glycogen synthase kinase-3 (GSK-3) signaling pathways. These effects are thought to play a critical role in modulating energy metabolism, neuroprotection, neuroplasticity, and mood stabilization. Additionally, the reduction in intracellular inositol levels is considered a significant contributor to lithium's clinical efficacy.(10).

Pharmacokinetically, lithium is rapidly absorbed after oral administration, reaching peak serum concentrations within 1 to 3 hours for immediate-release

formulations and within 4 to 6 hours for extended-release formulations (11). The volume of distribution (Vd) for lithium typically ranges between 0.5 and 1.2 L/kg. It is an ion that does not bind to plasma proteins and freely distributes in total body water. Lithium can accumulate in certain tissues, such as the brain, kidneys, and thyroid, at concentrations exceeding those in serum. Unmetabolized lithium is eliminated as a free ion through the kidneys. After glomerular filtration, approximately 80% of the filtered lithium is reabsorbed via passive diffusion in the proximal tubules. In individuals with normal renal function, the elimination half-life of lithium ranges from 16 to 30 hours. However, this duration is extended in elderly individuals due to age-related declines in renal clearance (12).

3. PATHOPHYSIOLOGY AND CLINICAL PRESENTATION

Lithium toxicity is a serious clinical condition resulting from the accumulation of the drug in the body and is classified into three main forms: acute, chronic, and acute-on-chronic toxicity. These forms differ in terms of the underlying pathophysiological mechanisms and clinical manifestations (13) .

3.1. Acute Lithium Toxicity

Acute lithium toxicity typically occurs following a single ingestion of a high dose of lithium and manifests with rapidly onset symptoms.

3.1.1. Gastrointestinal Symptoms:

Nausea, vomiting, and diarrhea are common early symptoms resulting from the irritative effects of lithium on the gastrointestinal mucosa. These symptoms generally appear within the first few hours of ingestion and can lead to fluid loss, increasing the risk of hypovolemia and renal dysfunction.

3.1.2. Neurological Symptoms:

Neurological manifestations, such as tremor, ataxia, agitation, and confusion, are associated with lithium's disruption of neuronal membrane stability. In severe cases, encephalopathy and seizures may develop. These symptoms usually follow the onset of gastrointestinal signs and correlate with the severity of toxicity. (9,14).

3.2. Chronic Lithium Toxicity

Chronic lithium toxicity typically develops during long-term lithium therapy due to overdose, impaired renal function, or drug interactions. Symptoms emerge gradually and often involve multiple organ systems (14).

3.2.1. Neurological Symptoms:

Neurological manifestations are prominent and include lethargy, cognitive impairment, cerebellar dysfunction, and extrapyramidal symptoms. These effects are associated with lithium crossing the blood-brain barrier and accumulating at the neuronal level (9,14).

3.2.2. Renal Dysfunction:

Complications such as nephrogenic diabetes insipidus (NDI) result from lithium's toxic effects on renal tubular cells. Symptoms like polyuria and polydipsia exacerbate fluid loss, further intensifying the severity of toxicity (15).

3.2.3. Endocrine Dysfunction:

Lithium's suppression of thyroid hormone synthesis can lead to hypothyroidism, which may exacerbate both neurological and systemic toxicity (16).

3.3. Acute-on-Chronic Lithium Toxicity

This form occurs when a patient receiving chronic lithium therapy experiences an acute overdose, combining features of both acute and chronic toxicity. Gastrointestinal symptoms tend to present earlier and more severely, while neurological symptoms develop rapidly, contributing to a more severe clinical presentation (14).

3.4. Variability in Clinical Presentation

The clinical manifestation of lithium toxicity varies depending on individual risk factors and underlying conditions. Elderly patients are more susceptible to toxicity due to decreased renal function and reduced total body water. Comorbid conditions, impaired hydration status, and the use of medications such as diuretics, ACE inhibitors, or NSAIDs further increase the risk of toxicity. Lithium toxicity is a complex clinical condition that can cause severe complications in the neurological, gastrointestinal, and renal systems. Acute toxicity presents with rapidly onset symptoms, whereas chronic toxicity progresses gradually with neurological and renal effects. The acute-on-chronic form combines features of both, resulting in a more complex clinical course. Clinical management requires careful assessment of the different forms of lithium toxicity and consideration of individual risk factors (9,14).

