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# Pure Tone Audiometry in Iron Deficient Anaemic Children

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## ABSTRACT

To study the effect of Iron deficiency anaemia on pure tone audiometry in children of age gp 5-12 yrs, 50 Anaemic children having Hb<12gm/dl were taken as cases and 30 healthy children were taken as controls. Anaemics were further divided in mild, moderate and severe anaemics. Recording was done by RMS Audiometry Platform version 1.0.0.390. The statistical significance of difference between groups was evaluated using unpaired student's t test. The hearing threshold values were more in all anaemic groups as compared to controls for both air and bone conduction but values were not statistically significant except at some higher frequencies. In left ear, significant change (P<0.05) was observed in air conduction values at 500 and 4k Hz while in right ear, significant changes were observed in air conduction value at 2k Hz and bone conduction value at 4k Hz while in right ear, significant changes were observed in air conduction value at 2k Hz and bone conduction value at 4k Hz. The results of the present study didn't support any significant hearing loss due to iron deficiency Anaemia but it can be said that there should be an increased awareness in the medical fraternity and the population in general, of the deleterious effects of iron deficiency.

**Keywords:** , Iron deficiency anaemia, air conduction, bone conduction.

## INTRODUCTION

Anemia is defined as a reduction in red cell mass or blood hemoglobin concentration. Iron deficiency anemia is the most common cause of nutritional anemia in the world. An estimated 20% of world's population has iron deficiency anemia.<sup>1</sup> In developing countries, iron deficiency anaemia is associated with low birth weight, malnutrition and unfavourable socio-economic conditions. Infants are at the greatest risk of iron deficiency anaemia because of rapid growth combined with inadequate diet. This risk also extends to pre-school and school children whose adverse living conditions and inadequate diet mean that iron deficiency is a common problem in this age group too.<sup>2</sup>

Iron deficiency has been shown to decrease activities of several important iron containing enzymes such as succinic dehydrogenase, monoamine oxidase and cytochrome reductase in a number of tissues including cardiac muscle, brain tissue and gastrointestinal mucosa. Adenosine triphosphatase activity in red cells decreases markedly in iron deficiency anaemia. In addition, iron deficiency increases RBC membrane stiffness, decreases

red cell deformability and increases blood viscosity. Consequently involvement of red cells in iron deficiency may produce various vascular effects in arteries of inner ear, resulting in disturbances of blood circulation in the cochlea.<sup>3</sup>

Audiometry determines the nature and degree of hearing loss of a person by measuring his hearing sensitivity. In audiological investigations, hearing sensitivity is tested for pure tones, speech or other sound stimuli. The result, when plotted graphically, is called an audiogram. The electronic instrument used for measuring hearing threshold level is called an audiometer. Test tones of different frequencies and levels are generated and presented to patient and hearing threshold are determined on basis of patient's response. Pure tone audiometry is a subjective test where cooperation of patient is needed for pushing a button or raising their hand, when they hear a tone.<sup>4</sup> So we undertook the present study to see if there was any effect of iron deficiency in the Pure tone audiometry recordings of anemic children.

## MATERIAL AND METHOD

The present study was conducted in the Department of Physiology; Pt. B.D. Sharma PGIMS, Rohtak in 30 healthy children and 50 children of iron deficiency anaemia (hemoglobin <12gm%)<sup>5</sup> of either sex before the onset of puberty in age group between 5-12 years attending Pediatrics OPD of Pt. B.D. Sharma PGIMS, Rohtak. Only proven cases of iron deficiency anaemia in which diagnosis was made by history, clinical examination, and investigations like Hemoglobin and Peripheral blood film were included in the study. Subjects with history of intake of ototoxic drugs, history of deafness or any other ear disease, any cardiovascular, respiratory, neurological disorders were excluded from the study.

### RECORDING OF AUDIOMETRY

Recording was done by RMS Audiometry Platform version 1.0.0.390. Audiometry was performed in a soundproof room. For air conduction testing, stimuli were presented to each ear independently with specialized earphones. For bone conduction testing, a bone vibrator was placed on the mastoid process of either right or left temporal bone; external auditory canal was not occluded. All equipments were calibrated.

Frequencies of 500, 1k, 2k, 4k Hz were tested for air and bone conduction in both the ears. Masking of air conduction was used when the difference between air conduction of ear to be tested and bone conduction of other ear was more than 40dB. Masking was used in all cases of bone conduction and results were plotted on a graph called audiogram<sup>4</sup>. An audiogram is a plot of threshold intensity versus frequency. Four separate curves were obtained – right ear air conduction (AC), right ear bone conduction (BC), left ear air conduction (AC) and left ear bone conduction (BC).

### STATISTICAL ANALYSIS

All data were presented as mean±SD. The statistical significance of difference between groups was evaluated using student's t test. The level of significance was set at p<0.05.

### RESULTS

Anaemic children were divided in 3 groups: mild anaemics (Hb:10-11.9gm%), moderate anaemics (Hb: 7-9.9 gm%) and severe anaemics (Hb<7 gm%). In Table I mean age, height, weight and hemoglobin of controls, mild anaemics, moderate anaemics and severe anaemics are shown.

**Table I: Mean age, height, weight and hemoglobin of four groups. Data are expressed as Mean±SD.**

Parameters	Controls (n=30)	Mild Anaemics (n=20)	Moderate Anaemics (n=20)	Severe Anaemics (n=10)
Age(yrs)	8.65±3.28	7.53±4.29	8.20±2.37	8.90±2.37
Height(cm)	118.82±4.54	117.72±3.65	126.10±12.84	130.60±12.85
Weight(kg)	21.06±2.25	20.74±3.21	25.37±6.81	26.80±6.72
Hemoglobin (gm%)	12.88±0.67	9.39±2.63***	8.72±0.75***	6.56±0.29***

Values are expressed as (mean±SD), \*\*\*p<0.001

Table II shows the Audiometry parameters (hearing intensities at different frequencies of air conduction and bone conduction) of right ear of different groups. When moderate Anaemics were compared with controls, the difference was highly significant (p<0.01) at frequency of 2k Hz. Same results were obtained when severe anaemics were compared with controls.



**Table II : Comparison of threshold intensity at various frequencies of air and bone conduction of right ear between different groups**

Parameters	Controls (n=30)	Mild Anaemics (n=20)	Moderate Anaemics (n=20)	Severe Anaemics (n=10)
<b>Air conduction</b>				
500 Hz	30.50±8.93	30.00±6.88	32.25±8.95	32.00±6.74
1000 Hz	29.60±8.08	30.50±6.26	32.50±5.73	32.00±9.18
2000 Hz	24.00±5.78	26.50±4.89	29.50±5.35**	29.50±6.85**
4000 Hz	23.83±5.52	25.00±5.61	26.25±5.59	27.50±7.54
<b>Bone conduction</b>				
500 Hz	19.00±6.74	17.00±7.14	17.15±6.38	18.00±10.05
1000 Hz	15.50±5.77	17.25±10.06	16.25±9.71	19.00±11.97
2000 Hz	15.00±6.15	17.00±6.56	18.50±11.7	19.50±6.85
4000 Hz	13.16±4.04	12.50±5.87	15.75±9.07	16.50±7.09

Values are expressed as (mean±SD), \*p<0.05, \*\*p<0.01, \*\*\*p<0.001

Table III shows the Audiometry parameters (intensities at different frequencies of air conduction and bone conduction) of right ear of different groups. When severe Anaemics were compared with controls, the difference was highly significant (p<0.01) for air conduction at frequency of 500 Hz. The difference was significant (p<0.05) for air conduction, highly significant (p<0.01) for bone conduction at a frequency of 4k Hz, when moderate anaemics were compared with controls.

**Table III : Comparison of threshold intensity at various frequencies of air and bone conduction of left ear between different groups**

Parameters	Controls (n=30)	Mild Anaemics (n=20)	Moderate Anaemics (n=20)	Severe Anaemics (n=10)
<b>Air conduction</b>				
500 Hz	31.00±10.20	35.50±9.85	31.25±11.20	39.50±10.10**
1000 Hz	35.83±6.95	36.25±8.56	36.25±9.15	40.50±7.24
2000 Hz	30.16±6.80	32.50±8.56	29.75±9.38	33.00±6.32
4000 Hz	26.50±6.179	27.00±8.49	31.00±6.19*	30.00±7.81
<b>Bone conduction</b>				
500 Hz	15.33±8.50	19.75±7.51	17.00±4.41	17.50±9.20
1000 Hz	16.33±6.93	18.50±5.87	16.25±4.25	18.00±9.77
2000 Hz	15.16±6.62	18.25±6.12	15.50±4.55	17.50±9.78
4000 Hz	10.66±4.86	12.50±4.72	14.50±4.26**	13.00±4.83

Values are expressed as (mean±SD), \*p<0.05, \*\*p<0.01, \*\*\*p<0.001



## DISCUSSION

Iron deficiency is the most commonly recognized form of nutritional deficiency in developing as well as in affluent societies. It is particularly prevalent among infants and young children because rapid growth imposes large iron needs and most of the infants diet contain a marginal supply of iron.<sup>6</sup> Decrease in cerebral iron content resulting from iron deficiency anaemia may decrease the activity of several neurotransmitters such as dopamine, serotonin, and noradrenaline, by interfering with iron deficiency enzymes, which are important in synthesis of these particular neurotransmitters.<sup>6,7</sup>

A decrease in the activity of aldehyde oxidase resulting from iron deficiency anaemia may interfere with degradation of serotonin and thus may cause a decrease in cognitive function.<sup>8,9</sup> These alterations in cognitive processes may affect auditory aspect of the CNS functions as well. So, we sought to determine whether auditory functions are negatively affected from iron-deficiency.

There was significant change ( $p < 0.05$ ) observed in air conduction value at 4k Hz when moderately anaemic children and control were compared. A significant change ( $p < 0.05$ ) was also observed at 500 Hz when severely anaemic children and controls were compared. Bone conduction value at 4k Hz showed highly significant ( $p < 0.01$ ) change when moderately anaemic children and controls were compared.

There were not much significant changes observed in right ear as in left ear. Air conduction value at 2k Hz was highly significant ( $p < 0.01$ ) when severely and moderately anaemic children were compared to controls respectively.

Sun et al performed electrocochleography and auditory brainstem response in 141 Wistar albino rats with iron deficient diet over a period of 100 days (14 wks). Threshold elevation of 15 dB were detected in 32% of animals, but it was unchanged in the controls. They stated that this hearing loss affected only high frequencies initially, but low frequencies were affected as iron deficient diet was continued. They also demonstrated association of presence of longer duration of iron deficiency anemia with higher incidence of hearing loss and with the more severe abnormalities of stria vascularis and spiral ganglion cells in growing rats.<sup>10</sup> In our study, there were some significant changes at

higher frequencies but in some cases, lower frequencies were also affected.

Unger et al in a study observed that early iron deficiency alters sensorimotor development and brain monoamines in rats.<sup>11</sup> In contrast, Delb et al investigated the hearing level of diet induced anaemic and control rats for upto 140 days (20 wks) using ABR and scanning electron microscopy and found no difference in ABR threshold and histocochleogram between anaemic and control groups.<sup>12</sup>

Ozturan et al found similar results. They performed distortion product otoacoustic emissions (DPOAEs) to investigate any type of cochlear insult at outer hair cell level and auditory brainstem response to detect any type of neural insult. Both tests indicated that in state of iron deficiency, hearing is not affected at cochlear or neural levels.<sup>13</sup> Sun et al showed the decreased activity of various enzymes in iron deficient rats and they have proposed that iron deficiency anaemia can cause auditory dysfunction by disturbing cell respiration and initiating peroxidative damage to inner ear cells.<sup>14</sup>

In contrast, Cetin et al analyzed the outer hair cell activity of the cochlea in patients with iron deficiency anaemia. Pure-tone audiometry (PTA) (250–6000 Hz) and distortion product otoacoustic emission (DPOAE) results of 42 patients with iron deficiency anaemia and 22 healthy, age and sex matched subjects for the control group were compared. PTA was normal in patients with iron deficiency anaemia and control subjects.<sup>15</sup>

The mechanisms for this alteration in the conductive process may be multifactorial involving a number of biochemical pathways in which iron is essential. It's not only the altered myelination but it also includes mitochondrial enzymes, various neurotransmitters etc. Studies document the alteration of dopaminergic functioning in iron deficiency.<sup>16</sup> Thus, iron deficiency could interfere directly with neurotransmission in the auditory pathway or indirectly by altering certain processes that modulate brainstem auditory activity.

## CONCLUSION

To conclude, we can say that there should be an increased awareness in the medical fraternity and the population in general, of the deleterious effects of iron deficiency, some of which are subtle and subclinical and will likely to have a long term effect.

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**Conflict of Interest -** Nil

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# Mean Platelet Volume and Mean Platelet Volume/Platelet Count Ratio Commensurate to Severity in Ischemic Stroke

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## ABSTRACT

**Introduction and background:** The mean platelet volume is an established indicator linked with platelet function and action. Increased MPV in thromboembolic disorder is reflected as a key risk factor. The aim of this study was to compare the MPV and mean platelet volume/platelet count (MPV/PC) ratio between ischemic stroke (cases) and control subjects and additionally, to also reveal their diagnostic value to help stratify risk in patients with ischemic stroke. **Method:** This is a cross-sectional analytical study conducted in Himalayan Institute of Medical Sciences, Dehradun encompassing 50 successive patients with acute ischemic stroke (cases), and 50 healthy volunteers (controls). Blood samples were used to measure MPV and MPV/PC ratio. **Result:** The ischemic stroke patients have significantly higher MPV and MPV/PC ratio compared to the control group ( $p < 0.01$ ). MPV was significantly higher ( $p < 0.01$ ) amongst cases as compared to controls. Receiver operator characteristic (ROC) curve analysis revealed that an MPV cutoff value of  $>7.5$  femtoliters provided 80.8% sensitivity and 78.5% specificity. The area under the ROC curve for MPV and MPV/PC ratio was 0.91 (CI: 0.893-0.921;  $p < 0.01$ ) and 0.84 (CI: 0.819-0.852;  $p < 0.01$ ) respectively, which indicates that both MPV and MPV/PC ratio predict ischemic stroke. **Conclusion:** MPV and MPV/PC ratio may be deemed suggestive laboratory markers for the risk of ischemic stroke.

**Keywords:** MPV; MPV/PC ratio, Ischemic Stroke prediction, Transient Ischemic Attack, ROC analysis

## INTRODUCTION

Mean platelet volume (MPV) is a routinely used laboratory marker, both prognostic as well as therapeutic, vis a vis platelet function<sup>1</sup>. Larger platelets have more granules than ordinary sized platelets<sup>2</sup> and have been established to produce a greater amount of pro-thrombotic factors as compared to regular platelets<sup>3</sup>. The platelet plays a major role in the pathogenesis of vascular disease, and mean platelet volume (MPV) is a physiological variable of hemostatic importance.

Higher mean platelet volume (MPV) values have been recognized in patients with stroke<sup>4,5</sup> and than in control subjects. Furthermore, MPV has been shown to be predictive of stroke, in patients with previous cerebrovascular accidents (CVAs) and is an independent predictor of the risk<sup>6</sup>. Additionally, a high ratio of MPV to platelet count (MPV/PC) is considered a risk factor for various diseases and is associated with myocardial infarction, anemia<sup>7</sup>, and hepatocellular carcinoma<sup>8</sup>. The connections between MPV and cerebrovascular accidents have been extensively studied but very few studies have adequately studied the role of MPV & MPV/PC ratio between cases of ischemic stroke and normal individuals (controls). The aim of this study was to compare the MPV and MPV/PC ratio between cases of ischemic stroke and an equal number of normal individuals and to find out their diagnostic value for risk stratification.

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## METHODOLOGY

This cross-sectional study analytical was conducted in Himalayan Institute of Medical Sciences, Dehradun comprising of 50 patients of ischemic stroke and an equal number of normal individuals (controls). The control group comprised 50 age and gender matched healthy volunteers with no quantifiable indication of any vascular disease, previous cerebrovascular disease, malignancy or infarction and not taking medications known to affect platelet function. The collected data includes baseline demographic parameters, previous history of ischemic heart disease or cerebrovascular accidents, biochemical parameters, medications, echocardiographic data along with neuro-imaging studies. Exclusion criteria were framed to exclude subjects with history of peripheral vascular disease, stroke, acute infection, positive C-reactive protein or inflammatory conditions, pregnancy, acute myocardial infarction, malignancies, cranial traumas, intracranial haemorrhage, hematomas, or Transient ischemic attacks (TIAs). The diagnosis of ischemic stroke was made clinically with the evidence of acute infarction confirmed by brain CT or MRI within the first 24 h of presentation to the emergency department.

### Estimation of MPV and platelet count:

Blood samples were taken from the Cubital Vein,

anticoagulated with EDTA and measured within the ensuing hour at room temperature. Platelet measurements were evaluated by flow cytometry in MS-9 automated hematology cell counter that provided MPV (in femtolitres) and platelet count (in millions).

**Sample Size:** The formula for comparing the difference of means between the groups (for two groups) was used with  $\alpha=0.05$ , power ( $\beta$ ) = 80% and effective size (es) = 0.6 which gives sample size  $n = 52^9$ . Verbal informed consent was taken from all the subjects prior sample collection.

### Statistical Analysis:

The data were analyzed using Statistical Package for the Social Sciences (SPSS) for Windows version 20.0, Illinois, USA. A comparison of normally distributed MPV and MPV/PC ratio among ischemic stroke patients and controls was done using Students' 't' test. As there were no exact data cut off values for MPV or MPV/PC ratio in ischemic cerebrovascular diseases sensitivity, specificity and AUC for these parameters at different cut-offs was estimated using Receiver Operating Characteristic (ROC) curve. Also, variable predictive parameters of PPV, NPV, LR(+), LR(-), Odds Ratio and Youden's Index were also calculated to enhance and further decipher accuracy of test.

## RESULTS

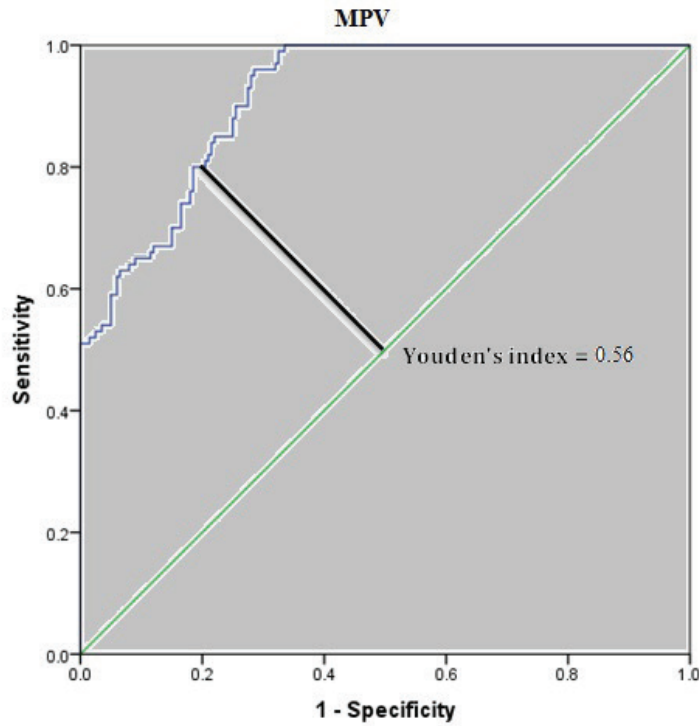
**Table 1: Comparison of baseline demographic parameters, platelet count (PC), mean platelet volume (MPV) and MPV/PC between control and ischaemic stroke cases**

Parameter	Control (n=50) Mean $\pm$ S.D.	Cases (Ischaemic stroke) (n=50) Mean $\pm$ S.D.	'p' value
Age (Years)	57.11 $\pm$ 5.26	58.11 $\pm$ 6.42	P > 0.01
Weight (Kilograms)	74.70 $\pm$ 7.42	75.45 $\pm$ 6.14	P > 0.01
Height (metres)	1.58 $\pm$ 2.32	1.62 $\pm$ 2.79	P > 0.01
BMI (Kg/m <sup>2</sup> )	25.89 $\pm$ 0.02	26.07 $\pm$ 0.02	P > 0.01
Platelet count (PC)	2.38 $\pm$ 0.78	2.47 $\pm$ 0.89	P > 0.01
Mean platelet volume (MPV)	6.89 $\pm$ 1.22	7.47 $\pm$ 1.29**	P < 0.01
MPV/PC	2.69 $\pm$ 0.56	2.91 $\pm$ 0.63**	P < 0.01

**Data was presented as mean  $\pm$  standard error of mean. Analysis was done using Students' 't' test. P < 0.05.**

Table 1 shows in a total of 100 subjects (50 cases and control each) baseline demographic parameters (age, weight, height and BMI), platelet count (PC), mean platelet volume (MPV) and MPV/PC are compared between controls and ischaemic stroke cases using Students' 't' test (Table 1). A statistically significant increase in mean platelet volume

(MPV) and MPV/PC is observed amongst ischemic stroke patients. No significant difference between the baseline demographic parameters between the cases and controls ensure optimum comparison avoiding bias.



**Figure 1: Specificity and sensitivity of MPV (mean platelet volume) in predicting ischemic stroke. AUC (Area under the curve) is 0.91 with Youden’s index of 0.56**

Fig 1 shows receiver operator characteristic (ROC) curve analysis elucidated that both MPV and MPV/PC ratio predicts ischemic stroke with AUCs (Area under the curve) of 0.91 (CI: 0.893-0.921;  $p < 0.01$ ) (Figure 2), 0.84 (CI: 0.819-0.852;  $p < 0.01$ ) respectively. The close proximity of the ROC curve to the top left corner as well as a greater area underneath (AUC) deciphers an accurate test. Furthermore, a Youden’s index value of 0.56, illustrated as a vertical height above the chance line gives an optimum MPV cut-off of 7.5. This index along with ROC curve definitely enhances the ischemic stroke probability keeping all predictions in mind.

**Table 2: Diagnostic test characteristics at different MPV cut-offs to predict ischemic stroke**

Cut-off values	7.1	7.3	7.5	7.7	7.9
Sensitivity (%)	94.3	84.3	80.8	75.8	65.1
Specificity (%)	60.8	68.4	78.5	82.3	89.4
PPV (%)	44.49	56.64	63.15	70.22	75.21
NPV (%)	94.22	90.54	86.56	83.04	79.29
Accuracy (%)	70.16	76.56	79.25	80.34	82.03
LR+	2.56	2.89	3.60	4.29	5.33
LR-	0.08	0.21	0.27	0.33	0.42
Odd’s ratio	29.32	24.88	16.32	11.03	09.33
Youden’s index	0.54	0.54	0.56	0.54	0.52



Abbreviations: PPV, Positive predictive value; NPV, Negative predictive value; LR+, Positive likelihood ratio; LR-, Negative likelihood ratio

Table 2 describes predictive accuracy of MPV at different cut offs in diagnosing ischaemic stroke using sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (LR+), negative likelihood ratio (LR-), Odd's ratio and Youden's index. A value of 7.5 appears to be the best suited cut-off keeping a legitimate balance amongst the above mentioned accuracy parameters. These values can be further vindicated from the AUC of 0.91; sensitivity: 80.8; and specificity 78.5 as depicted in Figure 1.

## DISCUSSION

MPV has been recognised to be of clinical relevance in thrombo-embolic affliction. Augmented mean platelet volume (MPV) level has been observed in patients with stroke<sup>10</sup> than in control subjects. In this study, we found that both MPV and MPV/PC ratio was significantly higher in subjects presenting with cerebrovascular stroke compared to a control group. Identically, O'Malley et al<sup>3</sup> appreciated MPV values in subjects with ischemic stroke as compared to control group. Despite the fact that this was not a blanket finding across all research substructures but most of the analyses proved that MPV levels were elevated in stroke patients<sup>11, 12</sup>. Indeed, the fact that MPV values may be elevated before an event of ischemia has clearly been demonstrated by the PROGRESS study<sup>6</sup> which professed an 11% rise of the relative risk of stroke for every femtoliter of MPV increment in 3134 individuals with prior cerebrovascular affliction, prospectively followed for a median period of 3.9 years.

