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A Study of Red Blood Cell Indices among Covid 19 Individuals in Tertiary Care Triage Centre

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Abstract

Background: SARS-CoV-2 infection is characterized by the development and progression of inflammatory responses. The exploration of prognostic predictors for patients with COVID-19 is vital for prompt clinical intervention. Our study aims to explore the predictive value of hematological parameters in categorization of patients with COVID-19.We aimed to investigate associations between hematological parameters and disease severity in patients with SARS-CoV-2infection.The red blood cell distribution width (RDW), an indicator of anisocytosis has emerged as a potential tool for risk stratification of critically ill patients. **Materials and Methods**: Retrospective study after getting approval from Institutional ethics committee was performed with data obtained from triage OPD in Chengalpattu Medical College & Hospital. The demographic, clinical, laboratory profile of COVID 19 positive patients who attended Triage OPD for a period of one month were collected. 3000 RT-PCR confirmed COVID positive patients of age group 20 to 70 years of both genders were included in the study. COVID 19 positive paediatric cases, antenatal and postnatal mothers, postoperative cases and sick cases admitted in COVID ICU were excluded from the study. Symptoms, associated co-morbidities and severity of COVID 19 were tabulated. Complete Blood Count measured by Automated Sysmex Analyser at the time of reporting to triage was noted. Red blood cell indices were analysed using SPSS 21.0 version. **Results:** There was statistically significant increase in RDW and decrease in MCV among patients with co-morbidity and severe illness compared to those with mild disease. There is no statistically significant difference in Red blood cell indices between patients with and without symptoms and patients without co-morbidities. **Conclusion:** RDW was found to be a screening tool to identify patients with severe COVID-19 and the results of this study suggest that RDW should be part of routine laboratory assessment and monitoring of COVID-19.

Key words: *COVID severity, Red cell indices, RDW, Triage OPD.*

Introduction

Corona virus disease 2019 (COVID-19) is asystemic viral infection presenting mostly asacuterespiratory illness with ahighrate of hospitalization and mortality. The emergence and rapid spread of the deadly COVID-19disease caused by severe acute respiratory syndrome coronavirus 2 (SARSCo2) is an evolving public health crisis worldwide.(1)

Covid-19 has significant impact onpulmonary, cardiovascular, renal, gastro intestinal, neurological and on hematopoietic system. The impact of Covid-19 disease on Immune system and hemostasis has been studied. Recent preliminary data following the Covid-19 out-break indicated an association of complete blood count (CBC) parameters(2) and coagulation profile (increased D-Dimer, fibrinogen and (FDP) with disease progression.(3)

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In the current COVID-19 scenario, it would be of utmost importance to explore if the most routine and cost-effective tests like CBC could serve as an aid in determining patient's clinical status. Emerging data raise the possibility that red blood cells (RBCs) might also be directly involved in the pathogenesis of the disease. An early report suggested that aprotein expressed on RBCs(CD147orBasigin) that serves as a receptor for plasmodium falciparum, might be an additional receptor for SARS-CoV-2[4] . These findings led some clinicians to ask whether anemia has impact on COVID-19 patient outcome. Red blood cell indices like MCH,MCV, and MCHC are useful in the morphologic classification of anemias. Anemias are classified, according to the size of the red cell, as being normocytic, macrocytic or microcytic.

Anisocytosis is a biological condition characterized by circulating erythrocytes with pronounced volume heterogeneity (i.e., large, small, or both). This parameter is typically expressed as red blood cell distribution width (RDW) and can hence be calculated as the SD of the mean corpuscular volume (RDW-SD) or more frequently as the coefficient of variation of erythrocyte volumes (RDW-CV) as follows: RDW-CV = SD of erythrocyte volumes/mean corpuscular volume × 100. RDW isused together with the mean corpuscular volume (MCV) in clinical practice to differentiate between causes of anemia.[5,6]Increased RDW can be used as a tool for early diagnosis, as an inflammatory marker, and a mortality indicator in hypertensive, diabetic and other co-morbidpatients due to its close relation to inflammation.[7]

The red cell distribution width (RDW) of patients with severe COVID-19 increases significantly. The role of erythroid cell parameters as risk indicators has not been studied in detail yet. As the investigation in to the novel COVID-19 continues to grow, we aim to report that the Red blood cell indices of the new viral disease will provide useful information to the treating physicians. This study aim sat investigating the significant changes observed in the Red blood cell parameters among COVID-19 patients.

Objectives

1. To compare Red blood cell indices in COVID 19 positive patients with and without symptoms.

2.To compare Red blood cell indices in COVID 19 positive patients with and without co-morbidities.

3. To analyse the association of red cell indices with severity of disease.

Materials and Methods

This study was performed after getting approval from Institutional ethics committee with data obtained from triage OPD in Chengalpattu Medical College& Hospital. The demographic, clinical, laboratory characteristics of RT-PCR confirmed patients who attended Triage OPD for a period of one month was collected and informed consent was obtained from all patients involved in the present study. The venous blood samples were collected on the day of reporting at triage centre for Complete Blood Count (CBC).CBC was analyzed on fully automated 6 part hematology analyzer (Sysmex XN 1000) and the ratios were calculated from hematological parameters. The parameters were statistically analysed using SPSS 21.0 version. The normal distribution measurement was expressed by mean ± standard deviation. The independent 't'test was used to compare the mean softwogroups and we considered $P<0.05$asstatistically significant.

COVID 19 positive patients of age group 20 to 70 years of both genders were included in the study. COVID 19 positive paediatric cases, antenatal and postnatal mothers, postoperative cases and sick cases admitted in COVID ICU were excluded from the study.

Symptoms of COVID 19 such as fever, cough, sore throat, myalgia, diarrhoea, decreased sensation of smell were noted. Co-morbidities such as Type 2 Diabetes Mellitus, Hypertension, Coronary Artery Disease, Bronchial asthma, Tuberculosis, Epilepsy, Chronic Kidney Disease on Dialysis and other medical conditions documented were noted.[8] Severity of the disease was classified into mild, moderate and severe based on the symptoms and lung involvement.

The patients were clinically categorised from the received data as,

1. Patients with symptoms and without symptoms.

2. Patients with co-morbidities & without co-morbidities.

3.Patients based on severity of disease.

In this study, severity was assessed by the presenting symptoms of the patient and lung involvement in the chest X-ray taken at the time of reporting. The number of mild, moderate and severe cases were 2662, 324 and 14 respectively. COVID19 severity was classified as follows;

1.Mild: without symptoms(2330) and very minimal symptoms with no lung involvement(332).

2. Moderate: Two or more symptoms and mild lung involvement(324).

3. Severe: Four or more symptoms with extensive lung involvement(14).

Tab.1; Normal values of Red blood cell indices[9]

Red blood cell Indices	Normal range
Red Blood Cell (RBC)	Male: 4.5- 6 millions/cu.mm Female: 3.9- 5.5 millions/cu.mm
Hemoglobin (Hb)	Male: 13-18 g/dl Female: 12 -16 g/dl
Haematocrit (HCT)	Male: 40- 52% Female: 36- 48%
RDW-SD	Male: 35.1 - 43.9fl Female: 36.4 - 46.3fl
RDW-CV	11.5-14.5%
Mean Corpuscular volume (MCV)	80-100fl
Mean Corpuscular Hemoglobin (MCH)	27-31pg
Mean Corpuscular Hemoglobin Concentration (MCHC)	30-36g/dl

Results

Results were analysed by Descriptive statistics, 't' test and ANOVA.

Table No: 2 - Comparison of Red cell indices in patients with and without symptoms.

Parameters	Without Symptoms n-2330	With Symptoms n-670	p value
RBC	4.52±0.61	4.53±0.58	0.705
HEMOGLOBIN	13.01±4.75	12.93±1.89	0.669
HCT	37.08±4.88	37.08±4.78	1.001
RDW-SD	41.85±3.99	41.86±3.87	0.954
RDW-CV	13.09±1.30	13.12±1.46	0.608
MCV	82.57±6.69	82.21±6.46	0.216
MCH	28.91±3.20	28.68±3.37	0.105
MCHC	34.71±1.99	34.69±1.83	0.815

Table No: 3 - Comparison of Red cell indices in patients with and without Co-morbidities.

Parameters	Without Co-morbidity n-2460	With Co-morbidity n-540	p value
RBC	4.37±0.60	4.52±0.61	0.454
HEMOGLOBIN	13.27±4.64	12.81±2.00	0.069
HCT	36.08±4.83	36.88±5.00	0.255
RDW-SD	41.67±3.17	42.21±3.75	0.002*
RDW-CV	13.12±1.20	13.24±1.42	0.042*
MCV	82.78±6.66	82.08±6.75	0.052*
MCH	28.86±3.21	28.64±3.43	0.072
MCHC	34.77±1.99	34.63±1.84	0.133

Table No: 4 - Relation between Red blood cell indices and severity of disease.

Parameters	Severity			p value
	Mild n-2662	Moderate n-324	Severe n-14	
RBC	4.52±0.61	4.42±0.51	4.31±0.15	0.09
HEMOGLOBIN	12.98±4.49	12.76±1.91	11.35±1.28	0.263
HCT	37.03±4.86	36.78±4.85	34.98±3.58	0.215
RDW-SD	41.82±4.28	42.07±2.90	45.16±2.43	0.007*
RDW-CV	13.12±1.34	13.14±1.57	14.18±0.87	0.01*
MCV	82.50±6.78	82.67±5.81	78.56±4.60	0.008*
MCH	28.88±3.29	28.81±2.94	27.41±2.98	0.22
MCHC	34.71±1.99	34.75±1.71	33.58±1.66	0.09

Discussion

In this cross-sectional study, 3000 patients with COVID-19 reported to triage centre at the tertiary care hospital were included. The mean age of study participants was41.25±14.14. Among them, 64.90% were males and 35.1% were females. This is consistent with studies done by Mukta et al[10] in ESIC MC and hospital, Faridabad, India.

Among them, 2330 were asymptomatic (77.6%) and 670 were symptomatic (22.4%). The Meta analysis study of symptoms in COVID-19 patients showed cough (38.50%), loss of taste/smell (11.79%), fever (10.59%), sore throat (2.53%) as the major symptoms followed by breathlessness(1.94%) and gastro intestinal symptoms(2.23%). Further, 29.55% had two to three symptoms and 2.83% presented with more than four symptoms at the time of reporting. Some are asymptomatic during full course of disease[11].

Table 2 shows there was no statistically significant difference in hematological parameters between symptomatic and asymptomatic patients. The probable reason could be that some study participants would have reached triage OPD at the onset of symptoms while some at the later stage of disease. Further, those patients without symptoms were not followed up and this has been also observed in studies done by R.E.A. Santos et al.

In Table 3; 82% were identified without any co-morbidity and 12% were with co-morbidity. We identified statistical differences in hematological parameters like RDW-SD(0.002), RDW-CV(0.0042), MCV(0.052) associated with co-morbid patients which is consistent with the study of Bilal et al[12].