The Syndrome of Irreversible Lithium-Effectuated Neurotoxicity (SILENT) is a clinical condition characterized by persistent neurological and neuropsychiatric symptoms following lithium toxicity. SILENT occurs in some

cases of lithium toxicity where neurological toxicity develops at high serum lithium concentrations, and symptoms persist even after successful elimination of the drug from the body. A review of 117 patients with SILENT reported that the most common acute neurological finding was an altered level of consciousness, ranging from confusion to coma. This condition is associated with lithium's long-lasting effects and neurotoxic mechanisms in the central nervous system, posing a significant clinical burden on affected patients.(17).

4. ROLE OF LABORATORY TESTS

The following laboratory tests play a crucial role in the diagnosis and management of lithium toxicity:

4.1. Serum Lithium Levels:

Serum lithium levels are a critical marker for both therapeutic efficacy and toxicity detection. The therapeutic range is typically defined as 0.6–1.2 mmol/L. Mild symptoms may occur when lithium concentrations exceed 1.5 mmol/L, while levels above 2.5 mmol/L are associated with moderate-to-severe symptoms such as neurological dysfunction, tremors, and altered consciousness. Concentrations exceeding 3.5 mmol/L generally indicate severe toxicity. However, in cases of acute lithium ingestion, high serum levels may be observed without toxic manifestations due to the slow distribution of lithium into the central nervous system (CNS). Therefore, the evaluation of toxicity requires consideration of both serum lithium levels and clinical findings (8,9).

4.2. Electrolytes and Renal Function Tests:

Given that lithium is excreted primarily through the kidneys, it is essential to evaluate renal function using tests such as serum creatinine and blood urea nitrogen (BUN). Electrolyte imbalances, including hyponatremia, hyperkalemia, and hypercalcemia, are also commonly observed and should be carefully monitored (9,18). Lithium therapy can lead to a reduction in the glomerular filtration rate (GFR), which reflects the kidneys' ability to filter blood. Additionally, lithium suppresses the kidneys' capacity to concentrate urine, resulting in conditions such as polyuria and polydipsia. Prolonged use of lithium is associated with an increased risk of chronic kidney disease and renal failure (7,19). Approximately half of the patients receiving lithium therapy develop polyuria within a few days to weeks of treatment initiation. Among these, about 20% may experience nephrogenic diabetes insipidus (NDI), a condition characterized by a loss of urine concentration capacity due to resistance to vasopressin. The clinical consequences of NDI include hypovolemia,

hypernatremia, hyperchloremic metabolic acidosis, and distal renal tubular acidosis. These effects result in significant disruptions in fluid and electrolyte balance, imposing a substantial clinical burden on affected patients. (9,15).

4.3. Thyroid Function Tests:

Lithium's suppression of thyroid hormone synthesis can lead to hypothyroidism. Therefore, monitoring thyroid-stimulating hormone (TSH) and free thyroxine (fT4) levels is essential in patients receiving lithium therapy (9,16).

4.4. Parathyroid Function:

Hyperparathyroidism, a complication of lithium therapy, is associated with hypercalcemia. The osmotic effects of hypercalcemia can lead to fluid loss and subsequent volume depletion, further complicating the patient's clinical status (8).

4.5. Electrocardiography (ECG):

Lithium toxicity can result in QT prolongation and cardiac arrhythmias. Given that cardiac complications can be fatal in severe cases, ECG monitoring is crucial for early detection and management (20,21).

5. MANAGEMENT OF LITHIUM TOXICITY:

Although lithium toxicity is not a common reason for emergency department visits, it is a serious clinical condition with a high mortality rate. Therefore, management must be rapid, effective, and tailored to the patient's clinical course and symptoms. It is important to note that clinical symptoms may be delayed even when toxic levels are reached, meaning symptoms do not always accurately reflect the severity of the toxicity. Consequently, measuring lithium levels is essential for devising an appropriate treatment plan. Currently, there is no specific antidote for lithium toxicity. Treatment is based on two fundamental principles: first, effectively reducing circulating lithium levels, and second, correcting fluid and electrolyte imbalances to minimize the multi-organ effects caused by lithium. These approaches are critical in the management of lithium toxicity (22,23).