In a Japanese study by Uchiyama et al a higher percentage of megathrombocytes was also noted in patients with cerebral infarct and TIA/RIND (Reversible Ischemic Neurological Deficit) than in the controls. The number of megathrombocytes was not elevated over 20% in controls while it was over 20% in 28 out of 76 (37%) patients with Transient Ischemic Attack(TIA)/ (RIND), 25 out of 101 (25%) patients with cerebral infarct, and 5 out of 14 (36%) patients with Rheumatic valvular heart disease (RVHD) accompanied by cerebral embolism<sup>14</sup>. Thus, people with enlarged platelets do have increased chances of ischemic stroke.

In the past various studies have found contradictory

finding regarding platelet count levels in ischaemic stroke. Some studies have repeatedly contended that patients with acute ischemic stroke had higher platelet counts than the control groups. This finding was mirrored in our result, despite the fact that we did not find any statistical significance<sup>5,13</sup>. Conversely, other studies have shown that since thrombus formation requires platelets, hence the platelet counts of subjects during acute stroke should be lower as compared to controls.

Our ROC curve analysis revealed that an MPV cut-off value of >7.5 femtoliters provided 80.8% sensitivity and 78.5% specificity. The area under the ROC curve for MPV and MPV/PC ratio was 0.91 (CI: 0.893-0.921; p<0.01) and 0.84 (CI: 0.819-0.852; p<0.01) respectively, which indicates that both MPV and MPV/PC ratio predict ischemic stroke. In comparison Nurettin et al<sup>15</sup> reported that an MPV cut-off value of 9.95 femtoliters had 46.2% sensitivity and 80.0% specificity. Furthermore, in an Egyptian study conducted by Elsayed and Mohamed ROC analysis verified that MPV (cut off value of >8.1 FL) and MPV/PC ratio (cut-off value of >0.031 FL 10 4 IL 1)) could be used as surrogate lab markers for pinpointing cerebrovascular stroke with 68% and 70% sensitivity and 80% and 75% specificity, respectively<sup>16</sup>. All these findings reiterate our results and indicate that both MPV and MPV/PC ratio are predictors of ischemic stroke.

In conclusion, raised MPV and MPV/PC ratio is related to ischemic stroke. Also MPV and MPV/PC ratio tests are minimally invasive, uncomplicated, economical and meaningful lab markers for timely discernment and risk stratification of ischemic stroke.

**Ethical Clearance:** Ethics committee of Himalayan Institute of Medical Sciences, Dehradun

**Source of Funding-** Self

**Conflict of Interest:** Nil

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# A Comparative Study of Cardiovascular Autonomic Function Tests between Pregnant and Non-Pregnant Women

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## ABSTRACT

**Background:** Normal pregnancy is associated with substantial changes in the cardiovascular system which begins to change after the first trimester. Failure of these adaptations may result in pregnancy related complications like PIH, Preeclampsia and Eclampsia.

**Objectives:** To evaluate and compare the cardiovascular autonomic function tests between First trimester pregnant women and controls.

**Method:** A cross sectional study was done by random selection of 67 first trimester pregnant women and 67 controls in the age group of 19-29 years. The cardiovascular autonomic function tests were carried out. The statistical analysis was done by using Unpaired 't' test.

**Results:** During isometric handgrip exercise the maximum rise in DBP, and during CPT the maximum rise in SBP and maximum rise in DBP, were significantly less in first trimester of pregnancy than controls. There was significantly less increase in the E:I ratio of First pregnant women when compared to controls.

**Interpretation and conclusion:** The cardiovascular autonomic nervous activity was decreased in first trimester of pregnancy when compared to controls.

**Keywords:** Cardiovascular autonomic function tests; Cold pressor test; Pregnancy induced Hypertension.

## INTRODUCTION

The response of human body to stress occurs by alterations in different biological functions especially autonomic functions like heart rate and blood pressure<sup>(1)</sup>. Subjects are at high risk of developing hypertension in future, whose cardiovascular system is very sensitive to a stressor and recovers slowly after its withdrawal<sup>(2)</sup>. Highly sensitive measure of parasympathetic cardiac function is heart rate variability with deep breathing<sup>(3)</sup>. Parameters like, blood pressure fluctuations during standing up and handgrip evaluate sympathetic activity<sup>(4)</sup>.

In first trimester of pregnancy significant changes are seen in arterial blood pressure after orthostatic variations and cold pressure test, reflecting higher sympathetic activity<sup>(5)</sup>. A comparative study of cardiovascular sympathetic activity in three trimesters of pregnancy showed that, there is decreased sympathetic activity more in second trimester, less in first trimester and least in last trimester of pregnancy that is, towards prepregnant

level<sup>(6)</sup>. No variations were found in parasympathetic activity in early stages of pregnancy<sup>(5,7)</sup>.

Thus, cardiovascular monitoring during pregnancy helps in early detection of cardiovascular abnormalities, especially in developing countries like India<sup>(7)</sup>. In preventing complications, the antenatal visits are very much helpful. So, the present study was undertaken to evaluate the non-invasive cardiovascular autonomic function tests in first trimester of pregnancy, and compare them with non-pregnant controls.

## AIMS AND OBJECTIVES

To evaluate and compare cardiovascular autonomic function tests between First trimester pregnant women and non-pregnant women.

## MATERIALS AND METHOD

The present study was undertaken at the Department of Physiology with collaboration of Department of

Gynecology and Obstetrics, Belagavi Institute of Medical Sciences (BIMS) College and Hospital, Belagavi, Karnataka. The pregnant women were selected by random sampling, attending BIMS Civil Hospital for routine antenatal care. Control group comprised of healthy non-pregnant women which included non-teaching staff, technicians and relatives of patients who were randomly selected from BIMS College and Hospital, Belagavi.

The study was conducted after obtaining the ethical clearance from Institutional ethics committee. The details such as purpose of the study, nature of the study and methods used, were explained to the subjects and controls, in their own understandable language. Written informed consents were duly signed by the subjects and controls.

#### **SAMPLE SIZE:**

- Study Design: Cross-sectional study.
- By referring the Article<sup>(7)</sup>, Sample size was calculated by using the formula,
- Formula: 
$$n = \frac{(Z \alpha + Z \beta)^2 \times S^2 \times 2}{d^2}$$
- Where
  - $Z \alpha$  - Z value for alpha Error = 1.96
  - $Z \beta$  - Z value for beta Error = 0.84 with 80% power
  - S - Common Standard Deviation between two groups = 5.02
  - d - Clinically meaningful difference = 2.43
  - Z - Standard normal deviate (deviation from the mean)
- By substituting above values in the formula,  $Z \alpha = 1.96$ ,  $Z \beta = 0.84$  with 80 % power,  $S = 5.02$  and  $d = (\text{mean 1} - \text{mean 2}) = (7.73 - 5.3) = 2.43$ , we get sample size as  $n = 67$  in each group.
  - First trimester – 67
  - Control – 67
  - Total – 134

#### **Inclusion criteria:**

1. SUBJECTS: 19 to 29 years healthy pregnant women of first trimester with singleton pregnancy were randomly selected.

2. CONTROLS: 19 to 29 years healthy non-pregnant women were randomly selected from population of non-teaching staff, technicians and relatives of patients.

#### **Exclusion criteria:**

All pregnant women in the age group of less than 19 or greater than 29 years, with multiple pregnancies, gestational diabetes, pre-eclampsia and eclampsia, diabetes mellitus, asthma, thyroid diseases, history of cardiovascular or lung diseases, smokers or on any drugs that might affect autonomic functions, e.g. adrenergic receptor stimulants and blockers and those with hemoglobin < 10 gm% were excluded.

#### **METHOD**

In the present study five simple, non-invasive cardiovascular reflex tests had been used to assess autonomic functions. All the tests were conducted between 10.00am and 4.00pm. The subjects had been instructed to abstain from coffee, tea, cola e.t.c. for a minimum period of 12 hours before the tests. After thorough examination of the subjects as per proforma, the subjects were asked to relax in supine position for 30 minutes. The resting heart rate (HR) was recorded on a standard ECG from lead II and blood pressure (BP) was measured from Omron digital blood pressure monitor. The standard cardiovascular autonomic function tests were performed, as per Ewing DJ and Edgar A Hines Jr criteria<sup>(8,9)</sup>.

The tests included,

1. Blood pressure response to standing.
2. Blood pressure response to sustained handgrip.
3. Heart rate response to standing.
4. Heart rate response to deep breathing.
5. Cold pressor test.

#### **Procedure:**

##### **1. Blood pressure response to standing:-**

Blood pressure was recorded when the subject is lying down quietly for 5 to 10 minutes. Three basal

readings were taken. The subject stood up suddenly, taking less than 5 seconds; then blood pressure was recorded at 30 seconds, 60 seconds and 90 seconds after standing. The postural fall in blood pressure is taken as the difference between the systolic blood pressure in lying and the lowest systolic blood pressure on standing<sup>(8)</sup>.

## 2. Blood pressure response to sustained handgrip:-

The subject was asked to exert maximal hand grip strength on hand grip dynamometer with dominant hand. The maximum voluntary contractions were first determined. Handgrip was then maintained at 30% of that of maximum for as long as possible (3 to 5 minutes). Blood pressure was measured three times before and at one-minute intervals during handgrip for 3 minutes in the non-dominant hand. The result is expressed as the difference between the highest diastolic blood pressure during handgrip exercise and the mean of the three diastolic blood-pressure readings before handgrip began<sup>(8)</sup>.

## 3. Immediate heart rate response to standing:-

The test was done with the subject lying quietly while the heart rate was recorded continuously for 30 seconds, on an ECG. The subject then stood suddenly and the point at starting to stand was marked on the ECG. ECG was recorded for 30 seconds after standing. The shortest RR interval at the 15<sup>th</sup> beat and the longest RR interval at around the 30<sup>th</sup> beat were measured. The heart rate response was expressed by 30:15 ratio<sup>(8)</sup>.

The ratio was expressed as follows:

$$\frac{\text{Longest R-R interval at around 30}^{\text{th}} \text{ beat}}{\text{Shortest R-R interval at around 15}^{\text{th}} \text{ beat}}$$

## 4. Heart rate variation during deep breathing:-

The subject sits quietly and was instructed to start deep breathing on verbal command as trained earlier (5 seconds deep inspiration and 5 seconds deep expiration) for 30 seconds and the ECG was recorded throughout the period of deep breathing. The mean of difference between maximum and minimum RR interval during each breathing cycle was measured<sup>(8)</sup>. The ratio was expressed as:

$$\text{Ratio} = \frac{\text{Maximum R-R interval}}{\text{Minimum R-R interval}}$$

## 5. Cold Pressor test:-

After the subject had rested supine for 5 to 10 minutes, resting BP was recorded with the subject sitting comfortably in the right upper arm. The left hand was then immersed to just above the wrist in cold water (3 to 5 degree centigrade) for 1 minute. Blood pressures were measured from right arm at 30 and 60 seconds after immersion. Maximum increase in SBP and DBP were noted<sup>(9)</sup>.

## Statistical analysis

The basic data was presented as mean  $\pm$  standard deviation. The statistical analysis was done by using unpaired 't' test (SPSS 22 Version) to compare the data and p value of  $< 0.05$  was considered as significant.

## RESULTS

The comparison of cardiovascular autonomic function tests (AFT) was done between the 67 first trimester pregnant women and 67 healthy non-pregnant women who were the controls.

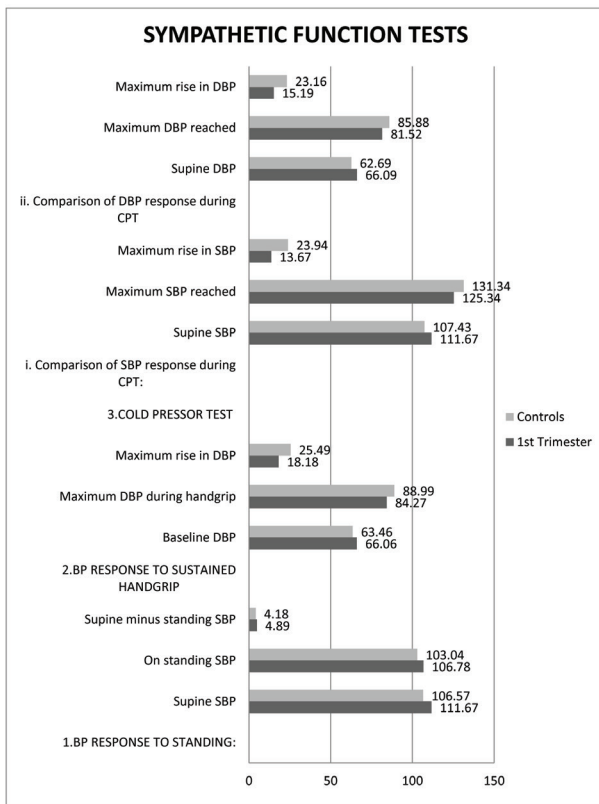
**Table 1: Comparison of cardiovascular AFT between first trimester pregnant women and controls.**

Parameters	1 <sup>st</sup> Trimester (Mean $\pm$ SD)	Controls (Mean $\pm$ SD)	Significance	
			t value	p value
<b>I. SYMPATHETIC TESTS :</b>				
<b>1. Blood pressure response to standing (mmHg):</b>				
Supine SBP	111.67 $\pm$ 7.86	106.57 $\pm$ 10.68	3.151	<b>0.002*</b>
On standing SBP	106.78 $\pm$ 12.34	103.04 $\pm$ 9.16	1.987	<b>0.049*</b>

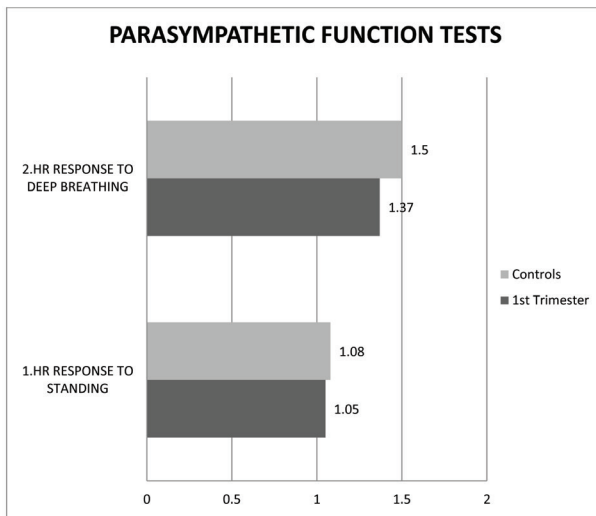
**Cont... Table 1: Comparison of cardiovascular AFT between first trimester pregnant women and controls.**

Fall in SBP	4.89±9.11	4.18±6.56	0.522	0.602
<b>2. Blood pressure response to sustained handgrip (mmHg):</b>				
Baseline DBP	66.06±6.68	63.46±8.79	1.925	<b>0.056*</b>
Maximum DBP during handgrip	84.27±12.36	88.99±15.77	-1.927	<b>0.056*</b>
Maximum rise in DBP	18.18±11.29	25.49±15.21	-3.160	<b>0.002*</b>
<b>3. Cold pressor test :</b>				
<b>i. Comparison of SBP response during CPT (mmHg):</b>				
Supine SBP	111.67±7.86	107.43±9.31	2.847	<b>0.005*</b>
Maximum SBP reached	125.34±13.83	131.34±16.22	-2.304	<b>0.023*</b>
Maximum rise in SBP	13.67±11.86	23.94±13.91	-4.597	<b>0.000*</b>
<b>ii. Comparison of DBP response during CPT (mmHg):</b>				
Supine DBP	66.09±6.66	62.69±6.45	3.003	<b>0.003*</b>
Maximum DBP reached	81.52±11.10	85.88±13.10	-2.077	<b>0.040*</b>
Maximum rise in DBP	15.19±8.27	23.16±12.63	-4.321	<b>0.000*</b>
<b>II. PARASYMPATHETIC TESTS :</b>				
<b>1. Heart rate response to standing - 30: 15 ratio</b>	1.05±0.13	1.08±0.18	-1.282	0.202
2. Heart rate response to deep breathing-ratio of max. to mini. RR interval	1.37±0.19	1.50±0.19	-3.959	<b>0.000*</b>

**FOOT NOTE: All the values in mmHg except ratios. \* Indicates significant value.**



**Graph 1: Comparison of sympathetic function parameters between first trimester pregnant women and controls.**



**Graph 2: Comparison of parasympathetic function parameters between first trimester pregnant women and controls.**

## DISCUSSION

In our study, the fall in SBP in response to standing was not significant in first trimester of pregnancy when compared to controls. Previous study had shown that, on orthostatic hypotension test, no significant change was found in BP among first trimester pregnant women, which correlates with our study<sup>(6)</sup>. In contrast to our

findings, a study showed that, the fall in SBP on standing was significantly increased during first trimester of pregnancy when compared to the controls<sup>(7)</sup>. A decrease in baroreceptor sensitivity, especially observed in early pregnancy may be attributed to this observed result perhaps signifying an incomplete adaptation of the cardiovascular system to the pregnant state. It has been noted that during second half of pregnancy, the increase in blood volume seemed to improve hemodynamic stability<sup>(10)</sup>.

In our study, the maximum rise in DBP during isometric handgrip exercise was significantly less in first trimester of pregnancy when compared to controls. This is in accordance with many studies<sup>(6,7,11,12,13)</sup>, which have shown that there is decreased sympathetic activity less in first trimester of pregnancy, which explains that the decreased peripheral vascular resistance in first trimester is mainly by decreased sympathetic activity<sup>(6)</sup>. The reduced BP response could be due to an antagonistic effect of products of uteroplacental unit such as progesterone, or a diminished contractile response of the blood vessels to adrenaline<sup>(14,15)</sup>. According to other study, BP decreases in early pregnancy, reaching a minimum in mid-pregnancy, which again correlates with our study<sup>(16)</sup>. In the early pregnancy an overall decrease in vascular tone leads to systemic vasodilatation and rise in arterial compliance, which may be directly responsible for down-regulation of baroreceptors. There is evidence that the release of vasopressin causes hemodilution and a reduction in viscosity<sup>(17)</sup>. A lower viscosity potentiates fall in vascular resistance. Both factors independently contribute to fall in afterload. These changes combined are responsible for decrease in BP in first trimester of pregnancy<sup>(6)</sup>.

In our study, the maximum rise in SBP and the maximum rise in DBP in response to CPT was significantly less in first trimester than compared to controls, indicating decreased sympathetic activity in first trimester of pregnancy than controls. In contrast, studies showed, the BP response to CPT was significantly increased in first trimester of pregnancy as compared to controls, reflecting higher sympathetic activity<sup>(5)</sup>.

In our study of HR response to standing, the 30:15 ratio on comparison between first trimester pregnant women and controls were not significant. This HR response is reduced during pregnancy, indicating a diminished Baroreflex induced slowing of HR which



suggests a rearrangement of autonomic tone takes place in normal pregnancy<sup>(18)</sup>. Many studies<sup>(5,7)</sup> showed HR response to standing remain unaltered in early stages of pregnancy<sup>(7)</sup> and in first trimester of pregnancy and controls<sup>(5)</sup>, which correlates with our study.

In our study of HR response to deep breathing, the ratio of maximum to minimum RR-interval during respiratory cycles that is, the E/I ratio of R-R intervals, was found to be significantly less in first trimester pregnant women when compared to controls. In accordance with our results, many studies<sup>(7,19,20,21,22)</sup> showed, the heart rate response to deep breathing expressed as deep breathing difference (DBD), a measure of cardiac parasympathetic function was significantly lower in pregnant women when compared to control group and followed a decreasing trend with increase in gestation<sup>(7)</sup>. This finding was in conformity with Ekholm EMK, et al who suggested a multifactorial basis for it<sup>(20)</sup>. A diminished parasympathetic input to the heart during pregnancy has been attributed to reduced baroreceptor sensitivity, impaired vagal afferents to brain and altered efferent signals to the heart<sup>(21)</sup>. A reduction in oscillation of right atrial distension arising from diminished pulsatility of venous return from the growing uterus has been described in pregnant subjects, accounting for the lowering of DBD in pregnancy<sup>(7)</sup>.

### CONCLUSION

From the above results, we can conclude that, on comparison of cardiovascular autonomic function tests between pregnant women and controls, the cardiovascular autonomic nervous activity was decreased during pregnancy than controls. Cardiovascular autonomic nervous variations starts from first trimester of pregnancy, there was decreased sympathetic and parasympathetic nervous activity in first trimester of pregnancy when compared to controls. Thus, cardiovascular autonomic function tests can be used as screening tests among pregnant women to detect any cardiovascular autonomic alterations at an early stage which may lead to complications like PIH, and thus may give valuable information in this regard.

**Ethical Clearance-** Taken from Institutional ethical clearance committee

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**Conflict of Interest -** Nil

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# A Comparative Study of Impact of Obesity on Dynamic Lung Function Test in Young Adult Women

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## ABSTRACT

**Background:** Obesity has become a global epidemic. The Prevalence and severity of obesity in young adult females is increasing worldwide. Along with other organs respiratory system is also compromised. Since the static lung volumes that are obtained by Spirometry are insufficient to conclude on whether the ventilatory abnormality is an obstructive one or a restrictive pattern, dynamic or forced Spirometry is performed. Hence the present study was done to know the Impact of obesity on Dynamic Lung Function Test in young Adult Females.

**Aims and Objectives:** 1. To Compare Dynamic Lung Function Test in obese adult females and non-obese adult female subject.

2. To evaluate the impact of obesity on Dynamic Lung Function Test.

**Materials and Method:** Dynamic Pulmonary Function tests (PFTs) of 50 normal, healthy, non-obese females and 50 healthy but obese females, age group 18-30 years of Hubli city were obtained and were compared. Criteria for obesity in our study were taken according to chart provided by WHO for categorization of BMI. The Dynamic Pulmonary function test was carried out with computerized Spirometer Eazy on-PC model. Dynamic Lung Function Test parameters were used as measure of lung function.

**Results:** The obese females had FVC ( litres ) of  $2.31 \pm 0.41$  whereas corresponding values in non-obese  $2.87 \pm 0.66$ . There was statistically highly significant differences between two groups ( $p < 0.01$ ). The obese females had  $FEV_1$  ( litres ) of  $1.94 \pm 0.38$  whereas corresponding values in non-obese was  $2.4 \pm 0.61$ . There was statistically highly significant differences between two groups ( $p < 0.01$ ). The obese females had  $FEV_1/FVC$  ( litres ) of  $0.83 \pm 0.08$  whereas corresponding values in non-obese was  $0.84 \pm 0.11$ , obese females had less  $FEV_1/FVC$  ratio compared to non-obese females.

**Conclusion:** In the present study Dynamic Lung Function Test parameters FVC,  $FEV_1$  values were significantly reduced in obese females compared to non-obese females, also obese females had lower  $FEV_1/FVC$  ratio compared to non-obese females. Obesity had significant impact on Dynamic Pulmonary function tests in young adult females of Hubli city.

**Keywords:** Obesity; Adult Women; Forced Vital Capacity(FVC); Forced Expiratory volume in One Second ( $FEV_1$ ); Ratio of Forced Expiratory Volume in one Second and Forced Vital Capacity( $FEV_1/FVC$ ).

## INTRODUCTION

Obesity can cause decline in pulmonary function which may lead to early morbidity and mortality in the

population. These changes in the lung function in obesity are caused due to extra adipose tissue in the chest wall and abdominal cavity, compressing the thoracic cage, diaphragm and lungs, which can lead to decrease in lung volumes.

Obesity can cause various hazardous effects on respiratory function, such as changes in respiratory

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mechanics, reduced respiratory muscle strength and endurance, decrease in pulmonary gas exchange, lower control of breathing, limitations in pulmonary function tests and exercise capacity and may cause impairment of an individual health and quality of life. [14]

Obesity may affect several body systems and therefore, lead to higher morbidity and mortality rates in the population. Of all those affected, the respiratory system derives special attention because obesity promotes important change in its mechanics, gas exchange, Changes in respiration pattern.