The abnormalities of RDW and MCV observed in patients with co-morbidities in this study are explained by the inability of the bone marrow to produce functionally effective RBCs. Due to inadequate RBCs, the ability to carry oxygen is affected, which makes gaseous exchange difficult resulting in breathing difficulty in these patients. Along with the lung involvement by Corona virus, these abnormalities of RBCs alsoexplains the symptoms of dyspnea (1.94%) observed in the COVID patients of the present study. On the other hand, the presence of co-morbid conditions of these patients might interfere with RBC production due to existing inflammation.[13]

RDW in inflammation;

An elevated RDW, a marker of anisocytosis has been implicated in a wide spectrum of diseases, particularly in patients with non-specific ARDS and also observed in diseases associated with acute and chronic inflammation. RDW as a marker of pre-existing, pro-inflammatory or chronic inflammatory state can be used as a predictor of COVID 19 disease progression. Inflammation plays a major role in the pathogenesis and severity of COVID 19 disease, culminating in cytokine release syndrome (CRS) or cytokine storm in its most severe form.[14,15] The inflammatory cytokines may induce profound alterations in the behavior of hematopoietic cells, mainly neutrophils, lymphocytes and monocytes and red blood cells. Neutrophil counts were significantly higher and lymphocytes were decreased in covid-19 patients with severe disease. This may be linked to persistent infection and prolonged hypoxia leading to release of more granulocytes as a compensatory mechanism by the bone marrow. These results are consistent with the findings of Chen et al.[16] However, the particular mechanism for altered RDW with SARS-COV-2 is still under evaluation. Sarkar et al[14] studied the relationship between elevated RDW with the severity in COVID-19 patients to predict the mortality of the disease. The presence of a sustained inflammatory state could trigger and sustain the marked anisocytosis observed in COVID-19 patients with severe illness in our study.

Red blood cell morphology in COVID;

Erythrocyte biology is strongly dependent on iron metabolism, and many inflammatory conditions are associated with markedly elevated erythrocyte size variability and impaired erythropoiesis with accelerated release of larger RBCs into the circulation, as well as oxidative erythrocyte injury, which would ultimately impair their flexibility, size, and morphology of red blood cells.[17]It has been reported that before aggravation of infection symptoms, the level of oxidative stress in the body increases significantly, and the release of oxygen free radicals increases. Also, insufficient circulating nutrients in patients may lead to an increase in RBC membrane instability, increasing RDW. All these factors might cause an increase in RDW and a decrease in MCV in severely ill patients.[18]

While a recent study reported that the structural change of lipids and proteins in the membrane of circulating RBCs is due to SARSCoV-2 infection and there are also reports of bone marrow injury secondary to SARS-CoV-2 infection. The development of micro-and macro-thrombi, due to intravascular coagulopathy which is commonly seen in critically ill COVID-19 patients leads to erythrocyte injury resulting in morphological abnormalities and large size heterogeneity within the circulation which implies elevated RDW.[19]

Table 4 shows significant increase in RDW-SD, RDW-CV and decrease in MCVin patients with severity of disease, which is similar to the study done by Wang et al.[18]. Also, Zinellu & Mangoni et al[20] found that the severely ill COVID-19 patients had significantly elevated RDW in comparison to the patients without severity which is also consistent with our study, that the elevated RDW is associated with disease severity.

Conclusion

The present study shows the abnormalities of RDW in severely ill Covid19 patients, which is consistent with the study of Lippi et al[7]. There is increase in RDW and decrease in MCV among COVID 19 patients with disease severity.[18] Clinicians should consider

theseparameterswhenreadingtheCBCofCOVID-19 patients. The results of this study support the use of RDW for assessing the risk of severity in COVID 19 progression.

Limitations of the study: Red blood cell indices at the time of reporting were only taken into account. Follow up of patients and monitoring of red blood cell indices could have given a clear picture regarding their role in COVID 19.

Implications: This study emphasize that evaluation of red blood cell indices can also be used as a screening tool asNLR, platelet and lymphocyte count interpretation in COVID 19.

Ethical Committee Clearance: Taken from Institutional Ethics Committee, Chengalpattu Medical College.

Financial Support: None

Conflict of Interest: None.

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A Study of Lipid Profile in Obese Hypertensive Subjects

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Abstract

Background: Obesity is a significant risk factor for metabolic syndrome in adults. Central fat distribution greatly alters the lipid profile and induces atherogenic dyslipidemia even in normoglycaemic, non-hypertensive subjects.

Aim and Objectives: Hence, the aim of the present study to assess lipid profile changes in non-obese hypertensive subjects. Obesity, hypertension and dyslipidemia are the three highly significant risk factor for the deranged lipid profile. Obesity can be defined as excess accumulation of body fat arising from a sustained or a periodic positive energy balance that when energy intake exceeds energy expenditure[1]. Indicators of overweight are useful in the diagnosis and management of obesity in both children and adults.

Material & Methods: This study was conducted on newly diagnosed cases of essential hypertension attending medical outdoor of M.G.M. Medical College, Kisangani. A complete clinical examination including laboratory investigation was done to exclude any systemic or other diseases which are likely to affect blood lipid levels directly or indirectly.

Results- The association between dyslipidaemia, obesity and hypertension is well establishedand all have been found to be major risk factor for the development of CAD, a leading cause of visits to physician and cause of death .

Conclusion: Our study was envisaged to know the effect of obesity on lipid profile profile only in hypertensive and not in general population, and the study found some definite but paradoxical effects. These are that in obesity on a background of hypertension, the total and LDL cholesterol as also the HDL cholesterol are decreased, but on use other hand, the value of VLDL cholesterol and triglycerides are grossly and significantly increased. These finding have two major Clinical implications in that obese hypertensives will be more prone to metabolic syndrome and type 2 diabetes mellitus, and steps should be taken to prevent them accordingly and also apart from statins one should treat the obese hypertensives with fibrates, fat restriction and physical exercise also.

Key words: *Obesity, Lipid profile,dyslipidaemia, Hypertension***.**

Background

Obesity, hypertension and dyslipidaemia is these are the three highly significant risk factors for overall human health, life expectancy and easily morbidity, particularly in relation to cerebrovascular and cardiovascular disease profiles. These factors are so extremely well known that they don't need my references to note. Obesity can be defined as excess accumulation of body fat arising from a sustained or a periodic positive energy balance that is when energy intake exceeds energy expenditure[1]. The most common method of classifying overweight and obesity is based on Body Mass Index (BMI) . According to the World Health Organization (WHO) classifies individual with BMI 25-29.99 Kg/

m^2 as overweight while individuals with BMI> 30 Kg/ m^2 are termed obese. Obesity can also be measured by knowing the body fat contest using various methods like Waist circumference (WC), Waist-Hip ratio (WHR), Skin and subcutaneous fat thickness in various areas of the body and also by measuring the Bio-electrical impendence of the body which is grossly affected by the body fat. Obesity is strongly related with hypertension dyslipidaemia and metabolic syndrome. In our thesis we are particularly interested in established the relationship of obesity with hypertension and lipid profile status. [1] Hypertension is the most common cardiovascular morbidity seen in the primary care and leads various fatal or severely morbid conditions like myocardial infarction, cerebral haemorrhage, cerebral thrombosis, renal failure, heart failure and death, if not detected early and treated appropriately. As per INC 812) report hypertension is regarded and is treatable in all adults with a B.P.> 140/90 mm Hg and in person with diabetes, CKD or related systemic co-morbidities the same values are maintained to define hypertension. Hypertension is a common disorder affecting 20 % of adult population[2] and 12 % of all deaths are caused by hypertension and its complications. We all know that.

Obesity has been described as an epidemic in many places throughout the world and its prevalence is of great concern. The World Health Organization (WHO) has defined obesity as having a body mass index (BMI) over 30 (body mass/ ht2). BMI does not directly assess how much fat a person has but is an indirect assessment that assumes that a higher body mass is due to an increasing percentage of the body's mass being fat 1191 Body mass index (BMI), which was defined by the World Health Organization (WHO), has been used for many years as a global index for assessing obesity [3].

India is undergoing rapid economic transition. At this stage in the associated epidemiological transition, the country is facing the double burden of communicable and non -communicable diseases. As in all such transitions, nutrition plays a central part. Obesity, representing one extreme of the continuum, is a preventable risk factor for chronic degenerative diseases while chronic energy deficiency (CED), though less directly " preventable," is associated with impaired physical capacity, reduced economic productivity, increased mortality, and poorer reproductive outcomes.

Under all these variable finds in manifold studies, it is assumed that the Lipid profile should be studied in detail in our context, that is, in Bihar (Kishanganj) district, India, in as much as obese hypertensive have already two risk factors, and we should know whether in most of the obese Hypertensive cases, The lipid profile is truly adverse and renders a third dimension in the issue, or in all hypertensive, the lipid profile shows no significant difference irrespective o f whether the hypertensive person is obese or not. This is why we have ventured to proceed on to the present study whereby the lipid profile is evaluated and compared between, obese and non obese hypertensive patients.

Although hypertension and obesity are both closely associated, there is no universal anthropometric marker of this association. This is probably due to distinct population characteristics, and in the case of Brazil, the highly heterogeneous population.[4,5]

The current estimated frequency of hypertension in India is 10-15% in rural and 25-30% in urban population as shown in different epidemiological studies. Due to the stress and tension inherent within the altering life patterns there is a changing trend in the overall prevalence of hypertension.[6,7]

Hypertension is also directly responsible for 57 percent of all strokes deaths and 24 percent of coronary heart disease death in India and also remains to be the leading cause of blindness, renal failure and congestive heart failure. Previously thought as a disease of urban well-off population, and a rural infringement in nowadays increasingly being reported. The probability of escalating cardiovascular diseases in rural India is a public health concern and not much research had been done to know about the burden and risk factors in rural areas.[8,9]

Hypertension is directly responsible for 57% of all stroke deaths and 24% of all coronary heart disease

deaths in India.[10]

The meta-analysis of eight studies carried out in urban areas gives a pooled prevalence rate of 164.18 per thousand and in rural areas was 15744 per thousand. 4 Pooling of epidemiological studies shows that hypertension is present in 25% urban and 100% rural subjects in India.[10]

Almost 30-65% of adult urban Indians are reported to be either overweight (BMI>=25) or obese (BMI>=30) or have central obesity.[10]

Apart from the age groups 25-34 and 65+, the mean BMI was significantly higher for women compared to men across age-groups The standardized prevalence of overweight (25Kg/m^2 ≤BMI <30Kg/m^2) and obesity (BMI ≥30Kg/m^2) was respectively 23.89% and 11.19% for the study population, 23.79% and 7.59% for men, and 28.8% and 21.2% for women. Abdominal obesity was present in 14% of men and 59.5% of women.[11]

Methods

MATERIAL & DETAILS OF EXPERIMENTAL STUDY:

1. Human volunteers:

a. This study was conducted on newly diagnosed cases of essential hypertension attending medical outdoor of M.G.M. Medical College, Kishanganj.

b. A complete clinical examination including laboratory investigation was done to exclude any systemic or other diseases which are likely to affect blood lipid levels directly or indirectly.