5.1. General Approach in Lithium Toxicity Management:

As with the management of all poisoning cases, the initial approach in lithium toxicity involves the assessment and stabilization of airway, breathing, and circulation ("ABCs"). Establishing appropriate monitoring conditions and evaluating the patient's level of consciousness are critical steps. As part of the initial assessment, measuring blood glucose via a fingertip test is essential to rule out hypoglycemia (8).

5.2. Fluid Replacement in the Treatment of Lithium Toxicity:

Fluid replacement plays a critical role in optimizing lithium elimination by enhancing renal perfusion. Intravenous infusion of isotonic saline (0.9% NaCl) is the preferred approach, especially in hypovolemic patients. Rapid rehydration is achieved by administering a bolus of 20 mL/kg isotonic saline, followed by a continuous infusion at a rate 1.5 to 2 times the maintenance rate, adjusted based on the patient's clinical condition. During treatment, urine output and serum lithium concentrations should be monitored regularly to assess the adequacy of renal perfusion. Serum sodium levels must also be frequently checked, considering the risk of hypernatremia due to arginine vasopressin resistance (AVP-R). Restoring sodium and water balance in hypovolemic patients is vital for maintaining glomerular filtration rate and promoting lithium excretion (8,9,24).

5.3. Gastrointestinal Decontamination in Lithium Toxicity Management:

Gastrointestinal decontamination plays a limited role in managing lithium toxicity but may be considered in specific clinical scenarios. Gastric lavage and the use of polystyrene resins are recommended in cases of acute toxicity or ingestion of extended-release preparations, though evidence supporting their efficacy is limited. Conflicting data in the literature suggest that while these approaches may serve as alternative options when renal replacement therapy is unavailable, their overall effectiveness is modest. Additionally, these methods generally demonstrate low efficacy in lithium elimination and carry the potential for adverse effects, making them unsuitable as replacements for standard treatment protocols. Thus, the use of gastrointestinal decontamination techniques should be carefully evaluated on a case-by-case basis and considered only as a supportive measure when more effective treatment options are not available. (8,25,26).

5.4. Hemodialysis in Lithium Toxicity Management:

Hemodialysis is an effective treatment for lithium toxicity due to lithium's small molecular weight, low plasma protein binding, limited volume of distribution (0.8–1.2 L/kg), and slow endogenous clearance (15–20 mL/min). It is particularly indicated in cases of severe neurological toxicity (e.g., altered consciousness, clonus, hyperreflexia, and seizures), life-threatening dysrhythmias, or renal impairment. Hemodialysis is recommended when serum lithium levels exceed 4.0 mEq/L or when adequate hydration cannot be achieved with intravenous fluids. Intermittent high-efficiency hemodialysis (PHD) is the first-line choice as it provides rapid and effective lithium elimination. Sessions

typically last 6–8 hours and are continued until serum lithium levels fall below 1 mEq/L. However, rapid removal of lithium from the plasma increases the risk of redistribution from intracellular to extracellular compartments (the “rebound phenomenon”). For this reason, post-dialysis serum lithium levels should be monitored for 6–12 hours, and additional sessions should be planned if necessary.

Continuous renal replacement therapy (CRRT) can be considered when PHD is not feasible. However, as CRRT provides a lower lithium clearance rate, treatment duration is typically extended to 24 hours. Complications of hemodialysis include issues related to vascular access, such as infection and thrombosis, as well as osmotic imbalances that can lead to altered consciousness. Despite these risks, hemodialysis remains a reliable treatment option for reducing the total body lithium burden and mitigating the risk of neurotoxicity. A patient-specific, individualized approach is essential for optimal management.(18,27–29).

6. DISCHARGE PLANNING:

The management of lithium toxicity requires an individualized approach based on the severity of toxicity, serum lithium levels, and accompanying risk factors. Asymptomatic patients who have ingested immediate-release lithium preparations should be monitored with serial serum lithium measurements for 4–6 hours. Hospitalization is required if serum levels exceed 1.5 mEq/L. Patients who have ingested extended-release preparations should be admitted for close monitoring of toxicity symptoms, regardless of initial serum lithium levels. Patients with mild symptoms can usually be managed with intravenous saline therapy over 6–12 hours. They may be discharged once serum lithium levels fall below 1.5 mEq/L and after a psychiatric evaluation.