### AIMS AND OBJECTIVES

The study was conducted in the department of physiology KIMS, Hubli, between December 2013 to October 2014. This study was done to attain following aims and objectives

To record and interpret Dynamic Lung Function Test parameters in non-obese adult females and obese adult females.

(i) Forced Vital Capacity (FVC)

(ii) Forced Expiratory Volume in one Second (FEV<sub>1</sub>)

(iii) Ratio of Forced Expiratory Volume in one Second and Forced Vital Capacity (FEV<sub>1</sub>/FVC).

2. To evaluate the impact of obesity on Dynamic Lung Function Test of adult women with no history of pulmonary disease.

### METHODOLOGY

The present study was done in the department of physiology, KIMS, Hubli. Dynamic Pulmonary Function tests (PFTs) of normal, healthy, non-obese females and healthy but obese females, age group 18-30 years of Hubli city were obtained and were compared. Criteria for obesity in our study were taken according to chart provided by WHO for categorization of BMI. The Dynamic Pulmonary function test was carried out with computerized Spirometer Eazy on-PC (model nddmedizintechnik AG), Zurich. The study and the control group were selected based on inclusion and exclusion criteria.

#### Inclusion Criteria:

Age 18-30 years

Individuals falling within the range of normal and obese Body mass Index.

Healthy Individuals

Sedentary

Non-Smokers

Exclusion Criteria:

1. Smokers

2. Patient showing obstructive or restrictive changes in the pulmonary function tests

3. Obstructive sleep apnea syndrome

4. History of hypertension, diabetes mellitus and cardiovascular diseases.

The protocol of the study was approved by the institutional ethical committee. The procedure was explained to the subjects and the importance of the test was also briefed to the subjects.

Then the selected group of subjects were categorized into non-obese and obese based on the chart provided by WHO for body mass index BMI(kg/m<sup>2</sup>).

<18.5	Underweight
18.5-24.99	Normal Weight
25- 29.99	Overweight
30 and above	Obese

**BMI:** was calculated based on the Quetelets index,

$$\text{BMI} = \text{Weight (in kgs)}/\text{Height}^2(\text{in meters})$$

The subject who had BMI < 30 kg/m<sup>2</sup> were considered as non- obese. The subjects with a BMI 30 kg/m<sup>2</sup> and above were considered as obese:

#### SPIROLYSER :

The evaluation of pulmonary function was performed by Spirometry using an Easy on- PC model, Computerized spirometer (niddmedzintechnik AG CH-8005) Zurich, Switzerland. It plugs directly into the USB port of a PC. It works on ultrasonic Doppler principle. The directly evaluated parameters were lung volumes, capacities, and Flow through the procedures of, Forced Vital Capacity (FVC) performed at least three times each,

according to the standards of American Thoracic Society (ATS) with the volunteers in the sitting position. Results were expressed as absolute values and as percentage of the reference predicted values. The FVC procedure allowed for the determination of the Forced Expiratory capacity (FVC), Forced Expiratory volume in one second ( $FEV_1$ ), Ratio of Forced Expiratory Volume in one second and forced vital capacity ( $FEV_1/FVC$ ).

## RESULTS

Evaluation of Dynamic Lung Function Test were carried out on both the groups by using computerized spirometer easy on-PC model. The obtained data was tabulated, analysed and expressed as Mean  $\pm$  Standard Deviation (Mean  $\pm$  SD) to assess anthropometric, and various Pulmonary Function Test parameters in the 2 groups. In order to compare the level of PFT parameters between the two groups, the unpaired student's 't' test was applied and statistical significance was indicated by 'P' value less than 0.05 ( $p < 0.05$ ). Statistical software, SPSS is used for the analysis of the data and Microsoft and Excel have been used to generate graphs, tables etc.

### ANTHROPOMETRIC DATA:

**Age (yrs):** The mean ( $\pm$ SD) age of obese females was  $24.82 \pm 3.2$  and of controls was  $22.98 \pm 3.3$ . There was no statistically significant difference between the two groups.

**Height (cm):** The mean ( $\pm$ SD) height in obese females was  $131.54 \pm 19.6$  and that in non-obese females was  $137.09 \pm 9.10$ . There was no statistically significant difference between the two groups.

**Weight(kg):** The mean ( $\pm$ SD) weight in obese females was  $75.44 \pm 9$  and in non-obese females was  $58.74 \pm 9.9$ . There was statistically significant difference between the two groups.

**Body Mass Index (BMI):** The mean ( $\pm$ SD) in obese females was  $33.72 \pm 3.54$  and in non-obese females was  $22.71 \pm 2.8$ . There was statistically highly significant difference between two groups ( $p < 0.001$ ).

**Forced Vital Capacity (FVC):** The obese females had FVC (litres ) of  $2.31 \pm 0.41$  whereas corresponding values in non-obese  $2.87 \pm 0.66$ . There was statistically highly significant differences between two groups ( $p < 0.01$ ).

**Forced Expiratory volume in First Second ( $FEV_1$ ):** The obese females had  $FEV_1$  (litres ) of  $1.94 \pm 0.38$  whereas corresponding values in non-obese was  $2.4 \pm 0.61$ . There was statistically highly significant differences between two groups ( $p < 0.01$ ).

**$FEV_1/FVC$ :** The obese females had  $FEV_1/FVC$  (litres ) of  $0.83 \pm 0.08$  whereas corresponding values in non-obese was  $0.84 \pm 0.11$ . There was no statistically significant differences between two groups.

**Table 1. ANTHROPOMETRIC DATA OF OBESE FEMALES AND CONTROLS**

Mean with SD

	No. of Subjects	Age Years	Height Cm	Weight Kg	BMI Kg/m <sup>2</sup>
<b>Obese Females</b>	50	$24.82 \pm 3.20$	$131.5 \pm 19.6$	$75.44 \pm 9.79$	$33.72 \pm 3.54$
<b>Controls</b>	50	$22.98 \pm 3.35$	$137 \pm 9.1$	$58.74 \pm 9.9$	$22.71 \pm 2.89$
<b>P Value</b>		$> 0.05$ (NS)	$> 0.05$ (NS)	$< 0.01$ (HS)	$< 0.01$ (HS)

**Table 2: DYNAMIC LUNG FUNCTION TEST PARAMETERS OF OBESE FEMALES AND CONTROLS**

	No of subjects	FVC Litres	$FEV_1$	$FEV_1/FVC$
<b>OBESE</b>	50	$2.31 \pm 0.41$	$1.94 \pm 0.38$	$0.84 \pm 0.11$
<b>CONTROLS</b>	50	$2.87 \pm 0.66$	$2.40 \pm 0.61$	$0.83 \pm 0.08$
<b>P value</b>		$< 0.05$ (S)	$< 0.05$ (S)	$> 0.05$ (NS)

## DISCUSSION

Many studies have demonstrated an association between obesity and ventilatory abnormalities in adult females. The present study showed, FVC, FEV<sub>1</sub> values were significantly reduced in obese females compared to non-obese females. Obese females had lower FEV<sub>1</sub>/FVC ratio compared to non-obese but not much statistically significant which rules out obstructive pattern of airway disease.

The cause of decrease in Forced Vital Capacity parameters may be due to decrease in distensibility of chest wall or limited expansion of thoracic cavity or limited expansion of chest cavity.<sup>[5]</sup> Intra-abdominal pressure had a mechanical effect on the diaphragm which was suspected of being major reason for the association of obesity with lung dysfunction.<sup>[3]</sup> FEV<sub>1</sub>/FVC ratio declines with age but abnormally reduced ratios suggest airway obstruction, normal or increased ratio does not rule out obstruction.

Forced Vital Capacity(FVC): Shashi Mahajan et al. in their study have found decline in FVC in Obese individuals. The cause of decline of FVC may be due to decrease in distensibility of chest wall or limited expansion of thoracic cavity or limited expansion of chest cavity.<sup>[5]</sup> D. Canoyl.R et al. have investigated the relation between abdominal pattern of obesity and respiratory function in European Prospective Investigation into Cancer and Nutrition-Norfolk (EPIC-Norfolk) found FVC were decreased with increasing waist: Hip ratio in women.<sup>[6]</sup> Chen et al. in their study have found negative association between BMI and FVC in obese subjects when compared to the normal weight subjects. Intra-abdominal pressure had a mechanical effect on the diaphragm which was suspected of being major reason for the association of obesity with lung dysfunction.<sup>[3]</sup>

Anuradha R Joshi et al. they have found that FVC was inversely correlated with body fat. The amount of body fat and a central pattern of fat distribution might be related to lung function via several mechanisms, such as mechanical effects on the diaphragm (impending descent into abdominal cavity) and on the chestwall primarily due to the changes in compliance and in the work of breathing and elastic recoil.<sup>[7]</sup>

Bharat Thyagarajan et al. have observed in their study that there is a strong association between lung function and BMI and that the FVC and FEV<sub>1</sub> generally

decreased over a 10 year period both with higher baseline BMI and with increasing BMI over 10 years of follow up. They have also observed that the lung functions were maintained at high levels in thinnest people even through age.<sup>[12]</sup>

### Forced Expiratory Volume to first second (FEV<sub>1</sub>):

In the present study obese females had lower FEV<sub>1</sub> compared to non-obese females. This was statistically highly significant (P<0.01). Lynell C. Collins et al. in their study have found lower FEV<sub>1</sub> in Obese individuals than non-obese. In Obese subjects, the intra-abdominal adipose pressing upwards on the diaphragm prevents full downward excursion during deep inspiration.<sup>[1]</sup>

H M Ochs-Balcom et al. in their study have found the association of total body adiposity and abdominal adiposity with FEV<sub>1</sub> and FVC in a random sample of the population in western New York state. They hypothesized that a specific effect of fat distribution on pulmonary function exists. In particular, they hypothesized that a greater accumulation of abdominal fat is associated with lower levels of FEV<sub>1</sub> and FVC, and that abdominal fatness is a better predictor of reduced pulmonary function than total body adiposity. Their results suggested that both overall and abdominal adiposity are negatively associated with FEV<sub>1</sub> and FVC<sup>[2]</sup>

Yu chen et al. In their study have found a consistent association between Waist circumference and pulmonary function in subjects with normal weight, overweight and obesity. The negative association between BMI and FEV<sub>1</sub> was observed only in the overweight and obese. BMI was positively associated with FEV<sub>1</sub> in normal-weight subjects. Intra-abdominal pressure that has a mechanical effect on the diaphragm is suspected of being a major reason for the association of obesity with decreased FEV<sub>1</sub>.<sup>[3]</sup>

Results of our study is consistent with the study done by Rasslan et al. who found that obese women had significant decline in FEV<sub>1</sub> than normal group.<sup>[4]</sup>

Sekhri et al. collected spirometric results of 433 obese patients from both genders with BMI more than 40 kg/m<sup>2</sup> and reported that BMI had a significant negative impact on FVC, FEV<sub>1</sub> in both men and women.

Sutherland et al. included 55 female aged 20–50 years in their study and grouped them into normal, overweight and obese. There was a trend for a decrease in



FEV<sub>1</sub>, FVC with increasing BMI.

In a recent Asian study of Saxena et al. reported that obese women had a significant lower FEV<sub>1</sub> and FVC than normal group and there was a significant negative association between BMI and these dynamic lung volumes.<sup>[8]</sup>

Zerah F et al. in their study have found Morbid obesity was associated with altered FEV<sub>1</sub> with preservation of FEV<sub>1</sub>% which indicates that FEV<sub>1</sub> were decreased to the same extent, therefore obesity is said to be associated with restrictive pattern of lung impairment. This may be explained on the grounds that obesity exerts a direct mechanical effects on the diaphragm and applying pressure on the thoracic cage and lungs causing mechanical limitation of expansion during the FVC maneuver.<sup>[9]</sup>

FEV<sub>1</sub>/FVC: In the present study obese females had FEV<sub>1</sub>/FVC 0.83±0.08 and correspondent controls had FEV<sub>1</sub>/FVC 0.84±0.11. Obese females had lower FEV<sub>1</sub>/FVC compared to non -Obese females, this was not statistically significant.

Kalpana B et al. in their study have found ratio of FEV<sub>1</sub>/FVC in obese individuals when compared to the control group was found to be reduced ,this FEV<sub>1</sub>/FVC ratio was within normal range (above 80%) and hence rules out any obstructive pattern of lung disease in obese group .<sup>[10]</sup>

The present study is consistent with the study done by Mohammed Al Ghobain et al. where they found no significant differences in FEV<sub>1</sub>/FVC between the obese and non-obese subjects.<sup>[11]</sup>

Shashi Mahajan et al.in their study have stated that obesity influences the respiratory function . The cause of decline of various respiratory functions in obesity may be due to decrease in distensibility of chest wall or limited expansion of thoracic cavity and is the cause for reduced ventilator volumes and total lung capacity. Because of increase in elastic recoil pressure, the ratio FEV<sub>1</sub>/FVC may be greater than normal. But these hazardous effects of gaining weight might be reversible and weight loss can improve lung function in obesity, so obese patients benefit from weight loss by improved pulmonary mechanics.<sup>[5]</sup>

## CONCLUSION

A comparative study of pulmonary function tests in obese adult women in and around Hubli was conducted in the department of Physiology, KIMS, Hubli,

This type of study is entirely new to this geographical area and this study intends to find the alteration in the pulmonary functions in young adult obese females as compared with the normal weight individuals, particularly in this part of the country.

The present study dynamic Lung Function Test Parameters FVC, FEV<sub>1</sub> values were significantly reduced in obese females compared to non- obese females. Obese females had lower FEV<sub>1</sub>/FVC ratio compared to non-obese but not much statistically significant which rules out obstructive pattern of airway disease.

Obesity had significant impact on Dynamic Pulmonary function tests in young adult females of Hubli city.

**Conflict of Interest:** Nil

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# Relationship between body Mass Index and Blood Pressure among Healthy Adult Individuals in Tirupati

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## ABSTRACT

**Aims and objectives :** This study was designed to see the relationship between body mass index and blood pressure among south Indian young healthy adults.

**Method:** The present study was carried out on 100 adults including 62 males and 38 females whose heights(meters) and weights(kg) were measured, and body mass indices were calculated by using Quetelet's index. The blood pressures were recorded, PP and MAP were calculated.

**Results:** The mean BMI levels were found to be significantly ( $P=0.007$ ; S) higher in male subjects ( $23.76 \pm 3.75$ ) compared to female subjects( $21.63 \pm 3.75$ ). The mean Systolic Blood Pressure levels were found to be significantly ( $P<0.001$ ; S) higher in males ( $121.62 \pm 10.43$ ) than in female ( $107.63 \pm 14.08$ ) subjects. The BMI showed a positive correlation with all types of blood pressure values like SBP ( $P<0.001$ ; S), DBP ( $P=0.048$ ; S), PP ( $P<0.001$ ; S) and MAP ( $P<0.001$ ; S). The correlation coefficients were also statistically significant.

**Conclusions :** These findings show positive relationship between BMI and Blood pressure among healthy adults of south India.

**Keywords:** Adult Individuals, Body Mass Index, Blood Pressure, Hypertension, Obesity.

## INTRODUCTION

Obesity is one of the greatest challenges being faced by global health experts today<sup>(1)</sup>. It exerts adverse effects on health through multiple organ systems of the body and reduces life expectancy<sup>(2)</sup>.

Its associated complications are type 2 diabetes mellitus, cardiovascular diseases and some cancers.<sup>1</sup> The growing burden of these complications is projected to result in substantial increases in health care expenditures and productivity loss<sup>(1)</sup>.

There is a positive association between measures of obesity and blood pressure in both developed and the developing countries. In developing countries the prevalence of obesity and hypertension are known to rise with increasing urbanisation<sup>(3)</sup>.

Body mass index (BMI) is positively and independently associated with morbidity and mortality from hypertension, cardiovascular disease, type II diabetes mellitus and other chronic diseases<sup>(4)</sup>.

Therefore, the present study is aimed to evaluate the association of Body Mass Index with blood pressure and to investigate the prevalence of hypertension in urban young adult population of Tirupati.

## Study Design:

The present study was carried out on 100 individuals comprising 62 males and 38 females in Sri Venkateswara

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Medical College, Tirupati. The subjects comprised of young males and females aged 18-30 years. The subjects were explained about the essence of the study and written consent was taken from them. After collecting their details according to the proforma, their heights and weights were measured with height-weight scale, and their BMI is calculated. Their blood pressures were recorded using a mercury sphygmomanometer with appropriate cuff size.

#### Inclusion Criteria:

1. Age between 18 to 30 years
2. Males & Females
3. Healthy and Volunteering

#### Exclusion Criteria:

1. Age < 18 years and > 30 years
2. Non Volunteering
3. Presence of cardiac, respiratory, renal, neurological and haematological disorders

## METHOD

The body weight of each subject was recorded while putting on light weighted clothing and shoes off. Height was measured with their shoes off and they were made to stand against the height scale with their Calcaneus, Gluteus and Occiput touching it. Body Mass Index was determined from weight (Kg) and height (meters). The subjects were classified into normal, overweight and obese based on their body mass indices according to new WHO guidelines of BMI for Asians.

#### BMI Nutritional status

Below 18.5	Underweight
18.5–23	Normal weight
23 – 25	overweight
25– 27	Obesity class I
27– 39.9	Obesity class II
Above 40	Obesity class III

The blood pressures were recorded by using manual sphygmomanometry. The blood pressures were recorded after resting the subjects for five minutes in sitting

position. Two readings were taken with a time gap of 5 minutes and their average was taken for accuracy. Pulse pressure and Mean arterial pressure were calculated from systolic and diastolic blood pressures. The subjects were classified into normal and hypertensive according to JNC VIIth report.

#### Recommendations of the joint national committee on the diagnosis, evaluation, and treatment of hypertension for classifying and defining blood pressure levels for adults (aged 18 years and older)

Category	Systolic(mmHg)	Diastolic (mmHg)
<b>Normal</b>	<130	<85
<b>High normal</b>	130-139	85-89
<b>Hypertension</b>		
Stage 1 (mild)	140-159	90-99
Stage 2 (moderate)	160-179	100-109
Stage 3 (severe)	180-209	110-119
Stage 4 (very severe)	≥210	≥120

## RESULTS

The present study investigated the correlation between Body Mass Index and Blood pressure in apparently healthy subjects aged 18 - 30 years and most of the study subjects belong to 18 – 21 years' age group (46.0%) followed by 22 – 25 years' age group (34.0%) and 26-30 years (20.0%) respectively.

**Table 1: Age distribution**

Age group (Years)	No. of study subjects	Percentage
18 – 21	46	46.0
22 – 25	34	34.0
26 – 30	20	20.0
<b>Total</b>	<b>100</b>	<b>100.0</b>

Height, weight and blood pressure were recorded. BMI was calculated by using Quetelet's index.

$$\text{Quetelet's index} = \text{Weight ( Kg) / Height ( m}^2\text{)}$$

The data was obtained from one hundred subjects including 62 males and 38 females.

**Table 2: Gender distribution**

Gender	No. of study subjects	Percentage
Male	62	62.0
Female	38	38.0
<b>Total</b>	<b>100</b>	<b>100.0</b>

A higher proportion of the subjects are males (62.0%) compared to females (38.0%) on the whole.

**Table 3: Certain Anthropometric data by Gender**

S. No	Variable (Mean ± SD)	Male	Female	Statistical significance
1	Height	169.77 ± 5.8	158.84 ± 6.03	t= 8.69; P<0.001; S
2	Weight	68.30 ± 9.82	54.73 ± 10.93	t= 6.42; P<0.001; S
3	BMI	23.76 ± 3.75	21.63 ± 3.75	t= 2.75; P=0.007; S

Table-5 is showing certain anthropometric data by gender. The mean height of the males is 169.77 ± 5.8 where as in females it is 158.84 ± 6.03. The mean weight of males is 68.30 ± 9.82 whereas in females it is 54.73 ± 10.93. The mean BMI in males is 23.76 ± 3.75 in females it is 21.63 ± 3.75.

The mean height, weight as well as BMI levels are found to be significantly higher in male subjects compared to female subjects.

**Table 4: Mean BMI levels by age group (Males & Females combined)**

S.No	Age group (Years)	No. of study subjects	BMI levels (Mean ± SD)
1	18 – 21	46	22.01 ± 4.75
2	22 – 25	34	23.57 ± 2.66
3	26 – 30	20	24.05 ± 2.93

$F=2.65; P=0.075; NS$

Table-6 is showing the mean BMI levels by age group in both the genders. In the age group of 18-21 years the mean BMI level is 22.01 ± 4.75. In the age group of 22-25 years the mean BMI level is of 23.57 ± 2.66. The mean BMI levels in the age group of 26-30 years is 24.05 ± 2.93.

The mean BMI levels steadily increased with increase in the age group of the study subjects being lowest in 18-21 years and highest in 26 – 30 years age groups. The differences are however not found to be statistically significant. (p = 0.075; NS)

**Table 5: Mean BMI levels by age group in males and females (compared)**

Age group (Years)	Males	Females	Statistical significance
18 – 21	23.55 ± 5.92	20.83 ± 3.26	t= 1.98; P=0.053; NS
22 – 25	23.84 ± 2.06	22.30 ± 4.63	t= 1.30; P=0.20; NS
26 – 30	23.90 ± 2.47	24.39 ± 4.07	t= 0.33; P=0.74; NS

Table-5 is showing the comparison of mean BMI levels by age group in both the genders. The mean BMI levels are found to be higher in males than in females among 18-21 years' and 22-25 years' age groups. In the 26-30 years' age group, the mean BMI level is found to be higher in female than in male subjects. The differences between male and female subjects are however not found to be statistically significant in any age group.

**Table 6: Mean SBP (Systolic Blood Pressure) levels by gender**

Gender	No. of study subjects	SBP (Mean $\pm$ SD)
Male	62	121.62 $\pm$ 10.43
Female	38	107.63 $\pm$ 14.08

$F=32.3$ ;  $P<0.001$ ; S

Table-6 is showing mean systolic blood pressure levels by gender. In males the mean systolic pressure is 121.62  $\pm$  10.43. In females the mean systolic pressure is 107.63  $\pm$  14.08. The mean Systolic Blood Pressure levels are found to be higher in male than in female subjects. The difference is also found to be statistically significant ( $P<0.001$ ; S).

**Table 7: Correlation between BMI and various levels of blood pressures (Overall)**

Variable	Correlation coefficient ( $\gamma$ )	F ratio	P value & Significance
Systolic Blood Pressure	0.62	62.12	<0.001; S
Diastolic Blood Pressure	0.2	3.97	0.048; S
Pulse Pressure	0.57	49.1	<0.001; S
Mean Arterial Pressure	0.42	20.9	<0.001; S

The BMI showed a positive correlation with all types of blood pressure values like SBP ( $P<0.001$ ; S), DBP ( $P=0.048$ ; S), PP ( $P<0.001$ ; S) and MAP ( $P<0.001$ ; S). The correlation coefficients are also statistically significant.

**Table 8: Correlation between BMI and various levels of blood pressures (in Males)**

Variable	Correlation coefficient ( $\gamma$ )	F ratio	P value & Significance
Systolic Blood Pressure	0.51	22.71	<0.001; S
Diastolic Blood Pressure	0.1	0.40	0.52; NS
Pulse Pressure	0.6	35.6	<0.001; S
Mean Arterial Pressure	0.14	1.36	0.24; NS

The BMI showed a positive correlation with all types of blood pressure values like SBP, DBP, PP and MAP. However the correlation coefficients are statistically significant with regard to Systolic blood pressure ( $P<0.001$ ) and pulse pressure ( $P<0.001$ ).