2) Group formation:

It contain 25 obese male and female hypertensive subjects with (Systolic B.P- 140-200 mm of Hg ,Diastolic B.P- 90-110 mm of Hg, B.M.I-Less than 25%)

Selection Criteria :

Inclusion Criteria :

1. Only Hypertensive subjects will be included.

2. Age- All subjects will be from 35-60 yrs age group.

3. Sex - Both male and female will be selected for the study.

4. Body weight - Body weight will be in kilograms.

5. Body Height - It will be in cms.

6. Body Mass Index (B.M.I.) - Body weight in kg Body height in m

Exclusion Criteria:

1) Other than hypertensive subject.

2) Any other disease which affect lipid profile

Study Period :- From November 2012 To April 2014.

Blood Collection: Blood samples analysis was done for total cholesterol, triglyceride, HDL-cholesterol,VLDL cholesterol, LDL cholesterol by using Fried Ewald's equation.

Results

SIGNIFICANCE OF DEMOGRAPHIC VARIABLES BY GENDER OF OBESE PATIENT

TABLE NO 1:- STATISTICAL SIGNIFICANCE OF DEMOGRAPHIC VARIABLE BY GENDER OF OBESE PATIENTS

Gender	Female (n=25		Male (n=25)		t	p
D. variable	Mean	SD	Mean	SD		
Age	49.16	8.42	44.36	7.52	1.90	>0.05
Ht	149.00	8.47	170.12	6.11	9.04	<0.001
Wt	64.24	7.80	79.92	8.51	6.06	<0.001
BMI	28.64	1.82	27.50	1.43	2.20	<0.05

Here again the mean age in the two sexes is similar and within significant difference (F=49.168.42years; versesM=44.36±7.52 years; verses M=44.36±7.52 years; p>0.5) the mean height is females and male are149.0±8.47as and 170.12±6.11 and respectively. The males are definitely and significantly taller (p<0.001).The mean weight in the two sexes is F: M=64.24±7.80kg vs 79.92± 8.51kg(p<0.001),proving again, that obese males are significant heavier than obese females. The BMI of the obese group of females is 28.69± 1.82 and that is obese male is 5.27±1.43.This is a bit paradoxical average heavier than female but the mean BMI of female is significant greater than that of male p<0.05.In our study WC in all category of male hypertensive is 96.34 ± 4.78 and which is higher than desired value (<95cm) but the WHR is 1.043 ± 0.096, which is well neither normal nor desired range (0.85 to 1.15). However ,the mean WHR in all categories of female is 1.169±0.125 which is a bit higher than ideally what is should have been.

COMPARISON OF SBP, DBP, WC, HC AND WHR BY GENDER OF HYPERTENSIVE PATIENT WITH OBESITY(TABLE NO 2)

TABLE NO 2:- COMPARISON OF BP, WC, HC & WHR BY GENDER OF OBESE HYPERTENSIVE PATIENTS.

Gender	Female (n=25		Male (n=25)		t	p
Parameter	Mean	SD	Mean	SD		
SBP	163.84	9.69	170.32	9.89	2.09	<0.05
DBP	96.32	3.15	99.60	4.73	2.58	<0.01
WC	106.52	5.72	105.96	4.59	0.28	>0.05
HC	83.20	4.70	93.32	5.09	6.53	<0.001
WHR	1.279	0.079	1.132	0.044	7.27	<0.001

In obese patients with hypertension, the mean SBP in female and male patients, in our study were 163.84± 9.69 mm of Hg and 170.32±9.89 mm of Hg respectively. The DBP were 96.32± 3.15 (F) mm of Hg and 99.60± 4.73 (M) mm of Hg. The sex difference is both the parameters (SBP & DBP) is statistically significant (p<0.05and<0.01 respectively). The WC, HC and WHR in female were106.52± 5.72 cm, 83.2+4.7 cm and 1.279z0.079 respectively. The same parameters in male in our study were 105.96± 4.59 cm, 93.32± 5.09 cm and 1.132±0.044 respectively. In obese parameters, WC was not statistically significant (p>0.05) in two sexes but the HC&WHR were both statistically significant between two sexes (p<0.001 in both). When we studied all these parameters in all hypertensive patients disregarding wither they were obese or not, then in females the mean value were for SBP 162.32±8.78 mm *of* Hg , DBP95.72± 3.02mm of Hg, WC 93.14z14.49 cm, HC 79.26z6.15 cm and WHR1.169± 0.125. The same parameters in male shows:- SBP 167.20± 10.64cm,DBP98.60± 5.60mmofHg,WC96.34± 11.78cm, HC 92.72± 4.99 and WHR 1.043 ± 0.096 respectively. All these parameters shows statistically significant differences (<0.05,<0.05,<0.001 and <0.001) respectively.

When hypertensive patients are compared to normal, one of major difference is an increased prevalence of obesity[12,13].Again conversely , weight again appears

to be a main determinant of the raise in BP that is commonly seen in aging.[14] It was estimated that excess body weight accounted for 26% of cases of hypertension is men and 28% in women[15]. Again in one study it was found that there is statistically significant relation between hypertensive and CRP[16]. While in another study it was found that CRP was strongly associated with BMI[17].In short obesity might cause an increase in CRP which then cause an increase in hypertension or the vice versecan help also. Whichever might be time , it is supported by our study also, that both systolic and diastolic blood pressure are slight significantly raised in obese person (both male and female) as compared to non obese individuals. The females have however a greater propensity of this size.

LIPIDPROFILE OF OBESE HYPERTENSIVE INDIVIDUALS :-(TABLE NO -3)

TABLE NO 3:- COMPARISON OF LIPID PROFILE BY GENDER OF OBESE HYPERTENSIVE PATIENTS.

Gender	Female (n=25)		Male (n=25)		t	P
D. variable	Mean	SD	Mean	SD		
CHOL T	239.36	14.92	235.96	26.73	0.49	>0.05
HDL	35.00	7.36	28.60	3.77	3.46	<0.001
LDL	152.93	21.86	154.00	27.91	0.13	>0.05
VLDL	51.03	6.73	54.05	6.81	1.41	>0.05
TG	255.20	33.59	271.24	34.07	1.50	>0.05

In case of comparison of lipid profile by gender of obese hypertensive patients, the mean total cholesterol, HDL, LDL, VLDL and triglycerides in female are 239.36±14.92 mg/dl, 35.00±7.36 mg/dl, 152.93±21.86 mg/dl, 51.03±6.73 mg/dl and 255.20±33.5 mg/dl, respectively. The same value in case of male obese hypertensive in our study are 235.96±26.73 mg/dl,28.60±3.77 mg/dl,154.00±27.91 mg/dl,54.05±6.81 mg/dl and 171.24±34.07, respectively. The sexual differences in all these value are statically significant ($p<0.05,<0.001<0.05<0.05$, and <0.05 respectively). However the value are higher in case of male in regard to total cholesterol, LDL-Cholesterol, VLDL-cholesterol and triglycerides but lower only in case of HDL-cholesterol than females. While comparing the lipid profile of hypertensive patients between two gender, irrespective of whether they were found, it was found that the mean values of total cholesterol, HDL, LDL, VLDL and triglyceride in a case of female are 239.34±14.03 mg/dl,35.46±6.87mg/dl,161.26±20.94 mg/dl, 42.22±10.23 mg/dl and 211.10±51.15 mg/dl respectively. The same value in order in case of male hypertensive (both obese and non obese together) are: 249.08±24.84 mg/dl, 29.82±3.65 mg/dl, 175.90±30.57 mg/dl, 43.30±12.15 mg/dl and 219.02±61.11 mg/dl, respectively.

These value are statistically significant (p value being $<0.05<0.001<0.01$) in case of total cholesterol, HDL-cholesterol and LDL-cholesterol, male value being

greater in case of total and LDL-cholesterol but lesser in case of HDL-cholesterol, than the corresponding female values. However, the value of VLDL-cholesterol and Triglycerides are though greater in male than the corresponding female values, yet this difference are not statistically significant (the p value being >0.05).

A study conducted by Castelli WP and Anderson KA[18] in 1986 had supported that blood pressure and serum cholesterol are correlated with "r" factor of 0.12 suggesting that those with higher blood pressure values tend to have higher serum cholesterol in their famous Framingham heart study

Kaare & Bonna[19] in 1991 supported the view that in both sexes non - HDL cholesterol levels increased significantly with increasing systolic or diastolic blood pressure and total cholesterol increase with age in women but decreased with age in men. Smoking, physical activity and alcohol consumption had little influence on the association between blood pressure and lipid profile. However, no mention was made regarding the effect of adiposity on lipid profile in hypertensive's.

Chen Y.D I, Wayne and Arthur[20] in 1991 in their study found that mildly hypertensive patients appear to have faster catabolic rate of APO - AI / HDL and hence lower HDL - C values.

Rost and Devis (1996) in the systolic hypertension in the elder program (S HEP study)[21] found that total plasma cholesterol, LDL - C and ratios of LDL - C / DHL - C as well as TC - HDL - C were significantly high in men and women with hypertension and (AI).

In a study from Calcutta[22] it is found that total cholesterol in other hypertensive was 231.20 mg / dl (compared to ours 237.66 mg / dl) and LDL - C (our values 163.46 mg / dl) and HDL - C= 30.90 mg / dl (compared to our value = 31.88 mg / dl). These values very similar to our compared to the values obtained in Mangalore which may be due to lower geographical proximity racial, cultural and dietary resemblances between Bengal and Bihar compared to there of Mangalore (which is in extremes South India).

In short, our studies have shown that despite the well established fact that lipid profile shows a deterioration both hypertension and obesity (deterioration means ↑TC, LDL - C, VLDL - C & TGL and HDL - C), but when both obesity and hypertension are concomitantly present, then the lipid profile assumes a typical pattern (i.e. ↑ Triglycerides) compared to non-obese hypertensive individuals. Our net - reaches have not revealed the existence of any study so far matching the goals and methodology envisaged as in ours, that is no further deterioration due to obesity in hypertensive in ours, that is no further deterioration due to obesity in hypertensive in the values of TC & LDL - rather than a slight betterment of those, and vis- a-vis a significant and notable deterioration (i.e. Increase) in the values of VLDL - C and triglycerides.

The cause behind these peculiar changes are matters of further and broader research and can only be hypothesized in the present stage, viz

(a) The presentation of increased free fatty acids to liver as a function of obesity in primarily responsible for over production of VLDL as well as triglycerides.[23]

(b) Increased fat levels in obesity increased insulin level which is turn increases VLDL and triglycerides.[24,25]

(c) LDL in obesity mean decrease HDL - C and increase VLDL- C and Triglycerides[26]

(d) Obesity increased inflammatory marker (eg hscp) which increase VLDL - C and reduce HDL- C

The findings of our study have also important clinical implications. The association between dyslipidmia, obesity and hypertension is well established[27,28], and all have been found to be major risk factor for the development of eVD, a leading cause of visits to physician[29] and cause of death[30]. The peculiar lipid profile pattern in obese hypertensive compared to non - obese hypertensive will definitely change our way of looking at these complications will cause a paradigm shift in the prevention, treatment and deeper understanding of these morbid states.