Hospital admission is necessary for patients with moderate toxicity. Patients with severe symptoms, such as altered consciousness, seizures, or cardiotoxicity, require intensive care monitoring and extracorporeal therapy (e.g., hemodialysis). Medications that increase the risk of lithium toxicity, such as thiazide diuretics, NSAIDs, and ACE inhibitors, should be discontinued. In cases of extended-release lithium ingestion, serial serum measurements should continue until levels are within the therapeutic range. Pregnant women taking lithium should be referred to an obstetrician for fetal evaluation and a risk-benefit assessment of lithium therapy. Patients without clinical toxicity symptoms, with a normal physical examination and serum lithium levels within the therapeutic range, can be safely discharged after appropriate psychiatric evaluation and follow-up planning (8,30–32).

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CHAPTER 26

Acute Appendicitis

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1.INTRODUCTION

Acute appendicitis is one of the most common general surgical emergencies worldwide, with an estimated lifetime risk of 8.6% in men and 6.9–12% in women. While it is most frequently observed in individuals aged 10–19 years, it can occur across all age groups and is the most common non-obstetric surgical emergency during pregnancy, affecting approximately 1 in 1,500 pregnancies (1–4). In the United States, 250,000–300,000 appendectomies are performed annually, whereas in Europe, this number reaches up to 700,000 (5,6). Approximately 69% of appendicitis cases occur in individuals under 30 years of age, with the highest incidence reported during the summer months. This condition represents a significant burden on modern healthcare systems (1,2,5).

The etiology of appendicitis remains poorly understood, and the lack of reliable biomarkers to predict disease severity complicates its diagnosis and management. Some of patients experience complications such as perforation, often associated with disparities in access to healthcare services. Despite advancements in laboratory tests and imaging techniques, achieving an accurate diagnosis remains challenging. Both missed diagnoses of appendicitis and unnecessary surgeries due to misdiagnosis can result in severe consequences. Therefore, appendicitis should always be considered in patients presenting with acute, non-traumatic abdominal pain (2).

Historically regarded as a vestigial organ, the appendix is now thought to play an immunological role in maintaining gut microbiome homeostasis. Bacteria-phage complexes within the appendix may help reseed the intestinal microbiota in cases where gut flora is disrupted by stressors, dietary changes, or antibiotics, thus contributing to intestinal equilibrium (1,7).

Acute appendicitis stands out due to its epidemiological significance and challenges in clinical management. The development of improved diagnostic tools, reduction of complication rates, and elimination of healthcare disparities are essential steps in optimizing the management of this common surgical emergency.

2. PATHOPHYSİOLOGY

The pathophysiology of acute appendicitis typically begins with obstruction of the appendiceal lumen. This obstruction may result from fecaliths (hardened fecal masses), lymphoid hyperplasia, gallstones, foreign bodies, parasites, or tumors. Obstruction prevents the normal outflow of mucus and bacteria from the appendix. The continued secretion of mucus by the luminal mucosa increases

intraluminal pressure, leading to ischemia, necrosis, and eventually perforation of the appendiceal wall. However, the absence of fecaliths in most cases of appendicitis indicates that obstruction is not always present, and the underlying causes of appendicitis remain incompletely understood (2,8,9).

The increase in intraluminal pressure compromises the vascular and lymphatic circulation of the appendix, threatening the integrity of the wall. The cessation of capillary perfusion leads to localized ischemia and inflammation. Over time, bacterial overgrowth becomes predominant within the lumen, with bacteria invading the wall and inducing neutrophilic exudation. Aerobic bacteria dominate in the early stages, while anaerobic organisms, particularly *Escherichia coli*, *Bacteroides fragilis*, and *Peptostreptococcus*, become more common in later stages. This process can lead to the spread of inflammation to surrounding tissues, culminating in diffuse peritonitis (10,11).

Acute appendicitis typically begins with periumbilical pain, associated with the activation of visceral nerve fibers entering spinal segments T8 to T10 (9). This pain is usually described as vague and poorly localized. As the inflammation progresses to involve the parietal peritoneum, the pain becomes localized to McBurney's point in the right lower quadrant. However, anatomical variations can alter the localization of pain. A retrocecal appendix may present with flank pain, while a pelvic appendix can manifest as pelvic pain, dysuria, or diarrhea. During pregnancy, the displacement of abdominal organs by the enlarging uterus can lead to tenderness in the right upper quadrant. Nevertheless, the right lower quadrant remains the most common site of pain in pregnant patients (4).