**Table 9: Correlation between BMI and various levels of blood pressures (in Females)**

Variable	Correlation coefficient ( $\gamma$ )	F ratio	P value & Significance
Systolic Blood Pressure	0.67	30.28	<0.001; S
Diastolic Blood Pressure	0.57	17.49	<0.001; S
Pulse Pressure	0.4	6.82	0.013; S
Mean Arterial Pressure	0.67	29.0	<0.001; S

The BMI showed positive correlation with all types of blood pressure values like SBP ( $P<0.001$ ; S), DBP ( $P<0.001$ ; S), PP ( $P=0.013$ ; S) and MAP ( $P<0.001$ ; S). The correlation coefficients are also statistically significant.

## DISCUSSION

Overweight and obesity represent a rapidly growing threat to the health of populations in an increasing number of countries. Indeed they are now so common that they are replacing more traditional problems such as undernutrition and infectious diseases as the most significant causes of ill-health. Obesity comorbidities include coronary heart disease, hypertension and

stroke, certain types of cancer, non-insulin dependent diabetes mellitus, gall bladder disease, dyslipidaemia, osteoarthritis and gout, and pulmonary diseases, including sleep apnoea. In addition, the obese suffer from social bias, prejudice and discrimination, on the part not only of the general public but also of health professionals, and this may make them reluctant to seek medical assistance<sup>(5)</sup>.

Body mass index is the frequently used measure of obesity or body fatness. Obesity has been frequently known to be associated with various cardiovascular diseases like hypertension, diabetes, dyslipidaemia, etc. as said above<sup>(6)</sup>. Hence, the relationship between

BMI and blood pressure is of crucial interest in evaluating both public health and the clinical impact of so-called obesity epidemic<sup>(7)</sup>. An attempt has been made to determine the relationship between body mass index and blood pressure among healthy adult individuals.

The results obtained are tabulated and analysed in the light of available data in similar situations. The present study was carried out to show the correlation between Body Mass Index and blood pressure in apparently healthy subjects of Tirupati, aged about 18 - 30 years so as to show the growing burden of obesity in this place which is moving towards urbanisation. The present study was conducted among 100 subjects.

### CONCLUSION

The results conclude that there is a relation between BMI and blood pressure in the young adult individuals. The present study shows that there is an increase in both BMI and Blood pressure at an earlier age which accounts for the changing life style and socio-economic patterns in Tirupati which may be responsible for severe cardiovascular and other complications of obesity in later ages and requires public awareness about obesity

and hypertension. Appropriate preventive measures and intervention programmes must be encouraged in the young individuals to reduce the prevalence of obesity and hypertension.

**Ethical Clearance:** Taken from Institutional ethical committee, Sri Venkateswara Medical College, Tirupati, Andhra Pradesh.

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# Comparative Study of the Dynamic Pulmonary Function Tests between Jeans Washer Men and Normal Individuals in Ballari

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## ABSTRACT

**Background:** Ballari, popularly known as Jean capital of India. Jeans washer men are exposed to chemicals that cause lung diseases in the long run. Till now there are very few international studies done on these populations regarding their respiratory health. So this study may give some insights of the occupational respiratory diseases in jeans washer men of Ballari. **Objective:** To study the variations in the dynamic PFT's in jeans washer men who were exposed to the harmful chemicals for more than 5 years and comparing them with the normal healthy individuals who were not exposed to the harmful chemicals. **Materials and Method:** The sample populations (100) were selected from both the rural and urban population of Ballari. Dynamic PFT's: FVC, FEV1, FEV1/FVC, FEF 25-75 % and PEFr were measured by using RMS Helios 401 spirometer. **Results:** There was a statistically significant decrease in the level of FEV1/FVC %, FVC, FEV1, FEF 25-75% and PEFr in cases compared to controls. Among cases 26% of them were suffering from early small airway obstruction & mild restriction, 22% of them from early small airway obstruction & mild obstruction & 84% of them were suffering from restrictive type of COPD severity. **Conclusion:** The lowering of all the dynamic PFT's compared to controls suggested a combination of restrictive & obstructive patterns in their lungs. Reduction in their dynamic lung functions was possibly associated with exposure to chemicals for more than 5 years duration.

**Keywords:** Occupational Exposure, PFT, COPD.

## INTRODUCTION

Blue jeans are big business, and in recent years fashion designers have created new demand for denim which comes with a pre-worn look<sup>1</sup>. Ballari, popularly known as Jean capital of India, is the second important garment cluster in Karnataka. It is known for its specialization in manufacturing jeans for men<sup>2</sup>.

Work related asthma continues to be one of the most common disorders among all occupationally induced

lung diseases throughout the world<sup>3</sup>. Occupational exposures to dust and gases are associated with increased prevalence of respiratory symptoms<sup>4</sup>. Exposure-response relations between occupational agents and chronic respiratory symptoms have also been reported<sup>5</sup>. Periodic testing in workers can detect pulmonary disease in its earlier stages when corrective measures are more likely to be beneficial. Till now there are very few international studies done on these populations regarding their respiratory health. Magazines circulated in China regarding the Deadly Denim indicated that the sandblasting & various chemicals used in the jeans factories caused respiratory problems in the long run<sup>1</sup>. Chemicals like Potassium permanganate used to spray the Jeans & others like artificial colours, acids are known to cause the chemical injury to the lungs. These chemicals also cause allergic reactions to skin according

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to the study done on these jeans washer men of Ballari <sup>6</sup>. Studies done in turkey have proved that sandblasting of jeans also leads to silicosis <sup>7&8</sup>. There are no comparative studies done on dynamic PFT's on these jeans washer men till now. So this study may give some insights of the occupational respiratory diseases in jeans washer men of Ballari, India. In view of the fact that various airborne particulate, dust, chemicals puts the workers health into jeopardy and most of the workers in India do not use protective measures and no earlier study in these jeans washer men has been reported in India till now. Hence this Case-control study was undertaken to assess the effect of chemicals and fumes exposure on PFT of jeans washer men. In the present study objective is to compare the dynamic PFTs between jeans washer men who were exposed to the chemicals and fumes for more than 5 years with the normal healthy individuals who were not exposed to the harmful chemicals.

## MATERIALS AND METHOD

The sample population for the above study was selected from both the rural and the urban population of Ballari. Total sample size was 100. Two study groups were selected from the above sample population. Written informed consent was taken from each participant. Detailed clinical history was taken, using a structured questionnaire to conduct the various examinations. Each group had a sample size of 50 subjects who were selected using simple random sampling. Group 1 is the case group, consisting of fifty male jeans washer men who were exposed to chemicals and fumes during working hours in jeans factory for more than 5 years <sup>9</sup>. Age group is between 25-45 years with minimal respiratory symptoms of occupational lung disease. The work is of three 8-hour shifts/day for seven days/week. Group 2 is the control group. For this group fifty healthy male volunteers were selected from the study population other than jeans workers, not exposed to the dust, chemicals and gases in their lifetime, who were working in VIMS campus, Ballari, as security guards. All the subjects were subjected to computerized PFT. The data collected was tabulated and subjected to statistical analysis using SPSS version.21 software. (Standard deviation, mean and unpaired student T test). Microsoft Word 7 and Microsoft Excel 7 have been used to create tables etc. Study design: Case-control study. Exclusion criteria: Persons with history of respiratory disease, cardiac disease, neurological diseases, abuse of psychoactive substances, habits of smoking, allergy to other than chemicals used in

Jeans factories. Persons with family and personal history of psychiatric disease & who has not given consent. The Instruments: Examination proforma used for recording history and clinical examination findings was designed and validated to collect data on smoking, socioeconomic status, past history of pulmonary diseases, current respiratory symptoms (chronic cough, chronic phlegm, wheezing and whispering, breathlessness, dyspnea), education, job exposure matrix, and other parameters. Portable weighing machine (Samsco personal weighing scale of capacity 150 Kg) - to record the body weight in kilograms. Measuring tape- to measure the standing height in centimetres. Mercury sphygmomanometer (Diamond deluxe) for recording the blood pressure. Stethoscope (Revival) to auscultate the heart and respiratory sounds. Portable computerized spirometer of RMS company, Model- Helios-401, attached to the laptop for recording the pulmonary function tests. Glutaraldehyde solution 5% (Korsolex Rapid) for sterilizing the turbine transducer of Helios 401 and mouth pieces to avoid cross contamination. Spirometer is a simple method for studying pulmonary ventilation and to record the volume of air moved into and out of the lungs. It is a safe and simple test and it is used to classify respiratory diseases (whether obstructive or restrictive), establish prognosis, suggest treatment and they are research oriented. Based upon the subject's age, gender, height and weight data, and the Spirometry equation selected, numerical values of the parameters were predicted. The spirometer equation selected for this study was according to ERS 93 (European respiratory society). The actual values obtained during a manoeuvre were listed under %PRED (Predicted) as a percentage of the predicted values. An interpretative result of the FVC manoeuvre was given by plotting the results. Interpretation was only performed for the FVC test. Depending on where the values of FVC% PRED and (FEV1/FVC)% PRED lie, the patient's lung condition was suggested to be: Normal (NORM), Restrictive (RES), Mixed (MIXED) & Obstructive (OBS) <sup>10</sup>. Recommendations for spirometer performance and validation have been published by the American Thoracic Society (ATS) <sup>11</sup> & European Respiratory Society (ERS). Flow Spirometer measures how quickly air flows past a detector and then derives the volume by electronic means. They record the flow rate at very brief intervals such as 30 to 300 times a second and use the data obtained to reconstruct the flow rate at each point in time and volume. This process is called as digitization. Nowadays

computerized spirometers are used for the assessment of pulmonary function test; it is useful to measure large number of functional respiratory parameters. The test results are compared with normal (or) predicted values. The transducer used with the RMS Helios 401 spirometer was a turbine type transducer. The transducer converted the flow of air, breathed by the patient against a frictionless rotating vane, into an electrical signal which was used to produce the relevant plots. Methods: Ethical clearance was taken from the VIMS; Institution before conducting this study. A detailed history was obtained and recorded from the case and control group in a prescribed pretested and semi-structured proforma on socio-economic and demographic variables. The tests were carried out on the subjects in a relaxed state and privacy was given utmost importance. Recording of physical anthropometry of subjects: Height (in cms) of the subjects was measured in standing and erect posture. Weight (in Kgs) was recorded using standard weighing machine both for study and control group in standing posture. BMI was calculated using the Quetelet index, is a measure of relative weight based on an individual's mass and height. IOTF-proposed classification of BMI categories for Asia were applied to the cases. BSA was calculated using the Dubois and Dubois formula. Recording of physiological parameters of subjects: Pulse rate: It was expressed as beats per minute. Right radial pulse was examined by compressing radial artery in the semi -pronated forearm and slightly flexed wrist

of the subject. Respiratory rate: It was recorded by inspection and palpation of chest and abdomen and was expressed as cycles per minute. Blood pressure: It was measured by mercury sphygmomanometer in mm of Hg by palpatory and auscultatory methods. Technique of pulmonary function tests: The pulmonary function tests: FVC, FEV1, FEV1/FVC, FEF 25-75 % and PEFr were measured by using RMS Helios 401 spirometer using the standard laboratory methods. A disposable mouth piece was inserted into the hand piece of Helios 401 firmly. The mouthpiece was inserted well into the mouth of the subject (beyond the teeth) and the mouth must be closed around the mouthpiece to ensure that air cannot escape from the sides of the mouth. A nose clip was fitted to the nose of the subject to ensure that no air can escape through the nostrils. The subject was asked to breathe at rest for a few moments, and then inspire slowly as much air as possible and then make a complete expiration as fast as possible, to complete the cycle by inspiring again as quickly as possible. Each subject produced at least three acceptable FVC curves based on ATS standards.<sup>11</sup> The equipment used for pulmonary function measurements was calibrated using a 3 L syringe, before and after each day's use without any significant differences being found. As the spirometer was a flow-measuring device, it was reasonable to neglect the body temperature pressure saturated (BTPS, temperature-37° C, ambient pressure, saturated with water vapour at 37° C) conversion under environmental conditions.

### Results: Table-1: Comparison of vital parameters

Vitals	Groups		P value
	Cases	Controls	
	(Mean±SD)	(Mean±SD)	
Respiratory rate (n/min)	17.6±1.3	14.8±1.3	<0.05 (S)
Pulse rate (beats/min)	83.5±4.2	78.4±6	<0.05 (S)
SBP (mmHg)	118.4±5.2	116.8±6.2	0.16 (NS)
DBP (mmHg)	77.4±6.8	75.6±5.2	0.12 (NS)

HS- Highly significant, S- Significant, NS- Not significant



**Table-2: Comparison of Lung volumes**

Dynamic PFT	Groups		P value
	Cases	Controls	
FVC	75.3±11.3	94±6.8	<0.05 (HS)
FEV1	73.2±9.5	96.6±8.7	<0.05 (HS)
FEV1/FVC	97.9±8.4	102.2±4	<0.05 (S)
FEF	66.2±19.3	89.1±12	<0.05 (HS)
PEFR	66.2±12.2	82±10.5	<0.05 (HS)

**Table-3: Pre medical report indicates**

Sl.No.	Report	Cases
1.	Normal	1(2%)
2.	Early small airway obstruction & mild restriction	13(26%)
3.	Early small airway obstruction & moderate restriction	5(10%)
4.	Early small airway obstruction & mild obstruction	11(22%)
5.	Early small airway obstruction & moderate restriction	2(4%)
6.	Early small airway obstruction & mixed blockage	8(16%)
7.	Early small airway obstruction & normal	2(4%)
8.	Mild restriction	5(10%)
9.	Moderate restriction	3(6%)
	<b>Total</b>	<b>50 (100%)</b>

**Table-4: Pre test COPD severity**

Sl.No.	COPD	Cases
1.	Normal	8(16%)
2.	Moderate	0
3.	Restrictive	42(84%)
	<b>Total</b>	<b>50 (100%)</b>

## DISCUSSION

Age, height, weight, BMI & BSA were significantly matched between cases and controls. Mean pulse rate and respiratory rate were significantly high in cases compared to the controls as shown in the Table 1. The mean of dynamic PFT were significantly low in cases compared to controls as shown in Table-2. Among cases 26% of them were suffering from early small airway obstruction & mild restriction & 22% from early small

airway obstruction & mild obstruction suggested an 84 % of restrictive type of COPD severity according to

European Respiratory Society 97<sup>10</sup> & 11<sup>11</sup>.

High pulse rate and respiratory rate were seen in asymptomatic cases compared to controls indicating early lung dysfunction. In this study, there was a statistically significant decrease in the level of FEV1/FVC % and statistically highly significant decrease in the levels of FVC, FEV1, FEF 25-75% and PEFR (Table

3) in cases. All the dynamic PFT's decreased values in cases representing mixed type of lung disorders & Pre test COPD severity according to ERS 97 shows 84 % of restrictive type of COPD severity. This restrictive type of COPD severity may be attributed to the exposure of chemicals in jeans factories causing chemical injury to the lungs. "*Acid fumes used in the manufacture of dyes cause bronchitis & reduced PFT. Exposure to certain highly reactive, low molecular weight agents used in the manufacture of synthetic polymers, paints and coatings are associated with high risk of occupational asthma.*"<sup>12</sup> In our study exposure to acidic fumes & potassium permanganate may be the causative factor for restrictive type of COPD severity. "*Although this occupational asthma manifests clinically as if sensitization has occurred, an IgE mediated antibody mechanism is not necessarily involved*"<sup>12</sup>. Similar finding like elevated absolute eosinophil count was reported in the jeans washer men, which may be attributed to occupational asthma.<sup>6</sup>

Till now there are very few comparative studies done, comparing dynamic PFT's among jeans washer men & normal individuals. In this study we have proved that jeans workers show a mixed (restrictive & obstructive) pattern of dynamic PFT's compared to controls. Sand blasting & chemicals used in the jeans factories were the main cause for silicosis among jeans washer men according to some studies done in China & Turkey<sup>7 & 8</sup>. Sandblasting technique is not used in jeans factories of Ballari but certain chemicals like potassium permanganate, artificial colours and acids are used in terms of spraying to the jeans denim clothes that caused mixed pattern of lung disorders which may be attributed to occupational asthma.

The present study would have been better with a larger sample size. Assessment of respirable particulate matter in and around the working place of workers that affected the lung functions were not measured because of non availability. Diffusing capacity of lung studies would have made the study better. Other investigations which would have made definite diagnosis of the affected workers like chest radiograph were not done. Hence further studies should be done on these jeans washer men to elaborate on the cause of the mixed type of lung disorders.

## CONCLUSION

Evaluation of the dynamic PFT by computerized spirometer in jeans factories of Ballari with paucity of related symptoms of early lung dysfunction constitutes an important feasible and reproducible screening technique. It should be included as a routine in the periodic assessment of jeans wash factory workers as it often uncovers early lung dysfunction even in the asymptomatic state. Even though the cases did not have many respiratory symptoms except tachypnea compared to controls they did have underlying mixed (restrictive & obstructive) patterns of lung functions. The lowering of all the dynamic PFT compared to controls suggested a combination of restrictive & obstructive patterns in their lungs. Majority of the cases (26%) had early small airway obstruction and mild restriction suggested a restrictive type of COPD severity (84%). This reduction in their lung functions was possibly associated with exposure to chemicals, artificial colors and acids for more than 5 years duration and further they need investigations to rule out silicosis.

**Source of Funding-** Self

**Conflict of Interest -** Nil

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# Evaluation of Quality and Domains of Sleep Using Pittsburgh Index in Hospital Shift Nurses: An Observational Study

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## ABSTRACT

**Context:** Exposure to shift works especially to night shift goes against the Circadian rhythm of the social man which brings about a multitude of disruptive effects on health like sleep disturbances, day sleepiness, decreased cognitive performance, fatigue, increased risk of accidents, poor quality of life and vigilance troubles.

**Purpose:** Evaluate Sleep quality amongst hospital shift working nurses using Pittsburgh sleep index.

**Methodology:** Fifty of each night, day shift workers & those who never exposed to shift work participated in this study (n=150). Sleep quality & seven domains of sleep, subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleeping medication and daytime dysfunction was assessed using the Pittsburgh Sleep Quality Index (PSQI) questionnaire. Inter and intra group analysis done using ANOVA & Tukey's post hoc analysis.

**Results:** Global score of PSQI, subjective sleep quality, sleep duration and sleep medication were statistically high amongst night shift workers suggesting poor sleep quality compared to day shift and controls (p =0.021\*, p=0.021\*, p=0.00\*, p=0.00\*\*). About 52% (n=26) and 25% (n=12) of night and day shift workers reports poor quality of sleep.

**Conclusion:** Night shift nurses have poor quality of sleep when compared to day shift workers and those who were never exposed to shift work.

**Keywords:** *Circadian rhythms, Shift work, Sleep deprivation, Sleep quality.*

## INTRODUCTION

Sleep is an important biological function; it plays a role in various physiological processes of organisms.<sup>1</sup> Restful sleep provides the basis for physical, mental, and psychological well-being in humans, and sleep deprivation is associated with less productive behavior.<sup>2</sup> Despite common wisdom that sleep is important for sustaining performance, in the daily bustle of life, sleep is considered to be something of a minor nuisance – a

perfunctory part of the daily routine, akin to bathing, oral hygiene and waste elimination. Insufficient sleep is often the norm among many professions such as medical professionalisms, military personnel and shift workers.<sup>3</sup> Technological, Economic and Social pressures have led to an increase in the number of shift workers.<sup>4</sup> The exposure to shift works and especially to night shifts goes against the Circadian rhythm of the social man which brings about a multitude of disruptive effects on health like sleep disturbances, day sleepiness, decreased cognitive performance, fatigue and increased risk of accidents.<sup>5,6</sup> Night-shift work influences the quality of sleep.<sup>7</sup> Several studies have reported that impaired sleep is a common problem among nurses.<sup>8</sup> Among the dramatic effects of night shift are sleep and vigilance disorders, the alteration of the mental health and the quality of life of the exposed person.<sup>9</sup> Among night

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workers, 60% complain of sleep disorders and 30% of insomnia.

The term "Sleep Quality Measurement" includes assessing the quantitative aspects of sleep, such as sleep duration, sleep latency, and number of arousals as well as subjective aspects such as depth and restfulness of sleep. The Pittsburgh Sleep Quality Index (PSQI) is a self-rated questionnaire which assesses sleep quality and disturbances over a month time interval.<sup>10</sup>

Studies done by Mari SM, Matthew S shows the relationship between shift work, sleep quality in career emergency physicians, their data indicate the high incidence of disturbed sleep in this population leading to decline in short-term memory after day and overnight shifts.<sup>11</sup> Reza and Colleagues demonstrated effects of night shifts on cognition in relation to melatonin levels amongst control room operators and their data suggested to plan for an appropriate number of consecutive night shifts for improving alertness and to ensure safety.<sup>12</sup> We hypothesized; altered circadian rhythm as seen in shift workers will affect the quality of sleep and various domains of sleep, so the following study was conducted.

## MATERIALS AND METHOD

It is a cross sectional case-control study which was conducted in the department of physiology and hospital after obtaining the institutional ethical clearance.

Sample size- The study was conducted on 3 groups of people. Group I were shift nurses doing night shift duty from past one week (n=50) of age group (25-50). Group II were shift nurses who are doing day shift duty from past one week (n=50) of the age group (25-50). Group III were those females who are not exposed to shift work (n=50) of the age group (25-50) considered as controls. Sample size was decided based on previous references. Group I & II were selected randomly from nursing department based on willingness to participate in study. Group III were selected randomly from general population. Females with History of psychoactive substance use, Pregnancy, H/O of metabolic disorder, history of consumption of CNS affecting drugs, history of color blindness were excluded from the study.

Study design: An informed consent was taken before conducting the study. The consent was provided in English and Local languages stating that the values and the report will be kept confidential and will be used only

for study basis. History of any sleep related disorders were taken. The test was explained to the subjects and usual history taken.

**Assessment of sleep quality:** Pittsburgh sleep quality index (PSQI) was used to assess subjective sleep quality.<sup>10</sup> Subjects were asked to assess their sleep condition in the previous month. The PSQI is a self-rated questionnaire and comprises questions to assess seven domains of sleep, including subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleeping medication and daytime dysfunction. Each domain is rated on a 4-point scale (0–3), which generates a total score ranging between 0 and 21. In the questionnaire, 0 points indicated "no problems" while 3 points indicated a serious problem. The scores for the 7 components were added to form one global score.

**Statistical analysis-** Statistical analysis was done by analysis of variance (ANOVA). Intra and inter group analysis was done using tukey's post hoc analysis. Values were expressed as mean  $\pm$  SD. P values <0.05 considered as significant.

## RESULTS

It is a cross sectional study done to assess quality of sleep amongst shift workers. Values are expressed as mean $\pm$ SD. Table 1 demonstrating the demographical characteristics of the participants. The mean age group of Night shift workers, Day shift workers & controls who were never exposed to shift work were 33.50 $\pm$ 4.00, 31.23 $\pm$ 5.70 & 32.67 $\pm$ 5.00 years respectively. The night shift workers were on night shift working for an average of 10.98 $\pm$ 1.43 hours since an average of 6.75 $\pm$ 2.65 days. The day shift workers were on day shift working for an average of 8.34 $\pm$ 1.32 hours since an average of 7.43 $\pm$ 1.23 days. All the three groups were age matched.

Table 2 Demonstrating quality of sleep and its various domains assessed using Pittsburg sleep quality index questionnaire amongst participants. Table shows that global score of PSQI is statistically significant amongst the three groups. Intra group analysis shows that global score is significantly high in night shift workers when compared to day shift and controls.

Table 2 also demonstrating the seven components of sleep compared between three groups. It shows that C1 subjective sleep quality, C3 Sleep duration & C6



Sleep medication is statistically significant amongst the three groups. Intra group analysis shows that C1 value is significantly high in night shift workers when compared to controls, C3 is significantly high in night shift workers when compared to controls & day shift and C6 is significantly high in night shift workers when compared to controls.