Summary & Conclusion

Our study was envisaged to know the effect of obesity on lipid profile profile only in hypertensives and not in general population, and the study found some definite but paradoxical effects. These are that in obesity on a background of hypertension, the total and LDL cholesterol as also the HDL cholesterol are decreased, but on use other hand, the value of VLDL cholesterol and triglycerides are grossly and Significantly increased. These finding have two major Clinical implications in that obese hypertensives will be more prone to metabolic syndrome and type 2 diabetes mellitus, and steps should be taken to prevent them accordingly and also apart from statins one should treat the obese hypertensives with fibrates, fat restriction and physical exercise also. Our studies have been done in Kishanganj, Bihar which comprises a semi urban and rural population. So, a more elaborate and multicentric study covering all categories of population is required to be done to establish the findings more definitely and conclusively.

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A Study on Neutrophil Lymphocyte Ratio in Covid 19 Patients in Tertiary Care Centre

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Abstract

Introduction: COVID-19 pandemic is spread by droplet infectionwith symptoms of fever, cough, diarrhea,dyspnea, fatigue, myalgia, headache, anosmia and ageusia. Morbidity and mortality is higher among elderly and in patients with comorbidities especially Diabetes Mellitus(DM). Biomarkers of inflammation are potential predictors for prognosis in covid patients.

Aim: To analyze the association of NLR in covid 19 patients with symptoms and diabetic status Objective:
1. To studyNLR in covid 19 patients with and without symptoms

2. To study the association of NLR between diabetic and non-diabetic covid 19 patients.

3. To study the association between symptomatology and NLR in diabeticcovid 19 patients.

Methodology: 3000 RT-PCR confirmed Covid 19 patients who attended triage op were included afterEthics committee approval.Patients were grouped as patients with and without symptoms and patients with and without DM.The diabetic Covid patients were also analyzed based on the symptoms. Blood samples onreporting were used for analysis of NLR andparameters analyzed using SPSS23.0 version.

Result: There is no statistically significant difference in NLR among Covid symptomatic and asymptomatic patients. But NLR is markedly raised in covid symptomatic patients with diabetes mellitus.

***Key Words**: COVID 19, Diabetes Mellitus, Neutrophil Lymphocyte Ratio*

Introduction

The new coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is a highly communicable infectious disease.WHOdeclared it as a pandemic in March 2020 and current evidences suggestthat SARS-CoV-2 spreads among people via direct contactroutes and by droplet, airborne and, fomite transmission.[1,2]. The common clinical features of COVID-19 include fever, dry cough, dyspnea, fatigue, myalgia, headache, anosmia, ageusia and diarrhea[3,4]. Patients with severe disease, may develop life threatening complications like, acute respiratory distress syndrome (ARDS), metabolic acidosis, coagulopathy, and septic shock[5]. The pathophysiology of complications in Covid 19 patients is under study and new theories are still evolving.One of the proposed mechanisms of complications in Covid infection is Cytokine storm. Cytokine storm syndrome can be triggered by direct target cell lysis with release of cytokines like interferon

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gamma (IFN-γ) or tumour necrosis factor alpha (TNF-α) or by activation of T cells with subsequent cytokine release[6]. These cytokines cause activation of innate immune cells like macrophages and endothelial cells with further cytokine release[7]. Inflammation, plays a key role in progression of COVID-19 pneumonitis and vasculitis. InCOVID-19 patients leukocyte count in the peripheral blood is normal ordecreased with reduced lymphocyte count in the earlystage.Neutrophils are the major constituent of leukocytes and are responsible for first line of defense against pathogens, whereas lymphocytes are regulatory and protective component of immune system[8,9]. However,Systemicinflammation destroys CD4+ T lymphocytes and increases suppressor CD8+ T lymphocytes, thereby resulting in an increased neutrophil-to-lymphocyte ratio (NLR), NLR has been universally accepted as an inflammatory marker[10,11]. In a study conducted byYang et al., NLR was found to be an independent prognostic factor in patients with COVID-19. This reinforces the belief in the relationship between hyper-inflammation and SARS-CoV-2.

Studies conducted by Zhuo F et al, have suggested that, Covid infection and its severity is higher amongst individuals with comorbidities, especially those with diabetes mellitus[12].Diabetics reportedfor12%–22% of patients with COVID-19, higher thanthe incidence in non-diabetic people 9.3%[13]. Diabetes, itself being a chronic inflammatory disease, presents with significantly higher incidence of mortality and multiple organ damages.[14][15].

Identifying early warning signs of covid 19 infection and providing treatment at the right time, helps in reducing the mortality and shorten the hospital stay with better cure rate. Various blood parameters showing immune status of human, have been identified as potential markers in assessing the severity of the disease. But these tests are mostly expensive and time consuming, which challenges the process of early identification and initiation of treatment. In such a case, Complete Blood Count and arriving at NLR is a specific and sensitive indicator of immune status, which is inexpensive with less turnaround time[16]. Hence this study was undertaken to find the association of NLR among symptomatic and diabetic patients in patients attending triage op where patients are segregated for different treatment modalities.

AIM: To analyze the association of NLR in covid 19 patients with symptoms and diabetic status

Objective:

1. To study neutrophil lymphocyte ratio in covid 19 patients with and without symptoms

2. To study association of NLR between diabetic and non-diabetic patients of covid 19.

3. To study the association between symptomatology and neutrophil lymphocyte ratio in diabetic patients of covid 19.

Methodology: The demographic, clinical and laboratory characteristics of RT-PCR confirmed, 3000Covid -19 patients who attended Triage OPD at Chengalpattu Medical college & Hospitalfor a period of one monthwas collectedafter approval from Institutional ethics committee.

Inclusion Criteria: COVID 19 patients of age group 18 to 70 years of both genders

Exclusion criteria: COVID 19 positive pediatric cases, antenatal and postnatal mothers,post-operative cases and sick cases admitted in COVID ICU

Symptoms of COVID 19 such as fever, cough, sore throat, myalgia, diarrhea, anosmia were noted. Comorbidities such as Type 2 Diabetes Mellitus, Hypertension, Coronary Artery Disease, Bronchial asthma, Tuberculosis, Epilepsy, Chronic Kidney Disease on Dialysis and other medical conditions documented were noted. NLR on reporting to triage was noted from Complete Blood Count measured by Automated Sysmex Analyzer.

The patients were clinically categorized from the received data as

1. Patients with symptoms

2. Patients without symptoms

3. Patients with Diabetes Mellitus

- Further subdivided as, symptomatic and asymptomatic

.The parameters were analyzed using SPSS 21.0 version.

Results

Results were analyzed by Descriptive statistics, 't' test, p value <0.05 was considered significant.

Table No: 1- Comparison of NLR in Covid patients with and without symptoms

S.no	Covid patients Total(n=3000)	NLR Mean ± SD	p value
1.	Covid patients with symptoms (n=620)	2.309 ± 1.68	0.09
2.	Covid patients without symptoms (n=2380)	2.06 ± 1.56	

Table No: 2- Comparison of NLR in Covid patients with and without Diabetes mellitus

S.no	Covid patients Total(n=3000)	NLR Mean ± SD	p value
1.	Covid with Diabetes mellitus (n=350)	4.45 ± 2.3	0.00004
2.	Covid without Diabetes mellitus (n=2650)	2.02 ± 1.2	

Table No: 3- Comparison of NLR among Diabetic Covid patients with and without symptoms

S.no	Covid patients with Diabetes mellitus Total(n=350)	NLR Mean ± SD	p value
1.	Diabetic patients with symptoms (n=132)	4.93 ± 2.56	0.00012
2.	Diabetic patients without symptoms (n=218)	3.19 ± 1.049	

Discussion

In our study, out of 3000 Covid patients, 620 were symptomatic. On analysing the NLR values among symptomatic and asymptomatic patients, table-1 shows, there was a mild increase in NLR among symptomatic patients (2.309 ± 1.68), compared to asymptomatic patients (2.06 ± 1.56), which was not statistically significant. However, the NLR in symptomatic patient was in higher limit of normal range.This is consistent with study conducted by Jumana Mansur Aljishi et al in Saudi arabia on symptomatic and asymptomatic covid patients.[17]According to Al-Tawfiq et al, and Graselli G et alit was foundthat elevated NLR was significantly associated with illnessseverity, taking into consideration the presence of comorbidities.[18][19]

Among 3000 Covid patients, 350 had Diabetes mellitus. On analyzing their NLR, from table-2, we found that it is significantly higher with a mean value of 4.45 ± 2.3, than that of the covid patients without diabetes mellitus, 2.02 ± 1.2. This is consistent with study done by Anurag et al, in Jharkand.[1]. Richardson et al, stated that the most common comorbidity associated with Covid -19 is Diabetes mellitus.[20]. Studies conducted by Samaras K et al, Marioni et al, showed that Diabetes mellitus is associated with low grade chronic systemic inflammation, presenting with increased inflammatory mediators.[21–23]Various studies conducted by Shiny A et al, Sefil F et al and Luo M et al, have reported that increased NLR in diabetics is linked with poor glycemic control, insulin resistance and cardiovascular events. [24,25][26,27]

Table-3 shows, out of 350 diabetic Covid patients, 132 patients were symptomatic and 218 were asymptomatic. On analyzing NLR among them, it was elevated in symptomatic diabetes mellitus patients (4.93 ± 2.56), compared to asymptomatic diabetic patients (3.19 ± 1.049), which was statistically significant. Guozhen li et al, conducted a retrospective study on clinical characteristics of diabetic patients with covid 19, showing that the patients with DM who landed up in severe category of Covid infection with moderate to severe symptoms, suffered from more complications, showing prominent lymphopenia with increased NLR, when compared with those diabetic covid patients without symptoms.[26,28].

This increased NLR in diabetic Covid patients, has been documented in various studies. But our study shows that, the NLR is markedly increased in diabetic patients with symptoms, compared to asymptomatic diabetic patients, which is also statistically significant. This proves the fact that NLR, can be used as a screening tool, for early identification of high risk cases amongst Covid patients with diabetes.

Significance of NLR in Covid:In covid, viral antigens cause activation of cell mediated immunity and humoral immunity. There occurs a marked interaction withmolecules like vascular endothelial growth factor (VEGF), interleukin-6, interleukin-8, tumor necrosis factor-alpha, interferon-gamma, and granulocyte colony-stimulating factor, resulting in profound inflammatory reactions and altered NLR.According to Ji P et al and Yang X et al, VEGF-A and VEGF-Care notorious contributors to inflammation in SARSCoV2[29][30].In another study conducted by Ciccullo et al, an NLR of greater than four was seen as a predictor of theadmission of COVID-19 patients to the intensive care unit.[31]

The potential mechanisms for increased NLR in diabetic Covid patients, is over-activated inflammation and imbalanced immune response in DM.