The mechanism of luminal obstruction varies by age. In younger individuals, lymphoid hyperplasia is a more common cause, often secondary to infections. In older patients, obstruction is more likely due to fibrosis, fecaliths, or neoplasms. In endemic regions, parasites may play a significant role in the etiology of obstruction across all age groups (2,12).

The pathophysiological process can lead to various outcomes depending on the severity and duration of inflammation. Inflammation following obstruction usually becomes evident within 24 hours of symptom onset, but perforation generally takes more than 48 hours to develop. Perforation can result in localized abscess formation or diffuse peritonitis. In rare cases, appendicitis may resolve spontaneously (2,8,12) .

Finally, appendicitis can occasionally be associated with unique conditions such as "tip appendicitis" or "stump appendicitis." Tip appendicitis refers to inflammation localized to the distal tip of the appendix, which carries a higher

risk of misdiagnosis due to its subtle clinical and imaging findings. Stump appendicitis, on the other hand, involves inflammation of the residual appendiceal tissue following an appendectomy. This rare phenomenon can occur anywhere from 4 days to 50 years after the initial surgery (13).

The progressive nature of acute appendicitis highlights the importance of early diagnosis, as delayed intervention can result in significant complications. Therefore, a thorough understanding of its pathophysiology is essential for the development of effective diagnostic and therapeutic strategies.

3. CLINICAL FEATURES AND HISTORY

Abdominal pain is the most common symptom of acute appendicitis and is reported in nearly all confirmed cases. The classic presentation includes right lower quadrant pain, anorexia, and nausea or vomiting. Patients often describe the initial pain as peri-umbilical and colicky, which intensifies over time and migrates to the right lower quadrant as the inflammation progresses. However, this pain migration is observed in only 50–60% of cases. Nausea and vomiting, when present, typically follow the onset of pain. Fever and systemic symptoms usually appear in the later stages of the disease (9,14,15).

In some patients, the initial symptoms may be atypical or non-specific, including indigestion, bloating, irregular bowel habits, diarrhea, or generalized fatigue. The vague nature of these early symptoms can lead both patients and clinicians to underestimate their importance. The clinical manifestations of appendicitis often depend on the anatomical position of the appendix. For example, a retrocecal appendix may cause dull abdominal pain, while a pelvic appendix may present with lower abdominal tenderness, dysuria, tenesmus, or diarrhea. In pregnancy, the growing uterus may displace the appendix, causing tenderness in the right upper quadrant, although the right lower quadrant remains the most common site of pain (15–17).

The patient’s age and the appendix’s anatomical location significantly influence the clinical presentation. Diagnosis is particularly challenging in infants, young children, and elderly patients. Infants and young children often exhibit non-specific signs such as withdrawal, while elderly patients may present with atypical symptoms, including confusion(16).

The patient’s medical history is an essential part of the diagnostic process, providing clues that may confirm appendicitis or suggest alternative conditions. A prior history of similar symptoms is often associated with other diagnoses, as appendicitis rarely presents as a recurrent or waxing-and-waning condition.

Appendicitis is typically a progressive disease, with symptoms worsening until perforation occurs. Temporary relief may follow perforation due to a decrease in intraluminal pressure, but this is usually followed by generalized peritonitis and more severe abdominal pain (12,14).

Classic symptoms such as pain migration are useful diagnostic clues, but their absence does not rule out appendicitis. The duration of symptoms varies widely and may not reliably predict the severity or risk of complications. A high level of suspicion is essential, particularly in patients with atypical presentations or those in age groups where diagnosis is more challenging (18,19).

4.PHYSICAL EXAMINATION

Physical examination plays a crucial role in the diagnosis of acute appendicitis, but early-stage findings are often subtle due to the lack of involvement of somatic nerve fibers in the visceral organs. The most common physical finding is abdominal tenderness, which is observed in over 95% of patients. This is typically localized to the right lower quadrant, particularly at McBurney's point (16).