## DISCUSSION

Our study demonstrates the quality of sleep assessed using standard PSQI questionnaire amongst shift workers. Sleep quality was evaluated by the overall score of Pittsburgh sleep quality index (PSQI) which is known to evaluate sleep quality during the month preceding the study. It was used in many other surveys.<sup>13</sup> Our study demonstrates global score is significantly high in night shift workers when compared to day shift and controls. It also demonstrates that various components of sleep like subjective sleep quality sleep duration and sleep medication also significantly high in night shift workers compared to those who were never exposed to shift work. Results of our study is comparable to work done by Kazemi et al who showed that mean final scores of PSQI related to the day and night sleep were 8.32 and 9.03, respectively, which are higher than the cut-off point reported by other scholars. This indicates low sleep quality in the Petrochemical Control Room Operators. But their study showed no significant difference between the day and night shifts with respect to the final PSQI scores but showed that sleep quality and quantity were lower during the day rather than the night shift.<sup>5</sup> Machi et al used PSQI questionnaire to evaluate sleep quality among shift emergency physicians and indicated that a significant percentage of the participants suffered from poor sleep quality. Sleep quality was worse in emergency physicians (mean PSQI = 4.8, SD  $\pm$  2.5) compared to the normal population, with 31% of subjects reporting poor sleep quality. Their data suggest that sleep disruption continues routinely beyond training years and may be a widespread issue among health care providers.<sup>11</sup> Study done by Reza Kazemi and colleagues set out to measure quality of sleep after different consecutive night shifts (7 vs. 4) among control room operators (CORs). Sleep quality and quantity as well as PSQI score were compared between the two shift types. There was no difference between participants in the two shifts in terms of sleep quality and sleep quantity ( $p > 0.05$ ). Nevertheless, the PSQI score in the 4N shift participants was higher than that in their 7N shift counterparts.<sup>12</sup> Pei-LiChien

and colleagues demonstrated Sleep Quality among Female Hospital Staff Nurses. The Chinese version of the Pittsburgh sleep quality index (C-PSQI) was used to assess subjective sleep quality. Among the staff nurses, 75.8% had a PSQI score of  $\geq 5$  and 39.8% had an inadequate stable sleep ratio on subjective measures.<sup>8</sup>

Sleep is the main function disturbed by night shift. There is reduction of sleep duration among night workers because of the obligation to sleep during the usual early hours, especially when the surrounding conditions are unfavorable (light, noise, temperature)<sup>14</sup> study done by Rutenfranz J. et al.<sup>15</sup> who found that almost 70% of night workers complain of sleep disorders, assessing their sleep as insufficient, unsatisfactory and little restorative. Light and REM sleep seem to be the phases most affected. Night workers' most reported subjective complaints are early morning waking and the sensation of un-refreshing sleep. Study done by Wided Boughattas showed Hospital Night Shift among Nurses leading to poor Quality of Sleep, the Quality of Life, and Vigilance Troubles because night shift goes against the circadian rhythmicity of the social man, which brings about a multitude of disruptive effects on health.<sup>16</sup> The suprachiasmatic nucleus (SCN), a paired structure in the anterior hypothalamus is the site of a master circadian clock. The endogenous circadian rhythm is synchronized or entrained to the 24 hour rhythm of the external environments daily by synchronizing agents, including light, physical activity, social behaviors and melatonin. Among them, the light is the most influential entraining agent.<sup>17</sup> Exposure of light in the biological evening or early night will delay the circadian pacemaker causing the circadian cycle to shift late relative to clock time. The SCN signals the pineal gland via the superior cervical ganglion to inhibit the production of melatonin, an important entraining agent produced by the pineal gland responsible for the induction of sleep.<sup>18</sup>

Limitation of study: Even though our study demonstrating poor quality of sleep amongst shift workers, work would have been more substantiated if we estimated melatonin levels and correlating with quality of sleep.

## CONCLUSION

Our study concludes that shift workers both night and day shows poor quality of sleep when compared to those who were never exposed to shift working



environment but quality of sleep is significantly poor in night shift workers. As quality of sleep is important for various cognitive functions of brain like judgment, memory, intellectuality, it is important to see that some measures will be taken like modifying shift working hours to improve the quality of sleep.

**Table 1: Demographical characteristics of the participants. (n=150)**

	Night shift nurses (n=50)	Day shift nurses(n=50)	Control (n=50)
Age (years)	33.50±4.00	31.23±5.70	32.67±5.00
Shift (No of days)	6.75±2.65	7.43±1.23	Nil
Duration of shift hours	8.00pm-8.00am (Average 10.98±1.43)	8.00am- 5.00pm (Average 8.34±1.32)	Nil

Values are expressed as Mean±SD.

**Table 2: Quality of sleep and its various domains assessed using Pittsburg sleep quality index questionnaire amongst participants. (n=150)**

	Night shift nurses (n=50)	Day shift nurses (n=50)	Controls (n=50)	F value	P value	Intragroup analysis
PSQI global score	7.02±1.31	5.36±1.54	4.40±1.03	3.955	0.021 *	N Vs D-0.03 * N Vs C-0.01* DV <sub>s</sub> C- 0.98
Subjective Sleep Quality (C1)	1.04±0.44	0.84±0.50	0.60±0.40	3.966	0.021*	N Vs D-0.07 N Vs C- 0.02* DV <sub>s</sub> C- 0.90
Sleep latency (C2)	1.68±0.62	1.04±0.49	1.00±0.00	2.098	0.126	N Vs D-0.28 N Vs C- 0.12 DV <sub>s</sub> C- 0.90
Sleep duration (C3)	1.00±0.67	0.50±0.48	0.36±0.50	12.313	0.00**	N Vs D-0.00 ** N Vs C- .00** DV <sub>s</sub> C- 0.75
Habitual sleep efficiency (C4)	1.44±0.57	0.76±0.54	0.54±0.65	2.098	0.126	N Vs D-0.98 N Vs C- 0.21 DV <sub>s</sub> C- 0.15
Sleep disturbances (C5)	0.90±0.50	1.06±0.73	0.92±0.27	1.299	0.276	N Vs D-0.30 N Vs C- 0.98 DV <sub>s</sub> C- 0.40
Sleep medication (C6)	1.10±.30	0.34±0.19	0.10±0.46	8.051	0.00**	N Vs D-0.65 N Vs C- 0.01* DV <sub>s</sub> C- 0.00**
Daytime dysfunction(C7)	1.42±0.49	0.52±0.1	0.66±0.47	2.679	0.072	N Vs D-0.60 N Vs C- 0.05 DV <sub>s</sub> C- 0.37

Values are expressed as Mean±SD. p<0.05 considered significant. N- Night shift workers:

D- Day shift workers: C- Controls.

**Conflict of Interest:** None

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**Ethical Clearance:** Obtained.

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# Comparison of AEP Changes among the Type 2 Diabetes Mellitus Patients Taking Metformin and Insulin

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## ABSTRACT

**Background:** The relationship between the Auditory Evoked Potential (AEP) and the glycemic levels were well studied in various studies. The Metformin and Insulin alters the glycemic levels which causes Auditory Evoked Potential (AEP) changes are understudied.

**Aim:** To compare Auditory Evoked Potential (AEP) changes in type 2 Diabetes Mellitus patients with Metformin and Insulin.

**Materials and Method:** In this study, the type 2 Diabetes Mellitus patients (n – 90) age, height and weight were recorded. Auditory Evoked Potential (AEP) was recorded by using RMS polywrite.

**Findings:** Auditory Evoked Potential (AEP) latencies were lesser in Insulin group (wave III –  $3.6 \pm 0.3$ ) when compared with Metformin group (wave III -  $4.2 \pm 0.2$ ).

**Conclusion:** With our results, we conclude that tight glycemic control helps in avoiding adverse side effects.

**Keywords:** Auditory Evoked Potential, type 2 Diabetes Mellitus.

## INTRODUCTION

According to the International Diabetes Federation (IDF), 61.3 million people in India had diabetes in 2011. That figure is projected to rise to 101.2 million by 2030. India places second rank in the world in diabetes prevalence, next to China. Many studies proved that in India there is an increase in the prevalence of Diabetes Mellitus and more in South India about 14% in urban population. South India is at high risk for the prevalence of diabetes especially Chennai due to overdependence on rice, tubers, certain fruits (which has high carbohydrate content), choice of oil and genetic predisposition<sup>1</sup>. In India onset of diabetes is mostly at

the younger age group and they die of heart – attack at very young age<sup>2</sup>. Diabetic patients are suffering from increased permeability in the endothelium of the blood vessels which leads to changes in auditory electrolyte homeostasis within the endolymph which causes interferences with the hair cell transduction and signal transmission<sup>3</sup>. Auditory Evoked Potential (AEP) is the potentials recorded from the ear and vertex in response to a brief auditory stimulation to assess the conduction through the auditory pathway up to midbrain.

## MATERIALS AND METHOD

This study was done in the Department of Physiology, Sree Balaji Medical College and Hospital. The patients from the Diabetology clinic, Sree Balaji Medical College and Hospital were recruited for this study. A total number of 90 patients of age  $42.6 \pm 4.2$  were recruited. The procedure was explained in the local language to every patient. Written and informed consent was taken from each patient. Institutional ethical committee approval

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was obtained. The complete history was taken from the patients. Patients with cerebrovascular accidents, hypertension were excluded. The values were expressed in mean  $\pm$  standard deviation. Student  $-t$  test was used.

## FINDINGS

**Table: 1. Physical characteristic feature in study population:**

Parameters	Patients (n = 90)
Age (years)	42.6 $\pm$ 4.2
Height (mts)	1.5
Weight (kg)	62.5 $\pm$ 9.4

Values are expressed in mean  $\pm$  standard deviation.

**Table: 2. AEP in DM patients taking Oral medicine and Parental medicine:**

	I	III	V	I - III	III - V
<b>Oral</b>	2.1 $\pm$ 0.2	4.2 $\pm$ 0.2	6.3 $\pm$ 0.5	2.5 $\pm$ 0.4	2.6 $\pm$ 0.5
<b>Insulin</b>	1.7 $\pm$ 0.2	3.6 $\pm$ 0.3	5.7 $\pm$ 0.4	1.9 $\pm$ 0.3	2.0 $\pm$ 0.5

Values are expressed in mean  $\pm$  standard deviation

## DISCUSSION

In this study, there was an increased latency in wave I, III and V and also increased interpeak latencies of I – III and III – V among the subjects on oral anti-diabetic drugs compared to the subjects on parental insulin stating that insulin has a better effect on glycemic control. The basic pathology in hearing dysfunction among subjects with glucose intolerance have been well identified compared to that in the retina or kidney because an intra-vital examination was not feasible but there are some histopathological studies which shows microangiopathic changes in the vessels of stria vascularis, the endolymphatic sac and the basilar membrane of the cochlea. Also there is capillary wall thickening found in the same. Along with this there is increased loss of outer hair cells in the cochlea (mostly in the lower basal turn) and also atrophy of stria vascularis<sup>4</sup>. In the organ of Corti, the presence of nitric oxide regulates the vascular endothelium. Nitric oxide is present in cochlear blood vessels, including

spiral modiolar, basilar membrane and spiral osseous lamina vessels as well as in spiral ganglion and inner hair cells and the outer hair cells<sup>5</sup>. The hyperglycemia reduces the production of nitric oxide synthase which causes the impairment of vasodilatory effects of nitric oxide<sup>6</sup>. In hyperglycemic patients the hearing becomes worse. There is a significant relation between diabetes mellitus and hearing loss. In the diabetic elderly patients, histopathological studies showed damage to the nerves and vessels of the inner ear. The other adjoining factors which aggravate the hearing loss are ageing process and noise exposure which affects the sensorineural hearing loss. This type of hearing loss does not respond to treatment<sup>7</sup>. As per Geert-Jan Biessels et al., insulin treatment has reversed the prolonged interpeak latency III – V of AEP towards normal<sup>8</sup>.

## CONCLUSION

In this study, prolonged latencies in AEP were observed in patients with Oral Medication when compared to Insulin. We concluded that many physiological and pathological variations alter the AEP in type 2 diabetes mellitus; intake of medicines might be the reason for minimal variation in our results. So, tight control on glycemic level helps to avoid the pathological changes in type 2 Diabetes Mellitus patients. Patients should be educated so that they overcome the side effects of hyperglycemia.

**Conflict of Interest:** Nil

**Source of Funding:** Self

**Ethical Clearance:** Received

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# Simple Tests for Assessing the Autonomic Status in Iron Deficient Anemic Adolescent Girls

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## ABSTRACT

**Introduction:** Adolescent girls are at high risk of developing iron deficiency because of increased iron demands during puberty, menstrual losses, and limited dietary iron intake.

**Method:** Adolescent girls between 17-19 years of age with similar socioeconomic background were recruited from the college of nursing for the study. Students having Hb > 12 gm/dl formed the control group i.e. Group I (n=30). Students having Hb < 12 gm/dl and S.Ferritin < 12µg/dl formed Group II i.e. iron deficient anemic (IDA) group (n=30). The following haematological parameters were studied Hemoglobin (Hb), MCV, MCH, MCHC (using Sysmex KX-21 autoanalyser), S.Iron, TIBC (Spectrophotometry), S.Ferritin (ELISA). The autonomic test battery consisted of resting heart rate and blood pressure, Valsalva ratio and cold pressor test.

**Results and Conclusion:** Resting heart rate was significantly higher in the IDA group. Resting systolic blood pressure was significantly lower ( $P < 0.001$ ) in group II- IDA whereas Diastolic blood pressure did not show any significant difference between the two groups. Rate pressure product and double product were significantly higher in the anemic group as compared to control group. Valsalva ratio in anemic group demonstrated a significant decline as compared to control group. There was a significant increase in systolic as well as diastolic blood pressure in both the groups due to Cold Pressor test.

The present study reveals the affliction of both sympathetic and para-sympathetic divisions of Autonomic Nervous System as well as increase in mechanical load on heart in iron deficient anemic adolescent girls.

**Keywords:** iron deficiency, anemia, autonomic, adolescent

## INTRODUCTION

Iron deficiency is most common nutritional disorder affecting at least one third of world population. It is the predominant cause of anemia across countries and in both sexes, with women more commonly afflicted<sup>1</sup>. Iron deficiency anemia is associated with impaired cognitive function<sup>2</sup> and increased audiovisual reaction time<sup>3</sup>.

Adolescent girls and young women are at a higher risk of developing iron deficiency anemia because of

increased iron demands during adolescent growth spurt, menstrual losses and limited dietary intake<sup>3</sup>.

Cardiac autonomic function-anemia relationship has been studied in various anemic patient populations including vitamin B<sub>12</sub> deficiency, sickle cell anemia and thalassemia major. The results of these studies have implicated that cardiac autonomic functions are impaired in anemia<sup>4</sup>. There are insufficient data about cardiac autonomic functions in anemic patients specially adolescents. This cross-sectional study was designed for detecting the autonomic status of adolescents with iron deficiency anemia. .

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## MATERIAL & METHOD

The present study was carried out in the Department



of Physiology and Pathology, Lady Hardinge Medical College and Associated Hospitals, New Delhi.

Apparently healthy adolescent girls between the ages of 17-18 years belonging to similar socioeconomic status were recruited for the study. Students having Hb > 12 gm/dl formed the control group i.e. Group I (n=30). Students having Hb < 12 gm/dl and S.Ferritin < 12µg/dl<sup>5</sup> formed Group II i.e. iron deficient anemic (IDA) group (n=30).

Each subject underwent detailed history taking and thorough clinical assessment. Girls with history of any disease/infection, history of blood transfusion, receiving iron supplementation within one month were excluded from the study.

The subjects were briefed about the study protocol and informed consent was taken. The clearance from the ethical committee of the institution was taken for the study. Age and anthropometric parameters were noted.

The study was done during the Post menstrual phase of the menstrual cycle.

Complete blood count was performed with the help of electronic counter Sysmex KX-21. Ferritin levels were determined by using direct immunoenzymatic colorimetric determination (Biomeda, REF DKO039). S.Iron was determined using Biosystems (Spain), COD 11509 was used and S.Total Iron Binding Capacity was determined by Spectrophotometry using Biosystems (Spain), COD 11554.

**Autonomic Status:** Participants were instructed to avoid nicotine, caffeine and any kind of physical exercise in the last 24 hours.

**Recording of Resting Heart Rate & Blood Pressure:** Heart Rate was calculated from electrocardiogram in lead II after 15 minutes of rest by means of student's

physiograph. Systolic and Diastolic blood pressure (mmHg) was recorded from right arm by auscultatory method using mercury sphygmomanometer. Three readings were taken. Two concordant readings were taken as actual reading.

**Valsalva Ratio:** The subject was asked to breathe out at the end of tidal inspiration into a mercury manometer to raise the mercury column to 40 mmHg and maintain it for 15 seconds. Three trials were performed at an interval of 5 minutes. Continuous ECG was recorded 1 minute before the maneuver (i.e. resting period), during the strain period of 15 seconds and 60 seconds subsequent to strain period. Valsalva ratio was calculated as longest RR interval after the strain/shortest RR interval during the strain. The maximum ratio of the three trials obtained was recorded.

**Cold pressor test:** The pre-test BP was measured in the sitting position. The right hand of the subject was immersed in ice-cold water maintained at 4-6°c for two minutes. BP measurements were made from the other arm at every half-minute interval for two minutes during immersion. The maximum increase in SBP and DBP was determined and results recorded. The rise in MAP with CPT was calculated.

**Statistical Analysis:** Results were expressed as mean + S.D. For intra-group comparison, paired 't' test were used and for intergroup comparisons students "t" test was used. Pearson Correlation was used for taking out correlations, if any. Adherence to 'P' values was followed.

## RESULTS

There was no statistical difference between the age, height, weight and BMI of the two groups and hence they were comparable for the study.

**Table 1: Comparison of Hematological parameters and Iron status between the control and IDA group**

Parameters	Group I (CONTROL) n = 30	Group II (IDA) n = 30	p-value (t test)
Hb (gm/dl)	12.93 ± 0.86	10.08 ± 0.51	P<0.001
MCV (fl)	86.98 ± 4.09	76.89 ± 3.07	P<0.001
MCH (pg)	28.09 ± 1.96	24.29 ± 2.38	P<0.001
MCHC (g/dl)	34.61 ± 0.57	29.37 ± 1.14	P<0.001
S.Iron (µg/dl)	88.70 ± 9.37	47.74 ± 1.93	P<0.001
S.Ferritin (ng/ml)	37.90 ± 6.04	10.27 ± 0.70	P<0.001
TIBC (µg/dl)	256.30 ± 2.49	478.20 ± 48.57	P<0.001

Data are expressed as mean  $\pm$  SD; n= number of subjects.

The table above shows that the mean hemoglobin level in anemic group was significantly lower ( $P < 0.001$ ) than the control group. MCV, MCH, and MCHC were

also statistically lower in anemic group thus, confirming their anemic status.

Significantly decreased S.Iron, S.Ferritin and increased TIBC confirm that iron deficiency was the cause of anemia in iron deficient anemic group.

**Table 2: Comparison of Resting heart rate, Blood Pressure, Valsalva ratio and Cold pressor test between the groups.**

Parameters		Group I (CONTROL) n = 30	Group II (IDA) n = 30	Group I vs Group II
RHR(beats/min)		78.73 $\pm$ 4.88	88.20 $\pm$ 4.05	P<0.001
SBP (mm Hg)		110.33 $\pm$ 8.87	102.33 $\pm$ 6.76	P<0.001
DBP (mm Hg)		72.47 $\pm$ 7.42	70.07 $\pm$ 4.44	P>0.05
Mean Arterial Pressure(MAP) (mmHg)		85.1 $\pm$ 7.26	80.8 $\pm$ 4.78	P<0.001
Rate Pressure Product (RHR*SBP)		8689.60 $\pm$ 921.75	9016.80 $\pm$ 602.48	P<0.001
Double Product (MAP * RHR)		6707.24 $\pm$ 771.18	7122.9 $\pm$ 452.40	P<0.001
Valsalva Ratio		1.70 $\pm$ 0.07	1.51 $\pm$ 0.07	P<0.001
Cold Pressor test SBP(mm Hg)	Before	110.33 $\pm$ 8.86	102.33 $\pm$ 6.76	P<0.001
	After	132.73 $\pm$ 8.98***	119.06 $\pm$ 6.59***	P<0.001
	Rise	22.40 $\pm$ 2.74	16.73 $\pm$ 2.85	P<0.001
	% Rise	20.46 $\pm$ 3.27	16.47 $\pm$ 3.26	P<0.001
Cold Pressor test DBP(mm Hg)	Before	72.46 $\pm$ 7.42	70.07 $\pm$ 4.44	P<0.001
	After	90.33 $\pm$ 8.04***	81.06 $\pm$ 4.54***	P<0.001
	Rise	17.86 $\pm$ 3.48	11.00 $\pm$ 1.46	P<0.001
	% Rise	24.95 $\pm$ 5.82	15.77 $\pm$ 2.41	P<0.001

Data are expressed as mean  $\pm$  SD

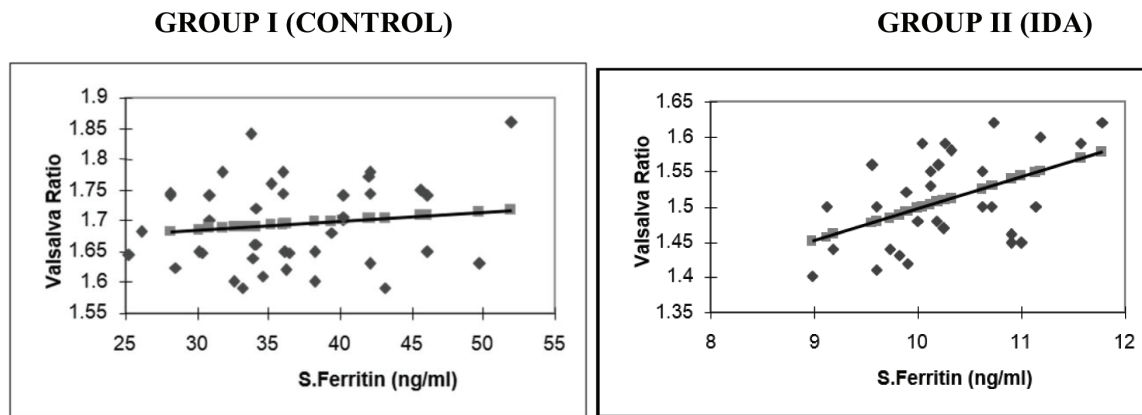
\*\*\* Before Vs after (Paired t-test)

P<0.001 Highly Significant

Resting heart rate was significantly higher in the IDA group as compared to the control group. Resting systolic blood pressure was significantly lower ( $P < 0.001$ ) in group II- IDA (102.33  $\pm$  6.76) than control group (110.33  $\pm$  8.87). Diastolic blood pressure did not show any significant difference between the two groups. Both Rate pressure product and double product were significantly higher in the anemic group as compared to control group.

Valsalva ratio in anemic group (1.51  $\pm$  0.07) demonstrated a significant decline ( $p < 0.001$ ) as compared to control group (1.70  $\pm$  0.07).

There was a significant increase in systolic as well as diastolic blood pressure in both the groups ( $p < 0.001$ ) due to Cold Pressor test. However, the percentage rise in systolic and diastolic blood pressure in anemic group was significantly less ( $p < 0.001$ ) than the control group.



**Figure 1: Correlation of S.Ferritin with Valsalva Ratio in Group I (control) and Group II (IDA)**

-S.Ferritin in anemic group showed significant correlation ( $P < 0.05$ ) with Valsalva ratio.

## DISCUSSION

Involvement of ANS in cases of anemia has been a matter of considerable speculation.

Normally, at rest, there is a moderate amount of tonic discharge in cardiac sympathetic nerves, but there is a good deal of tonic vagal discharge<sup>6</sup>.

In our study the heart rate was significantly higher ( $P < 0.001$ ) in iron deficient anemic as compared to control subjects, similar to observation of earlier studies<sup>4,7-13</sup>. Brinda et al did not observe any significant difference in resting heart rate in anemic patients<sup>14</sup>.

Increased Heart rate in anemics is due to a short circulatory time which occurs as compensatory mechanism in anemics to maintain tissue oxygenation<sup>10</sup> or may be due to depleted cardiac reserve<sup>8,11</sup>.