Diabetes is a condition related with proinflammatory stage, patients suffer from dysregulated immune response and were found to have altered cytokine profile, signifying the severity of Covid in them as reported in a study conducted by Yang J et al[32,33]A recent clinical study conducted byGuo et al,reported thatcompared to COVID-19 patients without diabetes, peoplewith diabetes had activated inflammatory response[34]. Hyperglycemia wasan independent predictor of a high neutrophil count. In a study conducted by Lim S et al, it showed there were moderatepositive correlations between Fasting Blood Glucose levels and NLRin the critical group of Covid patients with DM.[35,36]

Mehta N et al showed that, use of thiazolidinediones in diabetics, increased the expression of ACE2 receptors, to which corona virus binds.[37] This is reported in a study conducted by Gaoli Liu et al , on role of NLR in prognosis of type 2 diabetics with covid 19,[2]

Conclusion

In our study there is no statistically significant difference in NLR among symptomatic and asymptomatic Covid patients. NLR is increased in diabetic covid patients when compared with Non-diabetic and is statistically significant.The increase in NLR is more in symptomatic Covid patients with DM than in asymptomatic diabetic patients showing the marked increase in inflammation and chances for complication.NLR is a simple tool, which can be estimated at ease, in a triage centre, to assess the immune status and inflammatory response. It also helps in early identification of sick cases, which is ideally done at a triage centre, to categorize the patients, especially in patients with Diabetes mellitus and to refer them promptly, without delay, for initiation of treatment.

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Genetican Alysis of the Role of Connexin 37 Polymorphism in Spontaneous Abortion

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Abstract

Introduction: Among unique cardiovascular risk factors in women are complications during pregnancy, including miscarriage. Important risk factor is also genetic background. One of powerful candidate genes for cardiovascular disease of atherosclerotic origin (aCVD) is gene for connexin 37 (Cx37) with strong gene-environment interaction including smoking status, that is also strong risk factor for complications in pregnancy including spontaneous abortion (SA). **Material and Methods:** We analysed association between SA and Cx37 gene polymorphism (1019C>T; Pro319Ser) in 547 foetuses and its potential interaction with smoking status of mothers. Using genetic analyses from women from general population as controls, ORs for T allele, found in our previous studies to be protective against a CVD, were calculated. This study was a secondary analysis of a randomized controlled trial. **Results and Conclusion:** T allele carriers (foetuses), had OR 0.91 (95 % CI 0.72-1.14) and no interaction with smoking was observed. In conclusion, no significant association between Cx37 polymorphism and SA was observed and no modifying effect of smoking status on this association was detected.

Keywords: *Connexin 37 gene, Gap junctions, Spontaneous abortion, Protective factor, Smoking, Cardiovascular disease, Candidate gene. aCVD: Cardiovascular disease of atherosclerotic origin, CI: Confidence interval, Cx37: Connexin 37, DNA: Deoxyribonucleic acid, OR: Odds ratio, SA: Spontaneous abortion, VEGF: Vascular endothelial growth factor*

Introduction

Regarding cardiovascular disease of atherosclerotic origin (aCVD) the annual incidence of cardiovascular diseases is age- dependently increasing both in men and women. The prevalence is higher in men until midlife[1]. However, women are affected similarly or more than men in older age. Despite the main cardiovascular factors are shared by both sexes, several unique cardiovascular factors were described in women as is miscarriage and other complications during pregnancy and metabolic disorders as gestational diabetes mellitus[2]. Therefore, detection of determinants of atherosclerotic disease in women including metabolic ones may help to identify and to prevent atherosclerotic process and its clinical complications. However, also better understanding of the complications in pregnancy might have similar preventative effect especially when accompanied by genetic analyses. Indeed, genetic studies could help to identify not only inherited causes of early pregnancy loss, extremely serious and emotional problem both for affected women, their partners and their physicians, but also for aCVD. One of powerful candidate genes for aCVD is gene for connexin 37 (C1019>T) (Cx37) with

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strong gene- environment interaction including smoking status. Connexins are the main proteins in gap junctions. Connexin 37 (Gap junction protein alpha; OMIM ID 121012; Cx37) is the main gap junction representative in the vessel wall

expressed in endothelial cells, smooth muscle cells but also in monocytes, therefore in the main cells involved in the process of atherosclerosis. Gene polymorphism-association studies have detected a link between the C1019>T single nucleotide polymorphism (SNP) in the human Cx37 gene and its protective role in cardiovascular disease[3]. This polymorphism results in a non-conservative amino acid change in the regulatory C-terminus of the Cx37 protein (P319>S). It was recently demonstrated that Cx37 hemichannels control the initiation of atherosclerotic plaque development by regulating ATP-dependent monocyte adhesion.

In addition to cells responsible for atherosclerosis and aCVD, Cx37 is also present in ovary, in which gap junctions mediate metabolic cooperation between granulosa cells and oocyte. These interactions are mediated not only by paracrine factors but also by gap junctions including Cx37. Preimplantation embryos express multiple connexins and assemble them into gap junctions.

The suppression of connexin expression seems to play an important role in embryo implantation in several species. The gap junction induction is one of the earliest known signs for a blastocyst-derived signal, which may be involved in preparing the endometrium for implantation[4]. It could help to regulate the invasion process by a coordinated cell death of the endometrial cells replaced by the invading trophoblast. However, despite the importance of the different connexin channels expressed in the endometrium this topic was not evaluated[5].

Based on these facts, we analyzed data from fetuses from women with spontaneous abortion (SA) for Cx37 gene polymorphism (1019C>T; Pro319Ser) including its potential interaction

Material and Methods

This study was a secondary analysis of a randomized controlled trial. Based on these facts, we analyzed data from fetuses from women with spontaneous abortion (SA) for Cx37 gene polymorphism (1019C>T; Pro319Ser) including its potential interaction with smoking, frequent risk factor for early pregnancy loss. In particular, we analysed if T allele of Cx37 gene, found to be protective against ischemic heart disease in our previous studies, could be protective also against SA and if this protection could be modified by smoking status[6].

Women with verified diagnosis of SA participated in the study after giving written informed consent. The study was approved by the Institutional ethics committees and was conducted according to the Good Clinical Practice guidelines. Participating women filled in questionnaire focused on history of SA and physical and ultrasound examination were performed[7].

Inclusion Criteria: The healthy pregnant women who were willing to participate in the study were included after obtaining informed consent.

Exclusion Criteria: Severe blood pressure, heart disease, pregnancy- induced hypertension patients were excluded

Study Design: randomized controlled trial

Statistical Analysis

In women with spontaneous abortion 5.6 % were smokers/past smokers (mean age 27.2±6.5 years) and 94.4 % were non-smokers (mean age 32.3±4.6 years) (difference for age, $p<0.001$). In the population sample 44.8 % women were smokers/past smokers (mean age 57±2.7 years)and 55.2 % non-smokers (mean age 57±2.7 years)[8].

Cx37 T allele was present in 55.2 % (mean age 32.1±4.7 years) of aborted foetuses, and in 57.5 % (mean age 57±2.7 years) of women from general population. In smoking women at least one T allele was present in general population[9]. In non- smoking women at least one T allele was present in 55.1 % of the foetuses, and in53.4

% of the foetuses, and in 56.1 % of women from 59.0 % of women from general population. We compared distribution of Cx37 gene polymorphism between foetuses from women older than 32 years with women younger than 32 years (median age). No difference in the prevalence of T allele was detected (56.9 % vs. 54.2 %, p=0.294). We also tested difference in prevalence of T allele between foetuses of women with one SA (n=448) and foetuses of women with more than 1 SA (n=115), but also in this case no significant difference was detected (54.3 % vs.

60.0 %, p=0.161)[10]. The only difference found was that women with more than one SA were older than women with one SA (34.1±4.7 vs. 31.6±4.8 years, p<0.001)[11].

Results

Using general population as a control sample, ORs for T allele carriers were calculated for the whole populations under study and for smoking and non-smoking women including similar stratification to smokers/past smokers and non-smokers as in the control group[12]. Foetuses from women suffering from SA with T allele have OR 0.91 (95 % CI 0.72-1.14) and there was no interaction with smoking status of mothers, OR 0.84 (0.64-1.11) for non-smokers vs. OR 0.89 (0.42-1.9) for smokers (Table 1)[13].

Table 1. Cx37 polymorphism in foetuses and population control. Smoking status in foetusescorrespond with this characteristics in mothers.

Entiregroups						
Cx37		**Controls**		**Abortions**	**OR**	**P**
	N	%	N	%		
CC	281	42.5	239	44.8	1.00	
CT	300	45.3	239	44.8	0.93(0.73-1.19)	0.28
TT	81	12.2	55	10.4	0.80(0.54-1.18)	0.25
+T	381	57.5	294	55.2	0.91(0.72-1.14)	0.41
Smokers/past smokers						
Cx37		**Controls**		**Abortions**	**OR**	**P**
N		%	N	%		
CC	126	43.9	14	46.7	1.00	
CT	121	42.2	14	46.7	1.04(0.48-2.28)	0.92
TT	40	13.9	2	6.7	0.45(0.10-2.07)	0.29
+T	161	56.1	16	53.4	0.89(0.42-1.90)	0.77
Neversmokers						
Cx37		**Controls**		**Abortions**	**OR**	**P**
N		%	N	%		
CC	144	40.7	232	44.9	1.00	
CT	172	48.6	231	44.7	0.83(0.63-1.11)	0.21
TT	38	10.4	54	10.4	0.88(0.55-1.40)	0.28
+T	210	59.0	285	55.1	0.84(0.64-1.11)	0.22

In summary, in our study we found no significant differences in Cx37 gene variability between fetuses analysed after SA and general population of middle aged women[14]. These results were not modified by smoking status. Therefore, in contrast to our previous findings in women with acute coronary syndrome Tallele does not seem to be neither protective against SA, nor its effect is strongly modified by smoking status. Therefore, mechanisms leading to cardiovascular disease and complications during pregnancy including SA seem to be different regarding mechanism mediated by gap junctions involved in differentiation processes by mediating exchanges between mother and foetus cells, affecting the maternofetal blood flow interrelationships, trophoblast invasiveness and the formation of a syncytiotrophoblast[15]. Expression of some connexins, among them also Cx37, has been shown to reflect maturity of luteinized follicles in animal models and Cx43 expression was associated with better prognosis in in vitro fertilization[16]. Transcription factors of several connexins were detected in human embryos, e.g. Cx43 was indicated in all embryonic developing stages [17]. The expression of Cx43, together with VEGF, is significantly reduced in chorionic villi and decidua in women with spontaneous abortion. This may be caused by the influence on angiogenesis of placenta and developing embryo[18]. It was reported, that mouse embryos cultured with some gap junctions' inhibitors presented frequent collapses and developmental delay. Functional and structural abnormalities of Cx43 might also play an important role in heart diseases. Because of this finding and possible similarity with Cx43, we focused on Cx37 polymorphism on spontaneous abortion. Inaddition, Cx37 polymorphism is also supposed to play important role in cardiovascular events. Nevertheless, we did not found an association in the case of Cx37 gene as for other studies describing different connexins polymorphisms and spontaneous abortion [19].