4.1. General Findings

Patients may present with flushed skin, a dry tongue, and foul breath odor. Low-grade fever (up to 38°C) and tachycardia are common. A fever exceeding 38.3°C suggests progressive inflammation or the development of peritonitis. Percussion tenderness, involuntary muscle guarding, and rebound tenderness (Blumberg's sign) are among the most reliable clinical findings for acute appendicitis. However, rebound tenderness should not be routinely elicited to avoid unnecessary discomfort to the patient(9,16,18).

4.2. Specific Signs and Findings

These classic signs are helpful for diagnosing appendicitis but are not definitive. The absence of these findings does not exclude the diagnosis, as variations in the anatomical position of the appendix (e.g., retrocecal or pelvic) may result in atypical presentations.

Specific signs and findings helpful for diagnosing appendicitis include McBurney's point tenderness, characterized by maximal tenderness located 1.5–2 inches from the anterior superior iliac spine on a straight line to the umbilicus ; Rovsing's sign, where pain in the right lower quadrant elicited by palpation of the left lower quadrant suggests right-sided localized peritoneal irritation; the psoas sign, where right lower quadrant pain triggered by passive extension of the right hip indicates a retrocecal appendix; and the obturator sign, characterized by

pain in the right lower quadrant during internal rotation of the right hip while the hip and knee are flexed, suggesting a pelvic appendix. While these classic signs can support the diagnosis of appendicitis, they are not definitive, and their absence does not rule out the condition, as anatomical variations in the position of the appendix, such as retrocecal or pelvic locations, may lead to atypical presentations (12,16,19).

4.3. Rectal and Pelvic Examination

Rectal and pelvic examinations have limited diagnostic value for appendicitis and are not routinely recommended. However, they may be helpful in distinguishing appendicitis from pelvic or gynecological pathologies. In women, tenderness in the right adnexal region may be observed, but differentiating between pelvic and appendiceal tenderness can be challenging(20–22).

Physical examination findings are valuable for supporting the diagnosis of appendicitis but are not definitive on their own. A comprehensive evaluation, including imaging and laboratory testing, is essential for confirming the diagnosis.

5. LABORATORY FINDINGS

Although white blood cell (WBC) count is frequently used in the evaluation of acute appendicitis, it lacks sufficient sensitivity, specificity, and predictive value to definitively confirm or exclude the diagnosis. Peripheral WBC elevation is considered one of the earliest markers of inflammation. A study conducted prospectively and retrospectively on 722 children identified acute appendicitis as the most common diagnosis in children over 4 years old presenting with non-traumatic abdominal pain and leukocytosis. However, normal WBC levels are not uncommon, and leukopenic presentations have also been documented in the literatüre (23–25).

CRP, a systemic inflammatory marker synthesized by the liver, is typically elevated in acute appendicitis. CRP levels become more pronounced, especially when symptoms persist beyond 12 hours. However, CRP alone has limited diagnostic value (sensitivity 38–70%, specificity 65–85%). The combined evaluation of WBC and CRP can increase diagnostic sensitivity to as high as 97–100%. When both markers are within normal ranges, the likelihood of acute appendicitis is significantly reduced (26,27).

Urinalysis in acute appendicitis may reveal pyuria (7–25%) or microscopic hematuria, particularly when inflammation extends to the ureter or bladder. However, these findings are not specific and may also result from other

conditions such as renal colic or pyelonephritis. In female patients, pregnancy testing is recommended to exclude ectopic pregnancy or other pregnancy-related conditions. Tests such as electrolyte levels, liver function tests, and lipase may aid in evaluating alternative causes of abdominal pain but are not specific to acute appendicitis (28).

The combined evaluation of WBC, CRP, and neutrophil ratios can enhance diagnostic accuracy. The combination of normal WBC and CRP values yields a negative predictive value (NPV) of up to 88%. Some studies have shown that the diagnostic sensitivity of this combination can range from 97% to 100%. Research on novel biomarkers, such as procalcitonin and bilirubin, is ongoing, and further studies are needed before these markers can be implemented into routine clinical practice (29,30).

While laboratory findings alone are insufficient for diagnosing acute appendicitis, they provide valuable supportive information when combined with clinical and imaging evaluations. As such, laboratory tests remain an integral component of the diagnostic approach to acute appendicitis.