Yokusoglu et al<sup>4</sup> reported an impairment in global (SDNN, SDANN) HRV indices that might be caused by increased sympathetic or decreased parasympathetic activity and decreased PNN50 (a parasympathetic activity index) in iron deficiency anemia. Tuncer et al<sup>12</sup> and Gehlot et al<sup>15</sup> found no statistically significant difference between the healthy group and iron-deficient patients in regard to their HRV parameters.

The resting systolic blood pressure, in our study, was significantly lower ( $P < 0.001$ ) in anemic group. Nand et al<sup>7</sup>, Singh et al<sup>9</sup> and Jibhkate et al<sup>13</sup> found similar results in patients of severe anemia. Nagi et al<sup>16</sup> demonstrated decrease in systolic and diastolic blood pressure in mild and moderate cases of anemia.

Systolic blood pressure depends upon stroke volume and arterial compliance. Poor performance of the left ventricle due to inadequate myocardial oxygen supply or collagen content or alterations in NE and epinephrine metabolism, which has been shown to be reduced in iron deficient hearts<sup>16</sup>, could be the cause for reduced systolic blood pressure.

Brinda et al<sup>14</sup>, Jibhkate et al<sup>13</sup> and Singh et al<sup>17</sup> observed lower Systolic & Diastolic blood pressure in cases of anemia. Singh et al<sup>17</sup> found statistically significant lower values only in case of systolic blood pressure. Mani et al<sup>11</sup> observed resting systolic blood pressure to be significantly more in anemic children, Whereas Kapoor et al<sup>8</sup> observed no change in resting systolic blood pressure in mild, moderate and severe anemic cases.

Diastolic blood pressure did not differ significantly in our study, as was also observed by Singh et al<sup>9</sup>. Diastolic blood pressure depends upon peripheral resistance and viscosity of blood. Though viscosity is generally lower in anemics, & should reduce diastolic blood pressure. But in our study the DBP was not significantly altered as decreased viscosity would be countered by increased peripheral resistance induced by slight increase in sympathetic resting tone as also evident by increased heart rate<sup>6</sup>.

We observed a significantly attenuated cold pressor response indicating less pronounced sympathetic activation in anemics, similar to Bedi et al<sup>18</sup> observations in malnourished children. Such impaired augmentation could be due to already existing higher sympathetic

discharge to the blood vessels which leaves less margin for superimposed stimulation, a fact well established by Yokusoglu<sup>5</sup> by measuring HRV values in patients with iron deficiency anemia. Brinda et al<sup>14</sup> found that cold pressor test showed a positively abnormal response in 40% cases with a diastolic rise of less than 10 mm Hg.

Valsalva maneuver is dependent not only on parasympathetic but also on sympathetic innervation. A decrease in value of Valsalva ratio (though in range of normal value) in anemics as compared to control in our study, could be explained either due to a decrease in parasympathetic reactivity or decreased cardio-acceleration during straining phase of the Valsalva maneuver. Nand et al<sup>7</sup> observed abnormal Valsalva ratio and postural tachycardia index (PTI) along with normal atropine response implicating dysfunction of afferent limb of para-sympathetic reflex arc in cases of chronic severe anemia. Lakhotia et al<sup>10</sup> in their study found that Valsalva manoeuver did not show any significant difference in anemics and controls. Altered Valsalva ratio was encountered in their study by Brinda et al<sup>14</sup>.

The myocardial oxygen uptake reflecting myocardial oxygen consumption is estimated during exercise testing by the product of HR and SBP, the Double Product, also called modified tension time index (MTTI)<sup>11</sup>. Rate pressure product (RPP) is an easy measurable index of myocardial oxygen consumption and load on heart<sup>17</sup>. Mani et al<sup>11</sup> observed no significant difference in the Double Product (DP) of patients and controls. Kapoor et al<sup>19</sup> found that the mean values of gain in DP were significantly lesser in anemic children, probably due to decreased cardiac reserve in these children. Our study was in confirmation with study done by Singh et al<sup>17</sup> who also observed a significant elevation in rate pressure product ( $SP \times HR \times 10^{-2}$ ) and double product ( $MP \times HR$ ) in anemia versus controls, thus indicating increase in  $O_2$  consumption and imposing mechanical load on heart.

## CONCLUSION

The present study reveals the affliction of both sympathetic and para-sympathetic divisions of Autonomic Nervous System as well as increase in mechanical load on heart in iron deficient anemic adolescent girls.

**Limitations of the study:** Heart rate variability was not done in this study.

**Conflict of Interest:** None

**Sources of Funding:** Self-funded

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# Heart Rate Variability as a Tool for Early Diagnosis of Autonomic Neuropathy in Non Diabetic off Springs of Diabetic Parents

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**Abstract: Objective:** To evaluate the influence of parental diabetes on the heart rate variability of their non diabetic off springs. **Method:** The study was conducted in the department of Physiology SSIMS and RC after the approval of the institutional ethical committee. Thirty cases in the age group of 18 -25 years, who were non diabetic off springs of diabetic parents and age and gender matched thirty subjects with no family history of diabetes mellitus were recruited as controls for the study on the basis of predetermined inclusion exclusion criteria. Resting 15 minutes lead II ECG and heart rate was recorded by AD Instruments - Power lab 26T, Australia. Offline assessment of time and frequency domain parameters of heart rate variability (HRV) was done with Lab Chart 7 software. **Result:** Frequency domain analysis of HRV shows increased low frequency (p value 0.01) and a decreased high frequency (p value 0.02) in the cases as compared to that in the control group and the difference is statistically significant. Time domain analysis shows decrease in SDNN (standard deviation of normal R-R interval). **Conclusion:** Our results suggest that the autonomic functions of subjects whose parents are diabetic are affected and show a sympathetic over activity.

**Keywords:** Diabetes mellitus, heart rate variability, low frequency, high frequency

## INTRODUCTION

Non communicable diseases particularly cardiovascular diseases and type 2 diabetes mellitus (DM) are important determinants of mortality and morbidity.<sup>1</sup> According to international diabetic federation by 2030 the countries with largest number of diabetics will be in India, China and united states of America.<sup>2</sup> Diabetes is emerging as an epidemic in the developing countries of the world.<sup>3</sup> It is the 6<sup>th</sup> leading cause of death and people with diabetes have twice the risk of death as compared to nondiabetics of the same age.<sup>4</sup> Risk of becoming diabetic for a person with a positive family history of

diabetes increases by 2 to 4 folds.<sup>1</sup> Age at onset of DM in offsprings whose parents are diabetic is almost a decade early.<sup>5</sup>

DM is a state of hyperglycemia in which homeostasis of carbohydrate and lipid metabolism is improperly regulated by insulin.<sup>6, 7, 8</sup> DM is of two types. Type 1 is a result of complete or near total insulin deficiency. Type 2 is characterized by varying degrees of insulin resistance, impaired insulin secretion and increased glucose production.

Heart rate variability (HRV) is the variation between consecutive heart beats over a period of time which reflects the ability of heart to respond to various stimuli.<sup>9</sup> Analysis of HRV is used to assess cardiac autonomic activity.<sup>9, 10, 11, 12</sup>

Heart is under the influence of autonomic nervous system (ANS) and most of the behavioral and emotional patterns are exhibited through ANS.<sup>12, 13</sup> Heart Rate Variability (HRV) reflects the ability of the heart to

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respond to various stimuli. It is a very sensitive index of autonomic nervous activities. HRV analysis assesses the overall cardiac health and its regulation by ANS.<sup>9,11</sup>

The main purpose of our study was to determine whether parental DM affected the autonomic nervous activities in the non diabetic offsprings by the use of power spectral analysis of HRV.

**METHADODOLOGY**

The study was conducted in the department of Physiology SSIMS & RC after the approval of the institutional ethical committee .60 subjects of either gender, in the age group of 18 -25 years were recruited for the study (30 cases, 30 controls).Cases were non diabetic offsprings of diabetic parents and their age and gender matched healthy subjects with no family history of DM were taken as controls. Subjects with history of diabetes, hypertension, congenital heart disease, smoking, alcohol or substance abuse were excluded from the study. Subjects on long term medication that could influence cardiovascular and respiratory system were also excluded. The study protocol was explained and informed written consent was obtained from all the volunteers.

Resting 15 minutes lead II ECG and heart rate was recorded by AD Instruments - Power lab 26T, Australia (Serial number T26-2756, Model number ML856). Offline assessment of time and frequency domain heart rate variability was done with Lab Chart 7 software supplied by AD Instruments, Australia

**STATISTICAL ANALYSIS**

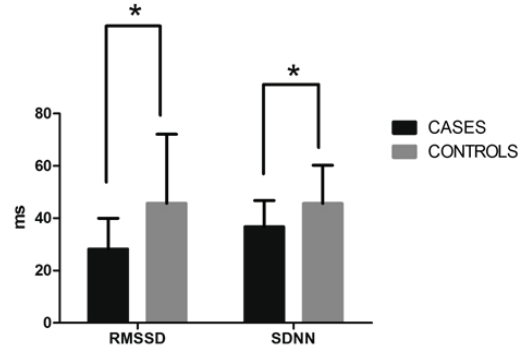
Students unpaired t test was used for analyzing the data.

**RESULTS**

Demographic details of the subjects are shown in table 1.The time and frequency domains of HRV are shown in figure 1 and 2 respectively. Our study shows statistically significant changes in the Low frequency component (LF) parameter of HRV in the cases as compared to that in the controls indicative of sympathetic over activity.

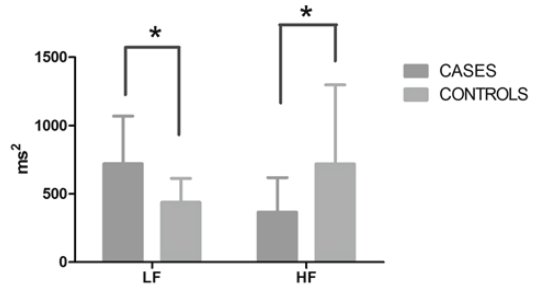
**TABLE.1: DEMOGRAPHIC DETAILS (n =60)**

	CASES	CONTROLS
HEIGHT (cm)	170.8 ±12.12	162.4±17.54
WEIGHT(Kg)	66.5 ± 10.73	59.5±10.13



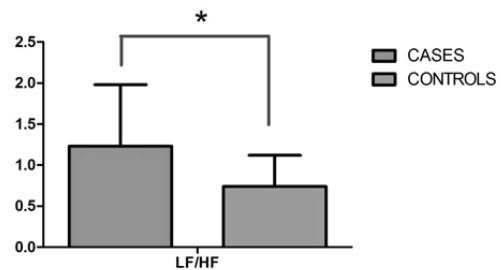
**Figure 1: TIME DOMAIN HRV PARAMETERS**

**Difference in SDNN and RMSSD in cases and controls is statistically significant (P=0.03\* and 0.01\* respectively).**



**Figure 2: FREQUENCY DOMAIN HRV PARAMETERS**

**LF and HF parameters show statistically significant difference between cases and controls (P = 0.01\* and 0.03\* respectively)**



**Figure 3: LF /HF ratio**

**Increased LF/HF ratio in cases as compared to that in the controls is indicative of autonomic imbalance with a sympathetic predominance.**

## DISCUSSION

HRV has time and frequency domains. Time domain HRV parameters include SDNN (standard deviation of NN interval), RMSSD (square root of the mean of the sum of squares of differences between adjacent NN intervals). SDNN reflects overall autonomic activity, whereas RMSSD is more indicative of parasympathetic activity. For short term HRV recordings (5 minutes) low frequency (LF) and high frequency (HF) parameters are considered. LF is 0.04-0.15 Hz and HF is 0.15-0.4 Hz.<sup>14</sup> LF component of HRV is indicative of sympathetic activity and HF reflects parasympathetic activity and LF/ HF represents sympathovagal balance.<sup>10, 14</sup>

Our results indicate that the autonomic functions of subjects whose parents are diabetic are affected and show a sympathetic over activity suggested by the analysis of frequency domain of HRV which shows increased LF (sympathetic) and decreased HF (parasympathetic) in the cases as compared to the controls. Time domain analysis also shows a decrease in SDNN.

Studies by Fiorentini et. Al have shown impaired autonomic activity with the familiarity for type 2 DM, demonstrating an increase in LF and LF/HF ratio and a reduction in the SDNN value.<sup>10</sup>

Laitinen et.al have also given similar results which show decreased HF and increased LF /HF ratio in non diabetic offsprings of diabetic parents .<sup>8</sup>

Some studies have shown early onset of type 2 diabetes among patients who's both the parents were diabetic as compared to other patients<sup>3</sup>. Others have concluded that nondiabetic offsprings of fathers with early onset of DM are leaner than the offsprings of mothers with early onset of type 2 diabetes or the control group.<sup>15,16</sup> However such relations still needs to be established probably with a larger sample size.

**Conflict of Interest** – None

**Source of Funding**- Self support

**Ethical Clearance** – Institutional Ethics Committee

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# Effect of Vegetarian and Non-Vegetarian Diet on Hematological Parameters among Young Adults of Uttarakhand

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## ABSTRACT

There is a conspicuous shift in the dietary habits of the population worldwide owing to economic status, technological advances and advertisement bombardment shifting this paradigm towards non vegetarian lifestyle affecting health in general. The present study was thus conducted to explore the effect of vegetarian and non-vegetarian diet on hematological parameters. 150 healthy adults were recruited and anthropometric measurements viz; height, weight, BMI was taken along with dietary intake pattern assessment through 24 hours recall method. Blood samples were taken for estimation of hemoglobin, red blood cell count, haematocrit, white blood cell count, platelet count. The results showed high prevalence of Hb, RBC count, WBC count and Haematocrit in non-vegetarianism as compared to vegetarian. Anthropometric profile of non-vegetarian was higher as compared to vegetarian but statistically not significant. Also non-vegetarians exhibited high Hb, RBC count, WBC count and haematocrit as compared to vegetarians which revealed deficiency of important nutrients like iron, Vit-B<sub>12</sub> among vegetarian population. Hence vegetarians must consume food fortified with the nutrients lacking in vegetarian diet to avoid the deficiency of the aforementioned nutrients in their body.

**Keywords:** Vegetarian diet, non-vegetarian diet, hematological parameters, Vit-B<sub>12</sub> deficiency, Folic Acid deficiency

## INTRODUCTION

There is an evident change in the dietary habits of the population worldwide. With the change in economic status the pattern of food consumption also changes noticeably. The vegetarian populations in most parts of the world do not consume meat owing to fiscal reasons. However, technological advances and advertisement bombardments shift this paradigm towards non vegetarian lifestyle<sup>1</sup>. Enormous social science literature is available which examines the factors that influence meat consumption behavior of individuals. Economic analysts devised sophisticated models used to project

future demand for eclectic food types, including meat. They found population growth, economically mediated lifestyle changes and urbanization form key factors influencing global food consumption trends. Popkin BM suggested that rapid shift in habitual dietary intake along with market globalization has significant and inevitable impact on an individual's lifespan. Most people tend to ignore healthy eating habits subtly until the catastrophic health hazards become ominous<sup>2</sup>.

Various studies have reported both the short terms as well as the long term advantages of adopting vegan lifestyle but most of them have chosen cohorts who are vegan for either religious reasons or differed in lifestyle habits from non-vegetarians. Moreover, most of the times vegans do not represent a homogenous group with cultural and religious nominations, moral beliefs related to animal rights, and health implications and environmental issues<sup>3</sup>. In India, dietary habits exhibit wide heterogeneity due to diverse socioeconomic and

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religious beliefs<sup>4</sup>. Also, vegetarianism has been attributed as a major cause of vitamin-B<sub>12</sub> deficiency. A well-planned varied vegan diet is perfectly consistent with good health potentially minimizing risk of many chronic ailments<sup>5</sup>. However, care must be taken during times of extra nutritional demand for instance adolescence to ensure sufficient intake of energy, calcium, iron, and vitamins B<sub>12</sub> and D<sup>6</sup>. The present study thus investigates the effects of vegetarianism and non-vegetarianism on different hematological parameters.

## MATERIALS AND METHOD

### Methodology

**Study design:** This cross sectional analytical study was designed to explore the effects of vegetarianism and non-vegetarianism on different hematological parameters. Volunteers; aged 16-25 years of both genders were selected from Himalayan Institute of Medical Sciences (HIMS), Dehradun. The research was carried out in the Department of Physiology, HIMS over a period of 12 months after obtaining written consent from all participants and approval by the institutional ethics committee.

**Study population:** Subjects were divided into two groups according to their dietary patterns; Non-vegetarians and vegetarians. Subjects were classified as non-vegetarians if foods of plant and animal origin, including meat, fowl, eggs, milk, other dairy products, and fish were included in their diet and vegetarians if foods of plant and milk and dairy products were included in their diet. Exclusion criteria includes unhealthy adults with any history of acute or chronic illness, bleeding and bleeding disorders, drug addiction and if they had donated blood within the previous 6 months were not included in the study. Pregnant women and those who had delivered within 3 months were also excluded.

**Sample size:** A sample size of 77 for each group was calculated through Cohen flexible algorithm using Fischer's 'F' distribution by comparing means between two group(s) with ' $\alpha$ '=0.05, ' $\beta$ '= 80%, ' $F$ '= 0.9 / 2 \* 1 = 0.45 and effect size (es) which is difference b/w the two groups / 2 \* within the group SD = 0.7

### Measurement of anthropometric and hematological parameters

Standing height was recorded with bare feet on a wall mounted measuring tape to nearest of centimeters. Weight was recorded with bare feet with light cloths on a weighing machine. BMI is measured as the weight in kilogram divided by square of height in meters (Kg/m<sup>2</sup>) based on Metric Imperial BMI formula. After taking antiseptic precautions samples were drawn from the ante-cubital vein were collected in 3ml EDTA vacutainers (Akuret, eastern medkit limited). The EDTA blood sample was processed using MS-9 automated hematology cell counter for obtaining various hematological parameters. Samples were processed on the same day within 3-5 hours of collection. Data collected was subjected to standard statistical analysis by SPSS software

### Statistical Analysis

Dataset was analyzed using SPSS (Statistical Package for the Social Sciences; version 20.0 for Windows). Student's t-test was used for comparing continuous variables which include weight, height, BMI, RBC, WBC, hemoglobin, hematocrit, and platelets between vegetarian and non-vegetarian groups.

## RESULTS

Table 1: compares the difference between anthropometric (weight, height and BMI) and hematological parameters (WBC count, RBC count, hematocrit, hemoglobin, platelets) amongst vegetarian and non-vegetarian subjects. Anthropometric measures showed mean weight, height and BMI of non-vegetarian subjects on a higher side as compared to vegetarians but only weight showed differences statistically significant difference (p= 0.02). For hematological parameters, the means values of total WBC count (p=0.004), total RBC count (p=0.003), hematocrit (p=0.001), hemoglobin (p=0.001) were significantly higher in non-vegetarian subjects as compared to vegetarian subjects. There was no statistically significant difference between the mean values of platelet count of non-vegetarian and vegetarian subjects (p=0.9).



**Table-I: Comparison of anthropometric and hematological parameters amongst vegetarian and non-vegetarian subjects**

S.No	PARAMETER	VEGETARIAN DIET (n=73) M ± SD	NON-VEGETARIAN DIET (n=77) M ± SD	'p' value
1.	Weight (Kg)	61.38 ± 9	65.01 ± 10.99	0.020
2.	Height (m)	1.59 ± 0.05	1.61 ± 0.05	0.090
3.	BMI (kg/m <sup>2</sup> )	24.05±3.02	24.88±3.21	0.100
4.	<b>White blood cell count (10,000 cells/mm<sup>3</sup>)</b>	6.73±1.58	7.43±1.37	0.004
5.	Red blood cell count (10,00,000 cells/mm <sup>3</sup> )	4.35±0.56	4.66±0.71	0.003
6.	Hematocrit (%)	38.18±3.70	42.24±3.42	0.001
7.	<b>Hemoglobin (gm/dl)</b>	12.85±1.14	14.26±0.96	0.001
8.	<b>Platelet count (1,00,000 cells/mm<sup>3</sup>)</b>	144.45±54.64	143.94±49.14	0.900

Data was presented as mean ± standard deviation (**M ± SD**). Analysis was done using Student's 't' test. P<0.05 is taken as statistically significant.

## DISCUSSION

The practice of vegetarianism involves exclusion of all meat and animal products from the diet which may confer few health benefits due to fiber rich content and minimal quantity of saturated fat. But for actively growing children a strict vegetarian diet however enhances the risk of micronutrient deficiencies as these diets are poor micronutrients source thus escalating demand of essential micronutrients in childhood. It's noteworthy once the diet becomes more restrictive in case of strict vegetarians it is empirical to select, diversify and plan meals. In vegetarian diets iron and vitamin-B<sub>12</sub> are present in traces as compared to non-vegetarian diets reasonably rich in meat, eggs and dairy products. It must be emphasized that although the iron content of vegetarian diet is typically similar to non-vegetarian diets, yet the bioavailability of iron is relatively on a lower side both due to unavailability of haem iron as well as high phytic acid content in plant food reduces it's availability. Globally iron availability is a prevalent nutrient deficiency and affects approximately two-third children in developing countries who typically

receive homemade complementary foods which are poor sources of bioavailable iron. Even a high dietary iron intake perhaps does not assure optimum bioavailability, it is paramount to promote dietary practices which encourage iron absorption from plant food<sup>7</sup>.

We found that non-vegetarians had significantly higher total white blood cell count as compared with vegetarians. This is in corroboration with Neubauerova E et al. who too discovered reduced white blood cell counts in younger as well as older vegetarians compared to non-vegetarians<sup>8</sup>. A similar significantly lower leukocyte count was elucidated by Haddad EH et al in vegans compared with non-vegetarians but vegans however did not differ from non-vegetarians in functional immunocompetence<sup>9</sup>. This lower total white blood cell count in vegetarian might either reflect deficiency or reduced bioavailability of certain minerals and micronutrients which might be responsible for their normal production. The National Institute of Health's Office of Dietary Supplements states that zinc is an essential mineral for the development and activation of white blood cells and even mild to moderate zinc deficiency can lead to a low white blood cell count<sup>10</sup>. The bioavailability of zinc from vegetarian diets is lower than from non-vegetarian diets. In addition, vegetarians



typically eat high levels of legumes and whole grains, which contain phytates that bind zinc and inhibit its absorption<sup>11</sup>.

Non vegetarians have higher hemoglobin concentration than ovo lacto and lacto vegetarians<sup>4</sup>. Experimentally a re-introduction of beef in the diets of beef eating group increased hemoglobin concentration and hematocrit as compared to vegetarian group during 12 weeks of resistive training ( $P < .05$ ). These changes were appreciated within clinically normal limits. During a 12-week period of resistive training Elderly men who consume beef containing higher-bioavailable iron diet have elevated hematological profile as compared to vegetarian taking lower-bioavailable iron diet<sup>12</sup>.

In general, people eating vegetarian diet had significantly lower red blood cell count in both young as well as old individuals as compared to non-vegetarian subjects<sup>8</sup>. We had similar results in our study depicting significantly high red blood cell parameters (RBC count, Haematocrit and Haemoglobin) in non-vegetarians as compared to vegetarians.

Dietary iron categorically exists in two forms: haem (haem iron is almost entirely found in foods of animal origin as haemoglobin and myoglobin) and non-haem. Rich sources of non-haem iron include cereals, vegetables, nuts, eggs, fish and meat. As a matter of fact dietary haem iron's availability for absorption is 2-6 times more than non-haem iron. On the contrary calcium, phytates in cereals and legumes, and phenolic compounds found in tea, coffee and other beverages bind with iron thus restricts its availability for absorption<sup>13</sup>. This subsequently reduces the delivery of iron to functional sites impairing iron-dependent functions such as erythropoiesis in vegetarian, leading to a decrease in hemoglobin concentration.