Regarding the (absence of) effect of smoking in our study, the idea was that adhesion properties of vessels wall caused by Cx37 gene polymorphism could favour not only macrophage accumulation in the atherosclerotic lesions but also endothelial dysfunction induced by smoking initiated by reduced nitric oxide bioavailability and further by the increased expression of adhesion molecules Therefore protective effect of the Cx37 T allele might be strongly modified by smoking in atherosclerosis. But according to our data the deleterious effect of smoking on pregnancy, particularly on SA seems not to be mediated through Cx37 gene[20].

The limitations of our study are incomplete data in women with history of SA and focus only on one polymorphism of one gene from rather large connexin family. On the other hand, the strengths of the study include high number of studied women and numerous representative control group from the population sample in Prague, which could reflect real population background for our findings in contrast to cases and controls usually used for such comparisons. In addition, selecting only Cx37 gene was based on knowledge, that connexin 37 is on one hand the main protein of gap junctions in cells involved in the process of atherosclerosis, but on the other hand it is also present in maternofetal organs[21]. Moreover, data indicated that the strongest protective effect of the Cx37 T allele was detected in the non- smoking patients without diabetes mellitus and hypertension and that effect could be mediated through stem cells[22]. Therefore, we have chosen Cx37 polymorphism as genepotentially covering both cardiovascular and pregnancy complications[23]. Another limitation is the lack of data from mothers/ fathers and absence of information regarding other potential modifying factors including diabetes mellitus; however, women with these characteristics were excluded to detect only the effect of this particular gene variability and smoking population, women (after exclusion of diabetics) with the history of one or more SA (n=51) were compared to women without history of SA with history of pregnancy (n=233) of similar age, no difference in the presence of T allele was observed (66.7 % vs. 60.1 %; p=0.67[24].

Conclusion

In summary, to the best of our knowledge, only a

few studies have demonstrated effect of connexins on spontaneous abortion and no studies analysed parallel effect of particular connexin gene polymorphism on cardiovascular disease and SA[25]. In our study, no effect of Cx37 polymorphism measured in foetuses was observed on abortion and no interaction with smoking status on this association was proved.

Conflict of Interest: Nil

Source of Funding: Self

Ethical Clearance: It was given by the institution.

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Depression, Anxiety and Stress Scores Inmale and Female Students

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Abstract

Psychological parameters play a key role in the daily life of young adults.It was reported that there was a high incidence of suicidal rate in these age groups when compared with others. The present study was undertaken to observe the selected psychological parameters that are depression, anxiety, and stress in young adults. A total of 50 (males =25, females =25) young adults studying undergraduate allied health courses both males and females were part of the study after obtaining the written informed consent. The following criterion was used to recruit the participants. Depression, anxiety, and stress levels were assessed using the DASS-42 questionnaire. Depression scores were significantly higher in females when compared with males. Anxiety and stress scores were not significantly different among males and females. Females were in severe depression where males were moderate. Anxiety scores of males and females were mild. Stress scores of males and females were severe. The study results support earlier studies and emphasize the need to assess psychological parameters in young adults. The study also recommends detailed studies in this area.

Keywords*: Depression, anxiety, stress, young adults*

Introduction

Psychological parameters play a key role in the daily life of young adults. The young adults in the age group of 18-24 were reported to be more sensitive as they are in the transition period of their life. It was reported that there was a high incidence of suicidal rate in these age groups when compared with others.[1] There may be a number of causes for higher levels of stress in these individuals. It can be education stress, especially high in students of professional courses. It can be financial stress or issues with friends etc. The major issue is that though these students are undergoing stress, they do not know to whom to approach for management. Earlier studies suggested establishing a student monitoring cell that assesses regularly the psychosocial behavior of young adults.[1] Unmanaged or poorly managed stress leads to depression and further suicidal thoughts. Hence, there is a need for regular monitoring of the psychological parameters of young adults and counsel them with proper management strategies. The present study was undertaken to observe the selected psychological parameters that are depression, anxiety, and stress in young adults.

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Materials and Methods

Study design: Cross-sectional study

Sampling technique: Convenient sampling

Study population: A total of 50 (males =25, females =25) young adults studying undergraduate allied health courses both males and females were part of the study after obtaining the written informed consent. The following criterion was used to recruit the participants.

Inclusion criteria: Apparently healthy individuals within the age group of 18-24 were recruited in the study.

Exclusion criteria: Participants with health issues or undergoing any treatment or therapy and unwilling participants were excluded from the study.

Assessment of psychological parameters: Depression, anxiety, and stress levels were assessed using the DASS-42 questionnaire which is a standard questionnaire to assess the negative psychological aspects stress, anxiety, and depression.[2]

Ethical considerations: The study protocol was approved by the institutional human ethical committee of the hospital. The study followed the guidelines issued by ICMR. Informed consent was obtained from all the participants before the study.

Data analysis: Data was analyzed using SPSS 20.0 version. Student t-test was applied to observe the significance of the difference between the groups. A Probability value less than 0.05 was considered significant.

Results

Results were presented in table no 1.The two-tailed P value equals 0.0016 for the depression score. By conventional criteria, this difference is considered to be very statistically significant. The two-tailed P value equals 0.1719 for anxiety score. By conventional criteria, this difference is considered to be not statistically significant. The two-tailed P value equals 0.0136for the stress score. By conventional criteria, this difference is considered to be statistically significant.

Table 1: Psychological parameters of participants

Parameter	Males	Females	P value
Depression	16±0.80	13±0.40	0.0016**
Anxiety	9±0.60	8±0.40	0.1719
Stress	21±1.20	17±1	0.0136*

Data was presented as Mean and SEM. (*P<0.05 is significant, **P<0.01 is significant)

Discussion

The present study was undertaken to observe the selected psychological parameters that are depression, anxiety, and stress in young adults. Depression scores were significantly higher in females when compared with males. Anxiety and stress scores were not significantly different among males and females. Females were in severe depression where males were moderate. Anxiety scores of males and females were mild. Stress scores of males and females were severe. The psychological health of the students is much-neglected area of consideration. However, there is a need to assess the psychological parameters of young adults and monitor them and counsel them for better mental health and quality of life. It was reported that young adults have higher levels of stress, anxiety, and depression levels.[3]

The reason for stress may be the academic pressures from parents and teachers or competition from classmates. This is true if the student belongs to a professional course especially medical or engineering. There is lots of academic stress in these students and they have absolutely no time to relax. The majority of students fail to adjust themselves to this excessive stress and enters into depression.[4] Earlier studies reported that depression and anxiety are more prevalent than stress. However, in the present study, it was observed that

depression and stress are severe and anxiety is mild in participants.[5]

Academic stress was reported to be the major reason for the mental health issues of young adults. Excessive stress due to academics leads to decline cognitive functions and quality of life of students.[6] Interestingly, there is a strong link between the stress and immune system and excessive stress deteriorates the immune system and increases the incidence of diseases.[7] When the student fell sick his performance in academics decreases which further acts as a stressor. This will act as a vicious cycle if not interrupted, which can increase suicidal thoughts in young adults.

Though a minimum amount of stress is required in life, excess stress is very deleterious for both physical and mental health. The young adults must be periodically assessed for psychological distress and monitored and counseled for management strategies for their better quality of life and academic performance.

Conclusion

The study results support earlier studies and emphasize the need to assess psychological parameters in young adults. The study also recommends detailed studies in this area.

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A Descriptive Study to Assess the Perceptions of Undergraduate Medical Students Towards Acceptance of E-Learning Vs Conventional Methods in An Integrated Curriculum in Physiology

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Abstract

Background: Institutions across the world have shifted to virtual education to keep the academic activities going due to the COVID-19 pandemic jeopardizing the academiccalendars. E-learning was being used as a part of distance learning courses but the preparedness, designing and effectiveness in using it towards conducting regular courses have been the main concern in countries where students are not used to virtual classes due to technical constraints like faculty training, suitability of devices and bandwidth availability posing a serious challenge. **Methods:** A cross-sectional, web-based study was done among 50 second year medical undergraduate students in 2020. A standardized, electronic, self-administered, Google Form data collection sheet was distributed. It included the seven components to evaluate; Course Schedule, objectives, time management, teaching methods, assessment methods, academic achievement and student concerns. Descriptive, inferential statistics were applied. **Results:** e-learning has been accepted very well by the students (48%) and they found it to add as a better learning resource (44%). About 25% of students find both e-leaning and conventional methods as convenient in learning.The major advantage in conventional methods were due to peer interaction, student-faculty interaction and active participation of faculty with feedback. **Conclusion:** Most of the students appraised that e-learning can be used as complementary to conventional methods. The conventional methods had an upper hand in long term; teaching concepts, peer interaction and faculty feedback with better planning and implementation of curricular activities holding the key for success in fulfilling the expected outcomes by either of the methods.

Keywords: *Conventional, E-learning, Perceptions, Covid-19, Pandemic, Curriculum,Virtual Education, Blackboard.*

Introduction

The outbreak of the COVID-19 pandemic led to the closure of conventional way of academic activities which made a way to the e-learning platform asan alternate method of learning across the world.

In view of the ongoing pandemic, many theories towardse-learning application have been framed. E-learning is considered as a modern and flexible mode of education and some studies have shown it to be a better alternative even though it was continuously used as a part of distance learning courses since more than a decade.

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Recent studies project that, effectiveness of e-learning is influenced by many more factors. Access to e-learning, sudden interchange from traditional to online teaching for regular courses, social and psychological wellbeing including student and pandemic specific demographic variables have been found to have a major influence on the effectiveness of online teaching and learning process.[1-5]

The uncertainties about the preparedness, designing and effectiveness of e-learning methodologies is still not clearly understood under diverse settings. Thereby, we did a web-based study among second year medical undergraduate students following integrated curriculum to assess their perceptions towards effectiveness of the E-learning platform in comparison with traditional methods of learning by using a validated questionnaire through electronic, self-administered, Google Form data collection sheet.

Materials and Methods

A cross-sectional design was conducted under the auspices of Department of Physiology targeting second year medical undergraduate students who utilized e-learning for Immune Blood & lymphatics (IBL) module during the Covid-19 pandemic closure time. The same set of students were taught Musculoskeletal system (MSS) module earlier using traditional methods.

An online written consent was taken from each participant through the invitation to participate in the study.An electronic, self-administered data collection sheet using a Google Form was used. Three experts assessed the face and content validity of the data collection sheet.