6. IMAGING

6.1. Plain Radiography

Plain radiography has limited value in diagnosing acute appendicitis and is not recommended for routine use due to its poor sensitivity and specificity. However, it may provide supportive findings in certain cases. Radiographic signs suggestive of acute appendicitis include appendiceal fecalith, gas within the appendix, distension or air-fluid levels in the terminal ileum, cecum, or ascending colon (indicative of localized paralytic ileus), loss of the cecal shadow, blurring or obliteration of the right psoas muscle, rightward scoliosis of the lumbar spine, increased density or haziness over the right sacroiliac joint, and free intraperitoneal air or fluid. These findings are not specific and may overlap with other inflammatory conditions. In cases of suspected perforation, an upright chest radiograph may be helpful in identifying free air under the diaphragm, a finding indicative of peritonitis secondary to perforation. Such findings warrant immediate surgical consultation for likely operative intervention (31) .

6.2. Ultrasonography

Ultrasonography is the preferred imaging modality for evaluating acute appendicitis, particularly in children, pregnant women, and young, non-obese adults. Its advantages include the absence of radiation, rapid implementation, non-invasiveness, and cost-effectiveness. However, the diagnostic accuracy of

ultrasonography is highly dependent on the operator's experience and the patient's anatomical characteristics (32–34).

Graded compression ultrasonography is a technique that enhances diagnostic accuracy by applying pressure with the ultrasound probe to reduce bowel gas and facilitate visualization of the appendix. A normal appendix appears as a lamellated, elongated, blind-ending structure on ultrasonography. It is typically compressible and measures less than 6 mm in diameter. In acute appendicitis, the appendix is >6 mm in diameter, has a thickened wall, and is non-compressible. Doppler ultrasonography can detect increased vascularity around the inflamed appendix. In cases of perforation, the appendix may exhibit an irregular contour, and periappendiceal fluid collections may be observed (32,35,36).

Ultrasonography is particularly advantageous in pediatric patients, as it avoids long-term radiation risks and benefits from lower obesity rates in this population. It is also useful in pregnant patients, although the visualization rate of the appendix tends to be lower during pregnancy. In women presenting with pelvic pain, pelvic ultrasonography can help differentiate appendicitis from other gynecologic conditions such as pelvic inflammatory disease (33,34).

The results of ultrasonography are operator-dependent and may vary based on the operator's expertise and the patient's anatomical characteristics. Inexperienced operators may have lower diagnostic accuracy. In cases of non-diagnostic ultrasonography, additional imaging or clinical observation is necessary. Even when ultrasonography strongly suggests appendicitis, the findings should be corroborated with other clinical parameters(18,27,32).

6.3. Computed Tomography (CT)

In adult patients with suspected acute appendicitis, particularly those with an ambiguous clinical presentation, abdominopelvic CT plays a primary diagnostic role. CT is characterized by findings such as a dilated appendix (>6mm), wall thickening, periappendiceal inflammation, and appendicolith/abscess. In cases of perforation, reduced luminal obstruction may complicate CT visualization (12,14).

CT demonstrates high sensitivity (>94%) and positive predictive value (>95%), exhibiting greater sensitivity (%96) compared to ultrasound (US). Preoperative CT is associated with reduced negative appendectomy rates. The American College of Radiology (ACR) recommends US as the first-line modality in children, but acknowledges CT as generally more accurate. Rectal contrast in

appendiceal CT offers an alternative to oral contrast and avoids adverse effects from IV contrast (37–39).

CT is the modality of choice in non-pregnant patients, offering high sensitivity, specificity, and a decreased negative appendectomy rate. It's a rapid and operator-independent examination. Ionizing radiation is the primary drawback. Low-dose CT protocols are effective in mitigating this risk (39,40).

6.4. Magnetic Resonance Imaging (MRI)

MRI is a significant alternative in evaluating acute appendicitis, particularly in pregnant patients and when ultrasound (US) is inconclusive. Its advantages include the absence of ionizing radiation and reduced operator dependence. However, cost, accessibility, and the need for specialized interpretation are limiting factors. MRI exhibits high sensitivity and specificity in pregnant women. While it eliminates radiation risk in younger patients, more data are needed regarding its use in acute abdominal pain. MRI is not superior to US in differentiating perforated appendicitis.