In our study, non-vegetarians had less platelet count than vegetarians but this difference between the two was statistically insignificant. Vitamin B<sub>12</sub> and folic acid are required for healthy red blood cell formation within the bone marrow. Deficiency of these vitamins not only results in immature, malformed red blood cells known as pernicious anemia but also reduces platelet cell production<sup>14</sup>. Thus, vegans have to plan their dietary intake carefully to get satisfactory amounts of vitamin B<sub>12</sub>. For instance ready to-eat breakfast cereals, fortified

soy and other nondairy milks, some nutritional yeasts and meat analogs often contains added vitamin B<sub>12</sub> should be consumed on a daily basis. With advancing age efficiency of vitamin B<sub>12</sub> absorption reduces and supplementation eventually becomes necessary. Zinc intake furthermore should be scrutinized for vegans as meat forms the most bio-available source of zinc. And despite the fact that zinc deficiency is not a major issue as such, vegans should strive to meet the RDA for zinc from cereals, wheat germ, legumes, soy products and some vegetables.

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# Introduction of a Pre-Medical Course Study Programme to Undergraduate Medical Curriculum – Students’ Perspective, Trichy, India

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## ABSTRACT

**Background:** It is important to analyse if an undergraduate medical student is psychologically fit and mature enough to take up the challenge of facing the subjects, understanding and developing an applicative knowledge from the subjects. In view of this, the present study was planned.

**Objectives:** To understand students’ perspective regarding the Introduction of a Pre-Medical course study program to Undergraduate Medical curriculum.

**Materials & Method:** The present study was conducted in Chennai Medical College Hospital & Research Centre, Trichy, from April-July 2016. After obtaining ethical clearance from the Institutional Ethics Committee, a cross-sectional study was conducted among 50 second year under graduate medical students of both genders between 17-19 years of age. Data was analyzed using the SPSS statistical program (IBM SPSS statistics 21).

**Results:** More than half (66%) of the students felt stressed during their first year in medical school. 54% of them stated the need for a pre-medical course program. 97% of the students suggested the inclusion of stress management classes and classes to develop their clinical applicative skills as part of their undergraduate medical curriculum.

**Discussion and Conclusion:** The overload of course materials creates a feeling of disappointment because of the inability to handle all the information at once and succeed during the first year of the medical course. Our study results will be helpful to manage stress and develop clinical and application skills to medical undergraduate.

**Keywords:** Elective Program, Medical curriculum, Medical student, Premedical course, Students opinion, Undergraduate study.

## INTRODUCTION

Pre-medical programs providing broad preparation are referred to as “pre-professional” and may

simultaneously prepare students for entry in to first professional degree or graduate school programs (MBBS) because a doctor will always keep practicing<sup>(1)</sup>. At most colleges and universities, students do not have the option of pre-medical major or minor. The first year in the medical undergraduate course is the most crucial, considering it forms the basics of all the disciplines taught and learnt in the successive years. So, it is imminent to assess the student’s state of mind and knowledge on the course of study before he/she actually starts on it. It is important to analyze if the candidate is psychologically fit and mature enough to take up the challenge of the subjects, understanding and developing

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an applicative knowledge from the subjects, because a doctor will always keep practicing<sup>(2)</sup>

Stress in medical students is stress caused by strenuous medical programs, which may have physical and psychological effects on the well-being of medical students. A significant percentage of medical students suffer from anxiety disorder because of the long term effects of stress on emotions and behavior<sup>(3)</sup>. There are three important issues to be considered the most relevant, in terms of stress development in medical students. They are required to learn a great deal of new information in a short period of time before taking exams and evaluations, the overload of information creates a feeling of disappointment because of their inability to handle all the information at once and succeed during the examination period<sup>(4)</sup> and even though an 18-year-old has adult cognitive abilities, they still don't have the life experiences to guide them in acting appropriately. In view of this, the present study was planned<sup>(5)</sup>.

### OBJECTIVES

To understand students' perspectives regarding the Introduction of a Pre-Medical course study programme to undergraduate medical curriculum.

### METHODOLOGY

The present study was conducted in the Department of Anatomy, Chennai Medical College Hospital & Research Centre, Trichy, from April-July 2016. After obtaining ethical clearance from the Institutional Ethics Committee, the volunteers were explained in detail about the study protocol and informed consent was obtained from them. Cross sectional study design was conducted among 50 second year undergraduate medical students of ages between 18-19 years of both genders and the attendance register was taken as the sampling frame. This study group was specifically chosen because they were the closest to the relevance of the title of the research and as they had just completed their first year, they would have a greater understanding and remembrance of the difficulties they had faced during their first year. Those who were not willing to participate in this study were excluded from this study.

#### Brief procedure:

A planned, designed and a structured questionnaire to students was used in the study. Data collection was

done by a personal interview of the study subject and the students were explained about the study protocol, along with a clear, detailed explanation of our study and an opinionated consent was taken from the subjects. The questionnaire discussed about their reasons in choosing a medical career, the mental stress at the beginning of the course, the time taken to orient themselves and their levels of understanding of concepts. Demographic parameters included family details, parents' education, occupation, hobbies, aspirations, learning efficiency and techniques, grasping capability and understanding efficiency in students.

### STATISTICAL ANALYSIS

The data collected was entered in Microsoft Excel. Both descriptive and inferential statistical analysis was used to analyze the data. Data was analyzed using the SPSS statistical program (IBM SPSS statistics 21). Chi square test was done to find the association between the two categorical variables

### RESULTS

More than half of the students under study deemed their first year stressed due to the course subjects, 73% of males and 58% of females. High number of volunteers (males-62% and females-79%) opinionated that the pre-medical course will be helpful. Out of the students who felt the need for pre-medical study programme, 97% of them also suggested the inclusion of stress management classes and classes to develop their clinical skills to their pre-medical programme. The results were not statistically significant.

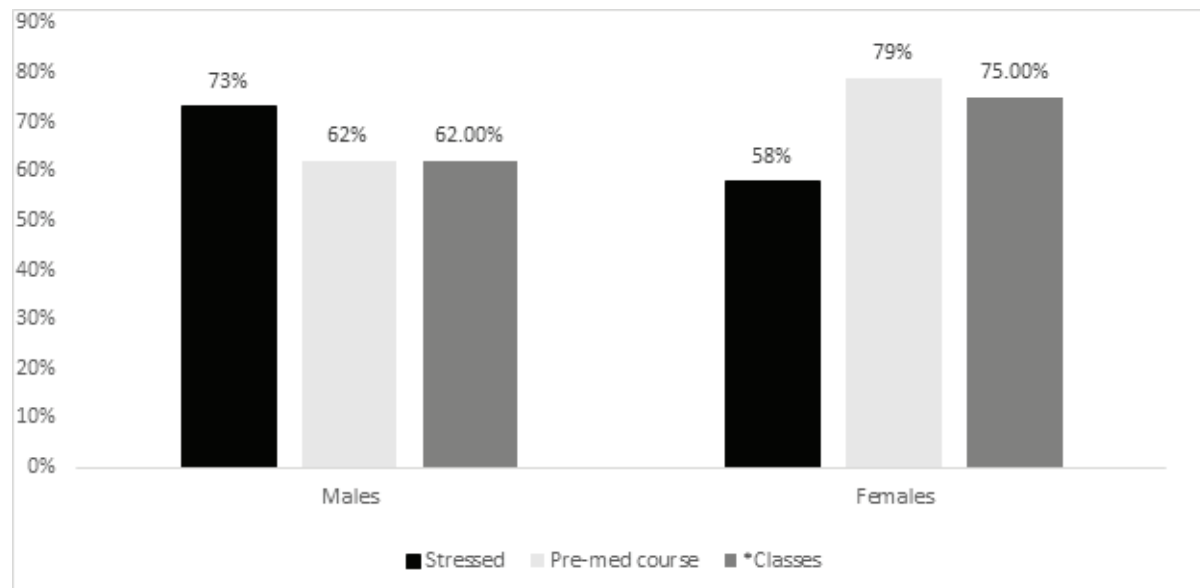
**Table 1: Shows students opinion in the pre medical course being recommended immediate after completion of high school.**

Student Group N=50	Agree	Not Agree	Total
Male	6	19	25
Female	14	11	25
<b>Total</b>	<b>20</b>	<b>30</b>	<b>50</b>

Values were expressed as numbers and frequency.

**Fisher's exact test. The two-tailed P value equals 0.0421\*.**

\*P≤0.05.,\*\*P≤0.01,\*\*\*P≤0.001



**Fig 1: A comparison between male and female students**

\*Classes to manage stress and develop clinical skills

## DISCUSSION

The pre-medical coursework is offered at many American colleges and universities; however, it is considered to be a “track” that follows a certain curriculum. Most pre-medical students major in the natural and applied sciences, such as agricultural science, biology, chemistry, or physics. Many colleges of medicine and undergraduate pre-medical advising offices have yet to formalize pre-medical curricular recommendations. In Australia **and the** United Kingdom, a number of universities offer a three or four year Bachelor of Medical Science, Bachelor of Health Sciences or Biomedical Science degree, which is similar in content and aim to pre-med courses in the United States. However, it is also possible to gain entry to a professional degree program in medicine (usually the Bachelor of Medicine and Bachelor of Surgery degree) directly from High School if the applicant achieves high grades upon graduation. This is the method followed in India as well. The aim of the study was to determine if the pre-medical programme made the understanding of the subjects easier and made students adapt better to the course as compared to students who pursue medicine after a pre-medical programme.(8)

## CONCLUSION

The overload of course materials creates a feeling of disappointment amongst the students because of

the inability to handle all the information at once and succeed during the first year medical course. Our study results will be useful to analyse pros and cons in a short period of time before introducing Pre-Medical course to Undergraduate medical curriculum.

### Implications:

This study will give an insight into the perception of the students to ensure better understanding of concepts, development of applicative ability and the management of pressure that comes with being a doctor and thereby affirming better doctors in the future.

- To explore the possibility of reducing mental stress to the students.
- Better Understanding of concepts and improvement of their grasping capabilities.
- Development Clinical Knowledge and application skills.
- To ensure Orientation to the study programme before they plunge into the core subjects of the course.
- Finally, to prepare them with Attitude and confidence towards the course, which is of crucial value in a course as intense as medicine.

### Limitation & Recommendations:

In the present study, our results can be considered to be preliminary and be viewed with caution of potential rater bias. We also recommend that, this study should

be carried out in different medical colleges across the country with a bigger study population before analyzing the significance of the study.

**Conflict of Interest:** Nil

**Source of Funding:** Nil

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# Study to Determine Prevalence of Obesity and its Association with Cardiometabolic Risk Factors in Young Adults

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## ABSTRACT

**Background:** The global prevalence of obesity has shown an increasing tendency in recent times particularly in young adults. Obesity is a key risk factor in the natural history of many chronic diseases and has a detrimental impact on the incidence of subsequent cardiovascular disease.

**Objectives:** The main objective of the study was to determine the prevalence of obesity and its association with cardiometabolic risk factors in young adults.

**Materials and Method:** A cross sectional study was done in 60 healthy young adults in the age group of 18 to 25 years. The subjects were screened for obesity by measuring their Body Mass Index and Waist Circumference, as defined by WHO screening guidelines. They were also evaluated for cardiometabolic risk factors [Blood Pressure, FBS and Lipid Profile]. Independent distribution and association of indices of obesity across each risk factor was analyzed statistically.

**Results and Conclusions:** The results obtained showed that based on BMI, 35% of them were overweight and 16.6% were obese and based on waist circumference 50% were obese. There was significant increase in blood pressure and serum cholesterol and decrease in HDL cholesterol values in overweight and obese individuals particularly among males. The study recommends the potential role of screening obesity at an early age to take measures to delay the progress of cardiometabolic disorders and prevent its complications in later life.

**Keywords:** Young adults, BMI, WC, Cardiometabolic risk factors.

## INTRODUCTION

In spite of the diverse efforts of many federal organizations towards reducing obesity, the global prevalence of overweight and obesity has shown an increasing tendency particularly among children and young adults in recent times making it one of the most critical public health issue. Indeed they are becoming so common that they are replacing more traditional problems such as undernutrition and infectious diseases as the most significant causes of ill health<sup>1</sup>. The World

Health Organization has called this worldwide rise in obesity, a global epidemic.

Obesity is a result of complex interplay of environmental, social, economic, and behavioral factors, acting on a background of inherent genetic susceptibility. It is a key risk factor in the natural history of many chronic diseases. The detrimental impact of obesity on the incidence of subsequent cardiovascular disease is mediated by increased levels of various risk factors, particularly hypertension, dyslipidemia, diabetes, proinflammatory and prothrombotic states.

The screening for Cardiometabolic risk factors is usually done in individuals after middle age, despite the fact that the risk of these disorders is elevating and becoming common even in the younger population. Moreover most of the current literature on obesity comes

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from studies done in developed countries and defining the extent of the problem in developing countries is also important considering the rise in global prevalence.

## MATERIALS AND METHOD

A cross sectional study was done in 60 subjects. Healthy young adults both male and female [each 30] in the age group of 18 to 25 years were selected. Institutional Ethical committee approval was obtained prior to study. Informed written consent for participation was taken from all the subjects. The study included a questionnaire by which their personal information, medical history, dietary history, level of physical activity and family history of obesity, hypertension, diabetes and cardiovascular disease were documented.

The subjects were screened for two indices of Obesity – Body Mass Index (BMI) and Waist circumference [WC]. Height was measured with a stadiometer. Fasting body weight was measured using an electronic weighing machine. BMI was calculated as weight (kg) / height (meter<sup>2</sup>). The waist circumference was measured as recommended by the WHO STEPS protocol<sup>2</sup>.

They were evaluated for various variables that are known to be associated with Cardiometabolic risk - Blood Pressure, Fasting Blood Glucose and Lipid Profile. Blood pressure was measured with the subject in the sitting position using a mercury sphygmomanometer after 10 minutes of rest. Venous blood samples were obtained in the fasting state and FBS was determined by

GOD POD End point and Kinetic Assay method. Lipid profile was measured by conventional enzymatic assays.

The prevalence of obesity based on BMI was determined and the subjects were divided into three groups as defined by WHO screening guidelines<sup>3</sup>. The prevalence of central obesity based on WC was determined as recommended by The International Diabetes Federation (IDF) which are gender specific<sup>4,5</sup> and the subjects were divided into two groups - Group A – Normal WC [Males - WC < 90 cm and females - WC < 80 cm] Group B – Increased WC [Males - WC ≥ 90 cm and Females - WC ≥ 80 cm].

Blood Pressure was classified according to the guidelines of the seventh report of the Joint National Committee<sup>6</sup>. *Dyslipidemia was defined as* total cholesterol >250 mg/dl, LDL cholesterol > 120 mg/dl, VLDL cholesterol > 30 mg/dl, HDL cholesterol <40 mg/dl, and triglycerides > 160 mg/dl as recommended.

**Statistical Analysis** - Statistical analysis was performed using Statistical Package for Social Sciences (SPSS) software version 23.0. for windows. Independent distribution and association of overall obesity [BMI] and abdominal obesity [WC] across each risk factor [BP, FBG AND LIPID PROFILE] was analyzed by One Way Analysis of Variance (ANOVA) and by Students t test to compare the means of each variable between the different groups. P values less than 0.05 were considered statistically significant and those less than 0.01 as highly significant.

## RESULTS

**Table – 1 - The prevalence of overweight and obesity based on BMI**

SUBJECTS	GROUP I - NORMAL BMI [BMI- 18.5 - 24.9 kg/m <sup>2</sup> ] NO. OF SUBJECTS [%]	GROUP II - OVERWEIGHT [BMI - 25 - 29.9 kg/m <sup>2</sup> ] NO. OF SUBJECTS [%]	GROUP III - OBESE [BMI - ≥ 30 kg/m <sup>2</sup> ] NO. OF SUBJECTS [%]
GENERAL [n = 60]	29 [48.3]	21 [35]	10 [16.6]
MALES [n = 30]	13 [43.3]	12 [40]	5 [16.6]
FEMALES [n = 30]	16 [53.3]	9 [30]	5 [16.6]

**Table – 2 - The prevalence of central obesity based on WC.**

SUBJECTS	GROUP A - NORMAL WC NO. OF SUBJECTS [%]	GROUP B - INCREASED WC NO. OF SUBJECTS [%]
GENERAL [n = 60]	30 [50]	30 [50]
MALES [n = 30]	16 [53.3]	14 [46.6]
FEMALES [n = 30]	14 [46.6]	16 [53.3]

**Table - 3 - The distribution of all the study subjects based on both BMI and WC.**

	<b>BMI Normal No. of Subjects [%]</b>	<b>Overweight No. of Subjects [%]</b>	<b>Obese No. of Subjects [%]</b>
WC Normal	24 [40]	7 [11.66]	0
WC Increased	5 [8.33]	14 [23.33]	10 [16.66]

**Table – 4 - One way ANOVA analysis of differences in Mean  $\pm$  SD of cardiometabolic risk factors in subjects classified based on BMI.**

<b>PARAMETER</b>	<b>GROUP I [n = 29] Mean <math>\pm</math> SD</b>	<b>GROUP II [n = 21] Mean <math>\pm</math> SD</b>	<b>GROUP III [n = 10] Mean <math>\pm</math> SD</b>	<b>P VALUE</b>
BMI	22.52 $\pm$ 2.26	27.20 $\pm$ 1.56	32.24 $\pm$ 1.52	0.00 <b>HS</b>
W C	78.27 $\pm$ 7.29	89.23 $\pm$ 7.40	95 $\pm$ 8.16	0.00 <b>HS</b>
SBP	115.10 $\pm$ 10.72	118.85 $\pm$ 11.34	122.40 $\pm$ 13.68	0.19 NS
DBP	76.96 $\pm$ 8.01	79.33 $\pm$ 7.98	81.40 $\pm$ 5.42	0.24 NS
FBS	81.79 $\pm$ 10.80	83.85 $\pm$ 15.42	83.80 $\pm$ 19.96	0.85 NS
CHOL	142.89 $\pm$ 19.57	164.14 $\pm$ 42.12	162 $\pm$ 23.37	0.05 <b>S</b>
HDL	49.68 $\pm$ 5.85	47.33 $\pm$ 8.69	43.30 $\pm$ 7.76	0.00 <b>HS</b>
LDL	86.06 $\pm$ 16.21	98.38 $\pm$ 37.61	92.20 $\pm$ 22.58	0.27 NS
VLDL	17.17 $\pm$ 4.95	18.42 $\pm$ 9.36	16.50 $\pm$ 3.02	0.70 NS
TG	85.62 $\pm$ 25.16	92.14 $\pm$ 47.15	82.30 $\pm$ 13.97	0.69 NS

**Table – 5 - One Way ANOVA analysis - gender wise of cardiometabolic profile in groups based on BMI.**

<b>PARA-METER</b>	<b>GROUP I</b>			<b>GROUP II</b>			<b>GROUP III</b>		
	<b>Males n = 13 Mean <math>\pm</math> SD</b>	<b>Females n = 16 Mean <math>\pm</math> SD</b>	<b>P value</b>	<b>Males n = 12 Mean <math>\pm</math> SD</b>	<b>Females n = 9 Mean <math>\pm</math> SD</b>	<b>P value</b>	<b>Males n = 5 Mean <math>\pm</math> SD</b>	<b>Females n = 5 Mean <math>\pm</math> SD</b>	<b>P VALUE</b>
BMI	22.57 $\pm$ 2.28	22.48 $\pm$ 2.31	0.917 NS	27.07 $\pm$ 1.69	27.37 $\pm$ 1.44	0.682 NS	31.26 $\pm$ 0.67	33.22 $\pm$ 1.53	<b>0.031 S</b>
SBP	122.92 $\pm$ 5.80	108.75 $\pm$ 9.57	<b>0.000 HS</b>	127.16 $\pm$ 5.07	107.77 $\pm$ 6.66	<b>0.000 HS</b>	126.80 $\pm$ 7.29	118 $\pm$ 17.88	0.338 NS
DBP	80.92 $\pm$ 6.14	73.75 $\pm$ 8.06	<b>0.014 S</b>	83.83 $\pm$ 3.12	73.33 $\pm$ 8.66	<b>0.001 HS</b>	84.80 $\pm$ 4.14	78 $\pm$ 4.47	<b>0.037 S</b>
FBS	88.38 $\pm$ 10.61	76.43 $\pm$ 7.71	<b>0.002 S</b>	89.58 $\pm$ 17.51	76.22 $\pm$ 7.62	<b>0.046 S</b>	79.6 $\pm$ 11.58	88 $\pm$ 26.80	0.538 NS
CHOL	144.46 $\pm$ 24.24	141.62 $\pm$ 15.53	0.705 NS	170.08 $\pm$ 48.14	156.22 $\pm$ 33.54	0.470 NS	166.80 $\pm$ 16.02	137.20 $\pm$ 20.62	<b>0.035 S</b>
HDL	40.92 $\pm$ 4.51	38.68 $\pm$ 6.73	0.315 NS	46.91 $\pm$ 10.63	47.88 $\pm$ 5.71	0.807 NS	44 $\pm$ 9.53	42.60 $\pm$ 6.58	0.794 NS
LDL	86.92 $\pm$ 20.86	85.37 $\pm$ 11.88	0.803 NS	103.33 $\pm$ 42.27	91.77 $\pm$ 31.52	0.500 NS	104.60 $\pm$ 7.76	79.80 $\pm$ 26.50	0.080 NS
VLDL	16.69 $\pm$ 4.75	17.56 $\pm$ 5.24	0.647 NS	20.25 $\pm$ 10.55	16 $\pm$ 7.38	0.316 NS	18.20 $\pm$ 2.48	14.80 $\pm$ 2.68	0.071 NS
TG	82.76 $\pm$ 24.06	87.93 $\pm$ 26.57	0.592 NS	101.25 $\pm$ 53.39	80 $\pm$ 36.74	0.319 NS	89.80 $\pm$ 10.96	74.80 $\pm$ 13.36	0.088 NS

**Table – 6 - t test analysis of differences in Mean ± SD of cardiometabolic risk factors of all subjects classified based on their WC.**

PARAMETER	GROUP A - Normal WC [n = 30] Mean ± SD	GROUP B - Increased WC [n = 30] Mean ± SD	P VALUE
BMI	22.86 ± 2.53	28.69 ± 3.13	0.000 HS
W C	78.43 ± 7.46	91.36 ± 7.76	0.000 HS
SBP	116.40 ± 11.36	118.93 ± 11.88	0.269 NS
DBP	77.06 ± 8.25	80.00 ± 6.94	0.082 NS
FBS	81.70 ± 10.96	84.00 ± 16.72	0.516 NS
CHOL	145.23 ± 19.04	158.46 ± 38.72	0.132 NS
HDL	41.93 ± 7.94	44.00 ± 7.94	0.304 NS
LDL	87.20 ± 17.42	95.60 ± 33.34	0.252 NS
VLDL	16.10 ± 5.01	18.90 ± 7.68	0.106 NS
TG	80.40 ± 25.51	94.30 ± 38.51	0.108 NS

**Table – 7 - t test analysis - gender wise of cardiometabolic risk factors of subjects based on their WC.**

PARAMETER	MALES			FEMALES		
	GROUP A Normal WC [n = 16] Mean ± SD	GROUP B Increased WC [n = 14] Mean ± SD	P VALUE	GROUP A Normal WC [n = 14] Mean ± SD	GROUP B Increased WC [n = 16] Mean ± SD	P VALUE
BMI	23.28 ± 2.51	28.72 ± 2.56	0.000 HS	22.39 ± 2.56	28.66 ± 3.65	0.000 HS
W C	83.25 ± 5.49	97.21 ± 5.87	0.000 HS	72.92 ± 5.32	86.25 ± 5.17	0.000 HS
SBP	123.87 ± 6.30	127.00 ± 5.12	0.151 NS	107.85 ± 9.74	111.87 ± 11.67	0.319 NS
DBP	80.75 ± 5.31	85.00 ± 3.39	0.016 S	72.85 ± 9.13	75.62 ± 6.29	0.337 NS
FBS	87.93 ± 10.42	86.78 ± 17.53	0.826 NS	74.57 ± 6.33	81.56 ± 16.14	0.140 NS
CHOL	144.25 ± 22.53	174.64 ± 42.59	0.019 S	146.35 ± 14.84	144.31 ± 29.48	0.816 NS
HDL	42.75 ± 8.16	45.07 ± 8.93	0.463 NS	41.00 ± 7.88	43.06 ± 7.13	0.458 NS
LDL	86.06 ± 22.24	108.28 ± 35.18	0.045 S	88.50 ± 10.15	84.50 ± 28.18	0.619 NS
VLDL	15.43 ± 4.36	21.71 ± 8.86	0.018 S	16.85 ± 5.73	16.43 ± 5.66	0.842 NS
TG	76.75 ± 22.11	108.00 ± 44.98	0.020 S	84.57 ± 29.20	82.31 ± 28.02	0.831 NS

## DISCUSSION

Obesity is a modifiable risk factor associated with many chronic diseases. The rise in its prevalence particularly in young adults is a major concern because of the associated long term health effects. The aim of this study was to determine the prevalence of obesity and its association with cardiometabolic risk factors in young adults. This cross sectional study was done in 60 young adults in the age group of 18 to 25 years.