Statistical Analysis

The data was analyzed using SPSS version 14. Descriptive statistics were done. The sample size was calculated using the formula for the calculation of samples from the cross-sectional study. At 95% Confidence interval, Z equals 1.96, and 0.05 margins of error was used. P value of ≤ 0.05 was considered significant. The sample size was calculated to be 80. About 50 students volunteered to participate in the study and the same sample was analyzed to see the effectiveness in the implementation of e-learning module.

Results

The students in the study group have observed that the course of the schedule had been framed appropriately (64%) and clearly defined (62%) in e-learning platform compared to conventional methods (18% & 30% respectively). There were complaints by majority of the students (62%) with overcrowding in the learning sessions by the conventional methods which significantly affected their learning process. The majority of the students (54%) gave positive response using e-learning towards course outline across the other courses in comparison to conventional methods (20%) (Table I).

The course objectives were clearly defined, represented well and matched the program expectations in the e-learning module but did not have any significant impact on learning behavior over the students by both the methods. (Table II).

e-learning has been accepted very well by the students (48%) and they found it to be a better learning resource (44%). Most were of opinion that e-learning can be used as complementary to conventional methods. About 25% of students find both e-leaning and conventional methods as convenient in learning. (Table III)

The assessment methods were clearly defined, familiar and students could complete the tasks without any help fulfilling the expected outcomes in both the methods. But, in e-learning students could submit the assigned tasks conveniently (40%) in comparison to conventional methods (18%). (Table IV).

Table I:Section A: Schedule of the Course and course objectives								
Q. No.	**Questionnaire**	**Module**	**Strongly Agree (%)**	**Agree (%)**	**Neutral (%)**	**Disagree (%)**	**Strongly Disagree (%)**	**P-Value**
1	The Course schedule has been framed appropriately	IBL	38	26	14	12	10	0.000*
		MSS	8	10	24	20	38	
2	The course outline is appropriate across the other courses	IBL	30	24	26	6	14	0.008
		MSS	10	10	34	10	36	
3	The course schedule is clearly defined	IBL	42	20	22	6	10	0.003*
		MSS	12	18	24	10	36	
4	The course schedule is congested	IBL	10	12	26	18	34	0.002*
		MSS	32	30	12	10	16	
5	I am able to follow the schedule on Blackboard	IBL	40	12	18	18	12	0.004*
		MSS	14	24	14	12	36	

Table II: Section B: Course Objectives								
Q. No.	**Questionnaire**	**Module**	**Strongly Agree (%)**	**Agree (%)**	**Neutral (%)**	**Disagree (%)**	**Strongly Disagree (%)**	**P-Value**
1	The course objectives are clearly defined	IBL	34	20	26	8	12	0.030
		MSS	14	12	30	10	34	
2	The course objectives are informed before the start of the course	IBL	28	26	8	20	18	0.004*
		MSS	12	16	26	8	38	
3	The course objectives match the expected outcomes of the course	IBL	34	14	28	10	14	0.012
		MSS	12	18	18	12	40	
4	I am able to follow the objectives in each lecture across the course	IBL	32	16	26	18	8	0.001*
		MSS	10	18	22	10	40	
5	All the course objectives are covered in the virtual lectures using blackboard	IBL	34	14	26	18	8	0.000*
		MSS	8	6	18	14	54	
6	All the teaching faculty defines and follow the objectives of concerned lectures	IBL	32	16	28	8	16	0.011
		MSS	10%	10%	26%	10%	44%	

Table III: Section C: Teaching Methods								
Q. No.	**Questionnaire**	**Module**	**Strongly Agree (%)**	**Agree (%)**	**Neutral (%)**	**Disagree (%)**	**Strongly Disagree (%)**	**P-Value**
1	Blackboard teaching is appropriate for my leaning skills	IBL	32	16	22	16	14	0.500
		MSS	28	24	18	8	22	
2	Virtual lectures are excellent teaching resource compared to traditional teaching methods	IBL	38	6	28	8	20	0.424
		MSS	34	16	18	12	20	
3	Virtual lectures should be taken as complementary to the traditional teaching methods	IBL	36	16	20	14	14	0.692
		MSS	30	10	28	12	20	
4	Blackboard teaching should be used for the entire course rather than complementary	IBL	32	18	30	6	14	0.162
		MSS	28	12	18	18	24	
5	All the course objectives were followed in the teaching methods using blackboard	IBL	32	22	22	12	12	0.015
		MSS	16	14	14	16	40	

Table IV: Section D: Assessment Methods								
	Questionnaire	Module	Strongly Agree (%)	Agree (%)	Neutral (%)	Disagree (%)	Strongly Disagree (%)	
1	The assessment methods of the courses are clearly defined	IBL	30	20	14	14	22	0.475
		MSS	20	18	14	10	38	
2	I am familiar with the assessment methods used on blackboard	IBL	28	16	16	20	20	0.182
		MSS	14	12	18	16	40	
3	The coordinator has informed me about the expected outcomes from courses	IBL	28	8	26	16	22	0.258
		MSS	18	14	16	14	38	
4	I am able to complete the assessment tasks without any help	IBL	32	8	22	18	20	0.072
		MSS	12	6	22	18	42	
5	I am able to submit the assessment tasks on time conveniently on blackboard	IBL	32	8	20	20	20	0.068
		MSS	14	4	20	18	44	

Table V: Section E: Feedback and Complaints									
Q.No.	Questionnaire	Module	Strongly Agree (%)	Agree (%)	Neutral (%)	Disagree (%)	Strongly Disagree (%)	Not Applicable (%)	P-Value
1	The internet speed was sufficient to follow virtual lectures	IBL	10	32	22	18	18	0	0.000*
		MSS	0	13	23	21	44	22	
2	I am able to upload the assignments at ease on the blackboard	IBL	32	22	16	16	14	0	0.000*
		MSS	0	3	24	18	55	24	
3	I am able to follow the discussion boards	IBL	34	16	24	16	10	0	0.000*
		MSS	0	8	23	18	51	11	
4	The tools provided in the blackboard are student friendly for use	IBL	28	18	26	16	12	0	0.000*
		MSS	0	3	26	15	56	22	
5	I am satisfied from the faculty participation in completion of their teaching activities	IBL	34	16	18	18	14	0	0.000*
		MSS	2	12	21	16	49	7	
6	The lectures were conducted in scheduled hours	IBL	34	16	24	16	10	0	0.000*
		MSS	5	10	26	12	48	8	
7	I am satisfied from the faculty participation in completion of their teaching activities	IBL	28	12	34	14	12	0	0.000*
		MSS	4	13	24	11	47	5	
8	I am able to clarify my doubts among the peers	IBL	28	16	26	18	12	0	0.000*
		MSS	7	7	33	11	42	5	
9	Overall, I am satisfied in the implementation of the course	IBL	32	14	24	12	18	0	0.000*
		MSS	2	14	16	14	53	7	

The students also had issues and concerns using e-learning pertaining to internet speed, uploading of assignments, follow-up of topics and active involvement of faculty which significantly affected their learning in comparison to conventional methods. The major advantage in conventional methods were due to peer interaction, student-faculty interaction and active participation of faculty with feedback for improvement. (Table V)

Discussion

Following the outbreak of the Covid-19 pandemic, there has been renewed interest on the role and utility of virtual and digital learning across the whole world. E-learning has appeared as a unique learning approach with major concern in medical education continuity during the pandemic. [6]A web-based study was done using a standardized, validated, electronic, self-administered, Google Form data collection sheet towards effectiveness of the e-learning tool. We also investigated significant challenges faced by the students during this unique learning experience.

In our study, we found that the e-learning was acceptable for the young generation as effective learning tool. Students' rating e-learning as not a bad experience, and their rating it as better or similar to on-campus learning found that younger students had a higher level of e-learning acceptance. A study by Toe, et al. also reported that age was negatively associated with all facets. [7]One of the studies among Polish students also showed better acceptance of e-learning as learning resource. [8]But these was completely contradictory to another study.[9]

It has been seen in providing equal access to education, guaranteeing equity and justice,ensuring timely delivery of need-based educational contents, engaging the learners through acarefully planned pedagogical support with the latest online learning technologies.[10] Gaining a better understanding of medical students' acceptance of e-learning can improve our knowledge of their computer-related behaviours and obstacles. [7] The results from a recent meta-analysis, 2019, found that none of the sixteen studies included in their analysis reported that online learning was less effective than offline methods. [11]

In our study, the students had concerns using e-learning pertaining to internet speed, uploading of assignments, follow-up of topics and active involvement of faculty which significantly affected their learning in comparison to conventional methods. Similar findings with varied reasons including the advantage of face-to-face interactivity, immediate feedback and sense of community amongst many other were found. One reason could also be related to difficulty inteaching skills, as in practical classes.[10]Similar findings were found in other studies too. [12]

In our study, students expressed that the major advantage in conventional methods were due to peer interaction, student-faculty interaction and active participation of faculty with feedback for improvement. Similar findings were observed in a study done in Saudi Arabian university and Sudan. [12, 14] One of the major driving forces for accomplishment of objectives and utmost satisfaction of participants of online classes is interactivity and collaborative learning.[15]

Limitations of the study:

Only 50 students volunteered for participation in the study. We could evaluate only two modules due to technical issues and time constraints. Evaluation across all the modules would give more beneficial outcome towards implementation and productive use of E-learning in the curricular activities.

Conclusion

E-learning can be used as complementary to conventional methods with better planning as using both in a timely fashion and as per the needs of the students have an upper hand in long term teaching concepts with peer and faculty interaction and feedback for improvement. Proper implementation of curricular activities holds the key for success in fulfilling the expected outcomes by either of the methods.

Conflict of Interest –Nil

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Study of Cardiovascular Parameters and Heart Rate Variability (Frequency Domain Analysis) in 1St Trimester of Normal Pregnant Women and Pregnant Women with Risk Factors for PIH in Western Rajasthan

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Abstract

Background: The role of sympathovagal imbalance (SVI) and CV risk in pregnancy-induced hypertension (PIH) has been reported, and their association during early trimesters of gestation in PIH has not been studied. Therefore, in the present study, we have investigated the maternal cardiovascular parameters and frequency domain indices of Heart rate variability (HRV) between normal pregnant women and women with risk factors for PIH in their 1st trimester of gestation.

Methods: Two hundred twenty subjects each (220 of normotensive pregnant women i.e., control group and 220 of pregnant women with risk factor for PIH i.e., study group) of 1st trimester of gestation were recruited from the obstetrics & gynecology department of Umaid hospital, associated with Dr. S. N. Medical College, Jodhpur, Rajasthan. Physical examination was done and anthropometric measurement like height & weight were taken. The collected data was statistically analyzed using HRV analysis software.

Results: Significant difference in body mass index was observed between the two groups. Systolic blood pressure, Diastolic blood pressure, Pulse pressure, Rate pressure product and Mean arterial pressure of study group was significantly higher than control group. Values of LF and LF/HF ratio components of frequency domain analysis of HRV were significantly increased but HF component is non significantly decrease in 1st trimester of the pregnant women with risk factor for PIH than normal pregnant women.