MRI is preferred in pregnant patients and situations where radiation is contraindicated. It can image the entire abdomen in multiple planes. Its use is increasing in pediatric patients, especially those over 5 years old, and when US is insufficient. MRI is often favored over computed tomography (CT) for appendicitis evaluation in the first trimester of pregnancy (12). Gadolinium is contraindicated during pregnancy and in patients with renal insufficiency. MRI's longer scan time makes it unsuitable for hemodynamically unstable patients. However, advancements in technology are enabling faster and non-contrast MRI scans (41). On MRI, a normal appendix is ≤6mm, while a fluid-filled appendix >7mm is considered abnormal; appendices measuring 6-7mm are deemed indeterminate (42).

7. MANAGEMENT STRATEGIES FOR ACUTE APPENDICITIS

In the evaluation of patients with suspected acute appendicitis, supportive care is paramount. Patients should be kept without oral intake, and intravenous (IV) fluid therapy with isotonic solutions such as normal saline or lactated Ringer’s solution should be initiated to ensure adequate hydration. Systemic signs of infection, more common in perforated appendicitis, warrant antipyretics and antibiotics. Although acute uncomplicated appendicitis rarely causes severe sepsis or septic shock, these complications can arise with delayed presentation or diagnosis (16,43)Antibiotic therapy should be initiated upon diagnosis of appendicitis or early in the management of septic patients with suspected

appendicitis. Antibiotics should cover gram-negative aerobes, enteric gram-positive streptococci, and anaerobes. Pseudomonal coverage is not necessary for patients with mild-to-moderate disease and no healthcare-associated risk factors. Surgical consultation remains vital in the management of acute appendicitis. The decision regarding operative management (OM) versus nonoperative management (NOM) is made jointly between the patient and surgeon, but emergency physicians should be knowledgeable about both pathways. Appendectomy is generally considered the initial treatment of choice. Nonoperative management with antibiotics alone is also an option, similar to the management of uncomplicated diverticulitis, salpingitis, and neonatal enterocolitis. A nonoperative approach is occasionally used for older, sicker patients considered high-risk surgical candidates. However, some studies suggest that these patients may have worse outcomes with nonoperative treatment. Randomized clinical trials have yet to provide sufficient evidence to support the routine use of NOM; while antibiotics alone may be feasible in selected, uncomplicated patients, surgical management remains the accepted standard (5,44–47).

Preoperative intra-abdominal or pelvic abscess occurs in 3.8% of appendicitis patients, suspected with a palpable mass. While delayed presentation has traditionally been considered a risk factor for perforation and abscess, some patients are at risk despite prompt treatment. Meta-analyses support conservative treatment with antibiotics and percutaneous drainage if needed. Early surgery is associated with increased morbidity. Malignancy can be found in 1.2% of patients with a conservatively treated abscess. Patients over 40, or with malignancy suspicion, should undergo follow-up with colonoscopy or CT. The rate of occult appendiceal malignancy after successful antibiotic treatment is unknown. Long-term evidence and follow-up is scarce; interval appendectomy may be considered based on age, symptoms, and radiological findings (2,16,43,48).

In summary, management of acute appendicitis requires a multi-faceted approach including supportive care, antibiotics, and potentially surgical intervention. While non-operative management shows promise, surgery remains the standard of care in many cases. Careful consideration of patient characteristics and clinical presentation is critical in determining the most appropriate course of action.

8. DISPOSITION

Management and disposition decisions for patients with suspected acute appendicitis are contingent upon the certainty of diagnosis, patient reliability, and clinical status. When the diagnosis is confirmed, IV antibiotics are initiated, and surgical consultation is obtained, while selected patients may be discharged with oral antibiotics and close follow-up. Reliable patients with a low probability of appendicitis who demonstrate clinical improvement and understand return precautions can also be discharged. In cases of inconclusive imaging or persistent suspicion, patients are either admitted for observation or, if reliable, discharged with close follow-up and return precautions. Although surgery remains the standard treatment for acute appendicitis, stable and reliable patients may be discharged provided they have a scheduled follow-up visit. Pain control should be achieved, oral hydration should be tolerated, and patients must adhere to discharge instructions (2,16,18,43).

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