Conclusion: The present study indicates that the cardiovascular parameters in 1st trimester of pregnant women with risk factor for PIH were increased highly significantly than the normal pregnant women. The highly significant (HS) ($p<0.000$) increase in the LF (nu) & LF/HF ratio and non significant (NS) ($p<0.552$) decrease in HF (nu) of pregnant women with risk factors for PIH was observed than normal pregnant women this indicate that sympathetic tone was increased in 1st trimester of pregnant women with risk factor for PIH. Vagal withdrawal and sympathetic exaggeration may be the possible cause of PIH in pregnant women with its risk factors.

Keywords: *PIH, Heart Rate Variability, pregnancy, autonomic nervous system*

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Introduction

Severe HTN during pregnancy raises the risk of heart attacks, cardiac failure, cerebrovascular accidents, and renal failure in the mothers [(1)]. In India, the incidence of PIH is reported to be 8-10% among

pregnant women. According to a study, the prevalence of PIH was 7.8% with preeclampsia is 5.4% of the study population in India.

The exact pathophysiology of PIH is not known but low circulating volume and high vascular resistance is well established charecteristics of this disease [2,3]

Impairment of the autonomic nervous system functions may be the cause of PIH. [4] Although there is still a debate regarding whether PIH is associated with disturbances in the sympathetic and parasympathetic functions of the autonomic nervous system [5] Heart Rate Variability (HRV) analysis test can be used to evaluate changes in ANS during different pathophysiological conditions.

In recent year heart rate variability (HRV) is powerful tool for quantitative assessment of cardiac autonomic function, as an indicator of autonomic nervous activity and index of cardiac autonomic regulation.[6-8] It is well established that high-frequency (HF) of HRV is mediated by parasympathetic nervous system (PNS) modulation [9-11] whereas low-frequency (LF) reflects both sympathetic nervous system (SNS) and parasympathetic nervous system (PNS) autonomic influences.[12,13] The ratio of low-frequency power to high-frequency power (LF/HF) has been used to reflect cardiac sympathetic modulation (SNS indicator)[13,14]

Decreased HRV is a marker of cardiovascular autonomic dysfunction and a predictor for cardiovascular risk and increased mortality [15, 16]; it can be used as a sensitive tool in the early detection of PIH in pregnant women with risk factors for PIH. But very few studies have been done on it, thus the main objective of our present project was to compare the maternal HRV (frequency domain parameters) changes between normal pregnancy and pregnancy with risk factors for PIH.

Material & Method

The present study was conducted in the Upgraded department of Physiology in Dr. S. N. medical college and hospitals, Jodhpur. Subjects (control group and study group) were recruited from the out-patient unit of the Obstetrics and Gynecology Department of UMAID hospital associated with Dr. S. N. Medical College. Before starting study all ethical consideration for the subjects were taken in accounts and written permission was obtained from institutional ethical committee. A written consent was obtained from each subject. Sample of the 440 women (220 normal pregnant women and 220 pregnant women with risk factor for PIH) were collected during the October 2019 to December 2019.

Subjects of study group included pregnant women who had risk factors for PIH so inclusion criteria for the study group included established risk factors for PIH such as

1. family history of preeclampsia,
2. preeclampsia in previous pregnancy,
3. extremes of reproductive age, and
4. BMI ≥30.

Subjects of control group included pregnant women who had none of above mentioned risk factors for PIH. All the subjects were examined and detailed personal history was taken with reference to smoking, alcohol intake, family history of hypertension, socioeconomic status, place of residence etc. All subjects had to fill a proforma. Physical examination was done and anthropometric measurements like height and weight were taken and BMI was calculated.

The subject was advised to take complete bed rest in supine position for 10 minutes in a cool and calm environment and not to take and perform any physical or mental activity. Blood pressure was recorded using mercury sphygmomanometer. The recording of short

term HRV was done according to recommendation of the task force. After 10 minutes of supine rest in Polygraph laboratory of physiology department which was established in OPD of Obs. & Gyne. Department of UMAID hospital, all leads of HRV was placed over the subject in requisite position. Lead II of ECG was recorded for 5 minutes during supine rest using Physio Pac Digital Polygraph- Physiograph PL-2008, Medicaid 6 channel Systems, Chandigarh.

The data was transferred from Medicaid machine to window based computer with HRV analysis software. Frequency domain indices such as low frequency (LF), high frequency (HF) and LF/HF ratio (ratio of low frequency and high frequency) of HRV were calculated. In the frequency domain, LF power indicates a mixture of action of sympathetic and parasympathetic components on heart rate with a predominance of sympathetic ones, whereas HF power reflects parasympathetic modulation of heart rate [17, 18].

Statistical analysis of data:

SPSS version 13 (SPSS Software Inc., Chicago, IL, USA) was used for statistical analysis. All data were expressed as mean ± SD. We used Student's unpaired t-test for the level of significance between the two groups.

Result

Table 1: The comparison of the cardiovascular parameters between normal pregnant women and pregnant women with risk factors for PIH.

Cardio vascular parameters (mm Hg)	1st trimester of Normal pregnant women (control group)	1st trimester of Pregnant women with risk factor for PIH (study group)	Normal v/s PIH risk factors women
	Mean±S.D	Mean±S.D	P value
SBP	104.99±5.89	120±9.913	<0.000↑
DBP	65.30±3.12	78.49±7.67	<0.000↑
MAP	78.53±3.50	92.32±7.78	<0.000↑
RPP	82.28±5.55	101.23±13.8	<0.000↑
PP	39.68±5.10	41.52±7.15	<0.000↑

The data presented are Mean±S.D. P value<0.01 was considered statistically highly significant

Table 1 is showing the comparison of SBP (mm of Hg), DBP (mm of Hg), MAP (mm of Hg), PP (mm of Hg) & RPP between the normal pregnant women and pregnant women with risk factors for PIH in their 1st trimester. The result shows the highly significant (HS) ($p<0.000$) increase in the SBP (mm of Hg), & DBP (mm of Hg), MAP (mm of Hg), PP (mm of Hg) & RPP of pregnant women with risk factors for PIH.

Table 2: Comparison of Frequency domain parameters between normal pregnant women and pregnant women with risk factors for PIH.

Frequency domain analysis	1st trimester of Normal pregnant women (control group)	1st trimester of Pregnant women with risk factor for PIH (study group)	Normal v/s PIH risk factors women
	Mean±S.D	Mean±S.D	P value
LF (nu)	30.55±4.91	39.27±5.8	<0.000↑
HF (nu)	33.37±9.88	32.84±8.8	<0.552↓
LF/HF	0.99±0.31	1.29±0.43	<0.000↑

The data presented are Mean±S.D. P value<0.01 was considered statistically highly significant.

Table 2 is showing the comparison of LF (nu), HF (nu) & LF/HF ratio between the normal pregnant women and pregnant women with risk factors for PIH in their 1st trimester. The result shows the highly significant (HS) ($p<0.000$) increase in the LF (nu) & LF/HF ratio and non significant (NS) ($p<0.552$) decrease in HF (nu) of pregnant women with risk factors for PIH.

Discussion

In the present study, the blood pressure measurement in the present study, had a highly significant increase in Systolic Blood Pressure (SBP), Diastolic Blood Pressure (DBP), Mean Arterial Pressure (MAP), Rate Pressure Product (RPP) and Pulse Pressure (PP) in the study group compared to the control group, which suggest that subjects having risks of developing PIH have altered CV parameters even in the early part of pregnancy, this may be due to their increased sympathetic discharge as PIH is first and foremost a state of sympathetic overactivity. So the cause of elevated blood pressure in PIH risk factor women in our study is may be sympathetic activation. This observation was supported by the study of Chaswal M. et al. (2018) [19], Subha et al (2014) [20] & G. K. Pal et al. (2011) [21].

The variables analyzed among the "Frequency Domain Measures" included in our study are "LF, HF, and LF/HF Ratio". As expected "Frequency Domain" variable LF which reflect both sympathetic and parasympathetic influence show highly significant ($p<0.01$) increase in 1st trimester of gestation of study group compared to control group, where as HF which mainly reflect parasympathetic influences was non significantly ($p>0.05$) lower in 1st trimester of gestation of study group compared to control group. "LF/HF Ratio" marker of sympathovagal imbalance (mainly depicts sympathetic dominance) was significantly high ($P<0.01$) in 1st trimester of gestation of study group compared to control group.

The current investigation likewise shows that in study group, patients have higher LF/HF ratio segments of "Frequency Domain Indices" which for the most part measures the sympathovagal balance to heart reflecting an increase in sympathovagal nerve action in PIH patients in starting of their pregnancy. The LF/HF ratio has been proposed to be a precise

proportion of the general sympathovagal balance of the autonomic nervous system in which higher values demonstrate an all the more sympathetically driven cardiovascular system. Same results were obtained by the study of Chaswal M. et al. (2018)[19], A. Hossen et al. (2013) [22] G. K. Pal et al. (2011) [21]. The changes occurs in preeclampsia is not fully understood but few studies observed that some biologically active factors like cytokines or reactive oxygen species from placenta which inhibit vascular relaxation pathway or facilitates vascular smooth muscle contraction, may be responsible for hypertension in pregnancy.

Reports from various studies indicate that these placental factors cytokines and reactive oxygen species [23-25] released peripherally cross the blood-brain barrier and influence activities of various brain centers and their estimation may be helpful for further research. Although no intervention has yet proven effective for the prevention of PIH, early identification of women at risk for PIH may improve maternal and perinatal outcome. Screening for PIH is believed to be most relevant during the first trimester because preventive interventions (such as anti-platelet agents, calcium and antioxidants) are more likely to be effective if initiated early in pregnancy when pathogenic mechanisms may be modified. Further confirmation of the risk of future PIH based on HRV may enable closer prenatal monitoring, earlier diagnosis and prompt and appropriate management.

Conclusion

The present study conducted with the objective to compare maternal cardiovascular and HRV changes (frequency domain analysis) between normal pregnant women & pregnant women with risk factor for PIH it clearly indicates that the sympathetic activation may be the cause of elevated blood pressure in preeclamptic pregnant women of Jodhpur district. This study adds further evidence for the dominant cardiac sympathetic modulations in patients with risk factors for PIH compared with normal pregnant women, probably due to parasympathetic withdrawal in the study group. The frequency domain analysis of heart rate variability proved as a good tool in the study of preeclampsia. Significant increase in the LF and LF/HF ratio component of frequency domain indices was observed in pregnant women with risk factor for PIH than normal pregnant women this indicates that sympathetic tone was increased in study group. Our study on frequency domain analysis suggested that vagal withdrawal and sympathetic exaggeration may be the possible cause of PIH in pregnant women. Our study could have been better if this study would be conducted in different trimester of pregnancy. Further this study could be better if we estimate the levels of placental factors cytokines and reactive oxygen species in both the groups along with the level of proteinuria.

Ethical Clearance: Research involves human participants so ethical approval is obtained from Institutional ethics committee of Dr. Sampurnanand Medical College and Hospital, Jodhpur-. (IEC No.-18/15753).

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