The results of distribution of subjects based on the history documented showed overlap between the family history and dietary habits in the groups suggesting the transition in lifestyle and the trends prevalent which are common to most of the young individuals.

The prevalence of obesity was determined by measuring the indices of obesity, Body Mass Index and Waist Circumference. The results obtained showed

that based on BMI, 35% of them were overweight and 16.6% were obese. Based on WC central obesity was prevalent in 50% of the subjects. Almost similar results were obtained when the subjects were analyzed based on gender. The prevalence of obesity in young adults is consistent with the studies done earlier<sup>7</sup>.

The results showed enormous degree of overlap in overweight/obese and abdominally obese subjects suggesting that either index to measure adiposity will identify the prevalence, but if only BMI is used to screen obesity, the group with normal BMI but increased WC would not be identified as WC which correlates with visceral adiposity is considered to be more predictive of obesity related health consequences independent of BMI.

The cardiometabolic variables were measured and its relation was seen with increase in BMI and WC. The increase in Mean  $\pm$  SD in serum cholesterol levels in overweight individuals compared to normal subjects and decrease in HDL levels in obese individuals compared to overweight and normal subjects indicate the possibility of an increased susceptibility for atherosclerotic diseases. Our findings are consistent with other studies<sup>8,9</sup>.

It also shows the increase in systolic blood pressure, diastolic blood pressure and fasting blood sugar in overweight and obese individuals compared to normal subjects, though not significant statistically but the blood pressure values may have clinical significance as they are falling in the prehypertensive group. Prehypertension could serve as an early marker of adverse cardiometabolic profile in apparently healthy adults and its early detection and control could potentially reduce the burden of Cardiovascular diseases in later life. Our finding of association of BMI with prehypertension is consistent with other reports<sup>10</sup>.

Similarly when association of these variables was analysed with increase in WC, all the parameters increased with increase in WC but the differences were statistically not significant.

Gender based comparisons based on both BMI and WC of these variables showed that the males had increased values of all variables and the systolic and diastolic blood pressure and serum cholesterol were significantly high compared to females and the increase in these variables with obesity was also more in males compared to females. The differences between male and

female subjects in this study are significant, suggesting that the cardiometabolic risk factors develop earlier in males. Alternatively, the same definitions and cut off values for males and females may not determine equivalent risk.

## CONCLUSION

The study results show the presence of overweight and obesity in young adults and that both BMI and Waist Circumference should be measured as each confer differential risk for every cardiometabolic marker.

Abnormal levels of Cardiometabolic risk factor variables were more prevalent among overweight or obese young adults than those within normal range particularly among males. This suggests obesity during adolescence is associated with significantly higher complications in later life as evident by the increased values of risk variables.

The findings of the study emphasize the potential role of screening and evaluating obesity at an early age for early detection and intervention to effectively prevent progression of cardiometabolic diseases and thereby reduce morbidity, improve quality of life and lower cardiovascular mortality in later life. In addition there is a need for education on healthier lifestyles.

The study would also recommend the need to establish gender based reference ranges for biochemical parameters. Further studies may be carried out on a larger sample size to identify the extent of the problem.

**Conflicts of Interest** - The authors declare no conflict of interest.

**Source of Funding** - Self

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# A Study on Saliva as an Indicator for Type II Diabetes Mellitus

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## ABSTRACT

**Objectives** – To Evaluate the diagnostic and monetary value of salivary glucose in patients with Type II Diabetes Mellitus

**Material & Method** - A cross sectional and observational study done on 30 subjects of which 20 were Type II diabetes and 10 control healthy age matched individuals , the subjects were instructed to collect fasting Saliva in a sterile beaker , the specimen was subjected to centrifugation and the glucose levels were estimated by GOD POD method

The Data collected was subjected to Statistical Analysis by using Student T test

**Results** - The correlation coefficient of salivary glucose in study and control group was calculated using unpaired T test and P value was found to be less then 0.01.

**Conclusion** – It is concluded that the fasting salivary glucose level can be used as anon invasive diagnostic as well as monitoring tool to access the Glycemic status of diabetes Mellitus subjects.

**Keywords** – Salivary Glucose , Diabetic Mellitus

## INTRODUCTION

Diabetes mellitus is the most common growing metabolic disorder in the world. it is mostly of two types non insulin dependent diabetes mellitus and insulin dependent diabetes mellitus. According to the American Diabetes Association, diabetes mellitus has been classified on the basis of etiology into Type 1 and Type 2, other specific types (which include genetic defects in  $\beta$ -cells, genetic defect in insulin action<sup>3</sup>

Currently 366 million cases are present world wide, India ranks second with diabetic population. It is estimated that by 2030 India will be the on top of diabetes list.with 552 million cases

Current diagnostic tool in diabetes is mostly by blood and urine glucose estimation, for the glucose to be

detected in urine levels should cross the threshold point, which leads to missing of many cases. and blood sample estimation of glucose levels is a invasive procedure. This invasive procedures causes anxiety in the individual causing difficulty in monitoring in long term, and there is a risk of infections and finger callus.

Saliva offers distinctive advantage as it can be collected noninvasively and with limited training, involves fewer complications, and is cost effective. <sup>7</sup>It is beautifully said by Mandel that ‘saliva lacks the drama of blood, sincerity of sweat and emotional appearance of tears’ Saliva acts as a mirror of the body and, hence, is a perfect medium to be explored for disease and health surveillance.

Considering the ease, painless procedure,[ and noninvasiveness of collecting salivary samples, and also the limited amount of studies that evaluated the concentration of glucose in saliva, this study was undertaken to authenticate the reliability of saliva as a biomarker for the prediction of glucose levels in Type II diabetes.

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In this study saliva was used as an indicator for estimation of glucose concentration and it is compared with the blood glucose levels. so that non invasive procedure is used for monitoring glucose levels in diabetes individuals.

**MATERIAL AND METHOD**

This was a cross sectional study done on 30 subjects in the age group of 40-50 years of age , samples were obtained from patients visiting medical out patient in Bhaskar medical college, individuals were divided into two groups.

Study group: 20 known uncontrolled diabetic cases

Control group: 10 normal healthy age matched cases, both the groups were instructed to collect fasting salivary sample 2ml in a sterile beaker by spitting and subjected to centrifugation and glucose levels are estimated by using GOD POD method. At the same time fasting blood samples was also collected from both the groups by venopuncture, and analyzed by GOD POD method inclusion criterion – diabetic patient in the age group of 40-50years control subject from same age with non diabetic & no other ailments

**Exclusion Criterion** – patients with following complications were excluded diabetic with other complication Bad oral health Tobacco chewer & smokers Dental Problem Gastro intestinal diseases

**RESULTS**

In the control group, the serum glucose levels ranged from 73.91 to 101.0 mg/dL, with a mean of 81.4 mg/dl and SD of 9.70. The salivary glucose levels ranged from 0.51 to 2.32 mg/dl, with a mean of 2.91 mg/dl and SD of 0.80. The correlation coefficient between serum glucose and salivary glucose was calculated and the *r* value was found to be 0.5216, which was statistically significant (*P* < 0.05).

In the patient group, the serum glucose ranged from 78.1 to 236.4 mg/dl, with a mean of 203.95mg/dl and SD of 51.73 The salivary glucose ranged from 0.31 to 18.16 mg/dl, with a mean of 11.29 mg/dl and SD of 3.82.

The correlation coefficient between serum glucose and salivary glucose was calculated and the *r* value was found to be 0.7686, which was highly significant (*P* < 0.01). It is worth noting that the significance of the study

group was much greater than that of the control group.

Comparisons of salivary glucose levels between the control and patient groups were performed with an independent *t*-test; a *t*-value of 3.21 was obtained and the difference was highly significant (*P* < 0.01),

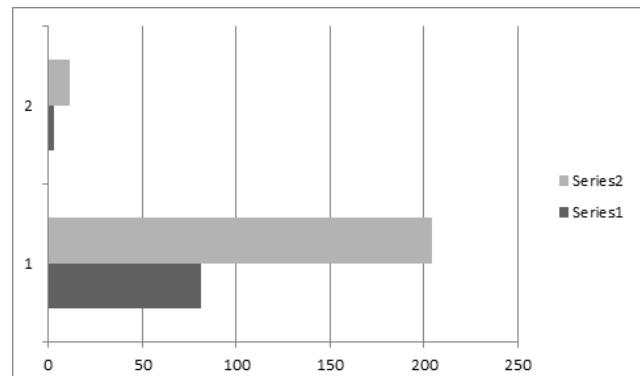
**RESULTS**

**Table I : Control group.**

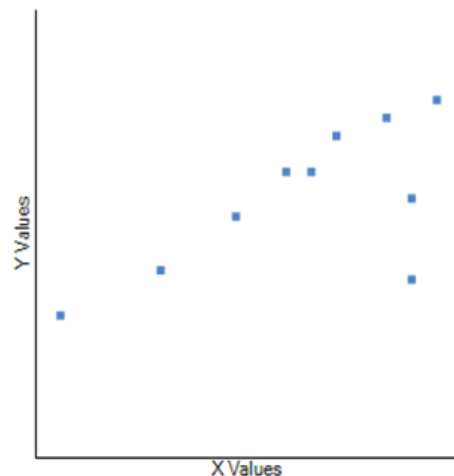
	mean	Standard deviation
Salivary glucose	2.91	0.80
Blood glucose	81.4	9.70

**Table II: Diabetes group**

	mean	Standard deviation
Salivary glucose	11.29	3.82
Blood glucose	203.95	51.73



**Graph 1 : Comparison of salivary and Blood Glucose in Both Groups**

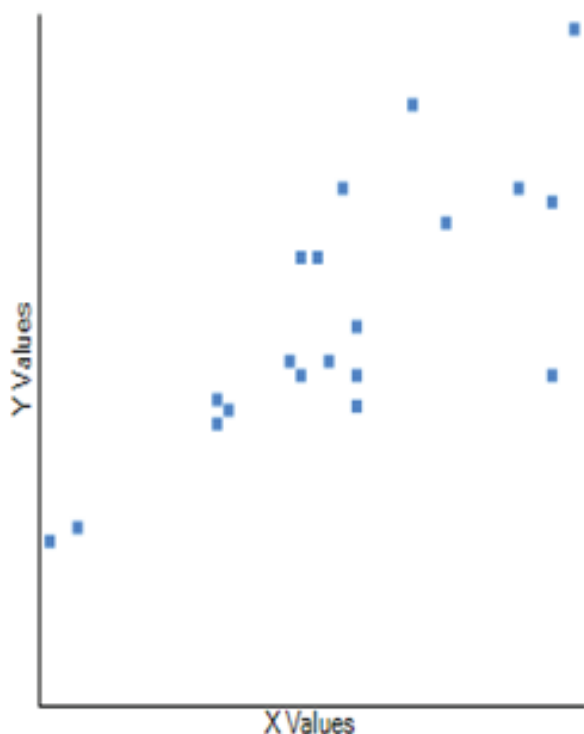


**Graph 2: Correlation coefficient in control group**

X axis-fasting blood glucose levels.

Y axis-fasting salivary glucose levels.

The value of R is 0.6641. This is a moderate positive correlation, which means there is a tendency for high X variable scores go with high Y variable scores (and vice versa).



**Graph 3**

### **Correlation of Coefficient in Diabetic group**

X axis-fasting blood glucose levels.

Y axis –fasting salivary glucose levels

The value of R is 0.7686. This is a strong positive correlation, which means that high X variable scores go with high Y variable scores (and vice versa).

The value of  $R^2$ , the coefficient of determination, is 0.5969.

## **DISCUSSION**

Diabetes mellitus is a group of metabolic disorders that share the common underlying feature of hyperglycemia. Hyperglycemia in diabetes results from defects in insulin secretion, insulin action, or, most commonly, both. Chronic hyperglycemia and the attendant metabolic disturbance may be associated with

secondary damage in multiple organ systems, especially the kidneys, eyes, nerves, and blood vessels. Data indicates that in 2011, 366 million people worldwide were affected by diabetes and the number is continuing to climb steeply. By 2030, predictions suggest that the number of people with diabetes will reach 552 million. Currently, India is in the second position in the chart<sup>3</sup>

Invasive procedures are the risk for infections and anxiety to the individuals in long run, So non invasive procedure like saliva glucose estimator can be used as an indicator for glycemic control as it is collected easily<sup>6</sup>, Glucose is a small molecule which can easily diffuse through semi permeable membrane and hence can be detected in the saliva .due to defect in the basement membrane which causes leak of glucose.(diabetic membranopathy)

In this study a significant correlation was found between fasting blood glucose levels and fasting salivary glucose levels for both diabetic and healthy individuals., Supporting that saliva can be used as a non invasive diagnostic tool in management of type II diabetic individuals. correlation between glucose measured during a tolerance test and the associated spectral response has been established and used to predict in vivo blood glucose concentrations during a tolerance test. The common sources of false correlation, such as instrument drift and major blood analysts, have been eliminated as contributors to the prediction results.<sup>7</sup>

The salivary glucose level was significantly elevated in diabetics. This could be attributed to the altered glucose homeostasis. It was stated by Chatterton RT et al., that salivary glands act as filters of blood glucose, that would be altered by hormonal or neural regulations<sup>8</sup>. Many other authors have also found higher glucose salivary levels in diabetic patients than in non-diabetics<sup>9</sup>. Lopez et al., in their study, also observed that diabetic saliva glucose values were higher than those of the controls and also a negative correlation was found between salivary glucose levels and the Glycemic status<sup>11</sup>

The study results were also in agreement with those of Karjalainen et al., who also reported that the elevated salivary glucose levels in diabetics decreased after starting with insulin treatment. On the contrary, Sharon et al., did not report any difference in salivary glucose levels<sup>10</sup>

## CONCLUSION

This study showed significant elevation of salivary glucose levels in T2DM, thereby emphasizing the fact that the salivary composition was not just a reflection of the oral health state of a subject, but also of one's systemic state. Future studies can be conducted on a larger scale, taking into account the various limitations. This would help us in conclusively establishing the role of saliva in the screening and diagnosis of diabetes, as an alternative to blood. Thus salivary glucose can be used as tool as diagnostic tool instead of invasive blood glucose levels.

**Conflict of Interest** - Nil

**Ethical Clearance** – Taken From IEC Bhaskar medical college

**Source of Fund** – Self

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# Impact of BMI on Autonomic Function in Females

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## ABSTRACT

**Background** – Body mass index (BMI) serves as an index of adiposity, and adiposity has been postulated to influence the functioning of the autonomic nervous system (ANS). The World health organisation (WHO) and other regional agencies have put forward various classifications based on BMI.

**Methodology** – This study was done on 60 healthy females in the age group of 18-25 years who have been classified into three groups of 20 each based on WHO BMI classification. A battery of autonomic function tests (AFTs) as proposed by Ewing was done and the results were compared across the groups using unpaired t test, with significance set at  $P < 0.05$ .

**Results** – All the seven tests representing the parasympathetic and sympathetic nervous system were normal, but there was a statistically significant lesser fall in SBP to standing in underweight and overweight individuals compared to normal BMI subjects.

**Conclusion** – The Autonomic nervous system was normal in our study subjects of different BMI categories, as depicted by the results of the AFTs.

**Keywords** – BMI, Females, Overweight, Underweight.

## INTRODUCTION

Adolphe Quetelet (a Belgian anthropologist) proposed in the late 19<sup>th</sup> century the ratio of body weight to squared stature ( $W/S^2$ ) as a measure of body shape. But it was not until a century later that the term BMI entered the medical subject heading of the national library of medicine, where it is defined as “one of anthropometric measures of body mass; it has the highest correlations with skinfolds and body density”.<sup>1</sup>

The term autonomic nervous system (ANS) was proposed by the physiologist Langley to include the sympathetic, parasympathetic and enteric nervous system. The ANS acts as an interface between internal and external milieu, by controlling various body

functions to maintain homeostasis as well as produce adaptive responses.<sup>2</sup>

Visceral fat by producing cytokines like leptin has been postulated to cause activation of the sympathetic nervous system by increasing the sympathetic nerve activity.<sup>3</sup> No single test is capable of assessing the ANS in its entirety, hence normally a battery of tests are employed and the most preferred ones are those recommended by Ewing.<sup>4</sup>

Therefore this study was proposed to observe the autonomic nervous system, by employing a set of AFTs across three categories of BMI in female subjects (normal, underweight and overweight).

## MATERIALS AND METHOD

The present study was conducted in the department of physiology, Karnataka institute of medical sciences, Hubli after obtaining ethical clearance from the institutional ethical committee. Data was collected from twenty female subjects each (low, normal and high BMI

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groups) in the age group of 18-25 years, who satisfied the relevant inclusion and exclusion criteria. BMI less than 18.5 was considered as underweight, 18.5-25 as normal weight and those with BMI >25 as overweight. Inclusion criteria: Females in the age groups of 18 to 25 years with regular menstrual cycles and those not on any medication that may alter the autonomic function for the preceding two weeks of the test. Persons with age < 18 or > 25 years, those with history of any other disease that is known to produce autonomic dysfunction like diabetes mellitus/hypertension/ischemic heart disease/scleroderma/chronic bronchitis/peripheral nervous system disorders, those practicing yoga or any other physical training, males, individuals with history of smoking/drug abuse, those with history of irregular menstrual cycles/amenorrhea were excluded. Informed consent was taken and the detailed procedure/purpose of the study was explained to each participant.

The procedures were usually done between 10.00 am to 1.00 pm. Detailed history was obtained followed by measurement of anthropometric variables like height and weight using standard techniques. BMI was calculated from height and weight using Quetelet's index. Resting heart rate was obtained in lead II using the ECG machine (Hygeia) and resting blood pressures (SBP and DBP) were recorded using OMRON automatic blood pressure monitor model HEM 712 C. Seven standard cardiovascular autonomic function tests were performed

by the subjects:

- Resting heart rate.
- Heart rate response to Valsalva maneuver.
- Heart rate variation during deep breathing (Expiration/Inspiration ratio).
- 30:15 ratio (Heart rate response to immediate standing).
- S:L ratio (standing to lying ratio).
- Blood pressure response to standing.
- Blood pressure response to sustained handgrip.

The results of the tests were expressed as mean  $\pm$  standard deviation and were subjected to unpaired t test using Microsoft excel 2007 with significance set at  $P < 0.05$ .

## RESULTS

The anthropometric data of the study subjects are provided in table – 1. There were twenty subjects each in the three subgroups – normal weight (NW), underweight (UW) and overweight (OW). The three groups were well matched as far as age and height are concerned, whereas weight and BMI are representative of the respective groups.

Parameter	Normal weight subjects(mean $\pm$ SD)	Underweight Subjects(mean $\pm$ SD)	Over weight Subjects(mean $\pm$ SD)
Age(years)	19.45 $\pm$ 0.82	19.15 $\pm$ 0.58	20.45 $\pm$ 1.54
Height(cms)	152.6 $\pm$ 4.86	155.5 $\pm$ 7.13	154.4 $\pm$ 7.05
Weight(kgs)	48.7 $\pm$ 4.74	42 $\pm$ 5.04*	63.2 $\pm$ 5.87**
BMI	20.97 $\pm$ 1.22	17.34 $\pm$ 0.99*	26.55 $\pm$ 0.72**
* P < 0.001 between normal weight and underweight individuals. ** P < 0.001 between normal weight and overweight individuals.			

The mean and standard deviations of resting cardiovascular parameters (heart rate, systolic blood pressure and diastolic blood pressure) for the three subgroups are represented in table – 2. Barring a significantly higher DBP in overweight group (albeit within the normal range), the groups had similar resting heart rates and SBP.



**Table No. 2: Resting cardiovascular parameters of subjects**

Parameter	Normal weight subjects(mean±SD)	Underweight Subjects(mean±SD)	Over weight Subjects(mean±SD)
Heart rate (beats/min)	77 ± 9.03	72.8 ± 6.77	74.4 ± 5.88
SBP (mm of Hg)	108.75 ± 10.41	108.5 ± 7.37	113.35 ± 4.35
DBP (mm of Hg)	66.6 ± 7.35	64.75 ± 6.87	73.45 ± 5.89**
** P < 0.01 between normal weight and overweight individuals.			

The results of the parasympathetic functions of the three subgroups are depicted in table – 3 and are in the normal ranges and without any significant differences between the groups.

**Table No. 3: Parasympathetic function tests of subjects**

Parameter	Normal weight subjects(mean±SD)	Underweight Subjects(mean±SD)	Over weight Subjects(mean±SD)
E:I ratio	1.62 ± 0.21	1.64 ± 0.21*	1.58 ± 0.16**
30:15 ratio	1.46 ± 0.23	1.54 ± 0.29*	1.55 ± 0.17**
S:L ratio	1.45 ± 0.17	1.58 ± 0.26*	1.46 ± 0.11**
Valsalva ratio	1.58 ± 0.30	1.65 ± 0.43*	1.51 ± 0.16**
* P > 0.05 between normal weight and underweight individuals. ** P > 0.05 between normal weight and overweight individuals.			

Table – 4 shows the details of the sympathetic function tests in the study subjects, with the fall in SBP on standing being significantly lower in underweight and overweight individuals when compared to normal weight individuals. But the results are still in the normal range.

**Table No. 4: Sympathetic function tests of subjects**

Parameter	Normal weight subjects(mean±SD)	Underweight Subjects(mean±SD)	Over weight Subjects(mean±SD)
Fall in SBP on standing (mm of Hg)	5.1 ± 1.25	3.45 ± 1.50*	3.95 ± 1.70**
Rise in DBP to sustained hand grip (mm of Hg)	14.55 ± 2.76	14.65 ± 4.23	15.9 ± 2.77
* P < 0.001 between normal weight and underweight individuals. ** P < 0.01 between normal weight and overweight individuals.			

## DISCUSSION

In the present study the autonomic function tests assessing both the parasympathetic and sympathetic components was normal in underweight (BMI <18.5), normal healthy weight (BMI > 18.5-25) and overweight

(BMI >25) groups.

Specifically the various parasympathetic tests was not statistically significant when comparison was made across the BMI ranges in our study population. A cross-sectional study done on 159 diabetic individuals found

that there was no difference in Valsalva ratio across the various BMI ranges in individuals with type 1 or type 2 diabetes, thereby concluding that obesity is not a confounding factor in the performance of autonomic function tests.<sup>5</sup> Similarly the BMI status did not independently correlate with 30/15 ratio.<sup>6</sup>

The HRV (heart rate variability) which is a measure to evaluate the cardiac autonomic activity is also unaltered in underweight individuals when compared to people with weight in the normal ranges.<sup>7</sup> A study done on 204 young adults (98 men, 106 women) in the age group of 18-40 found that BMI in the range of 21.08 ± 3.41 kg/m<sup>2</sup> correlated weakly with various indices of cardiac autonomic function.<sup>8</sup>

Autonomic nervous system abnormalities have been noted by some studies in people who are overweight and/or obese.<sup>9,10</sup> Absence of differences in parasympathetic component of AFT between obese and normal individuals, may be due to factors like duration of obesity and physical activity profile of the subjects as these could modify the parasympathetic activity.<sup>11</sup>

The sympathetic function tests (fall in SBP on standing and rise in DBP on sustained handgrip test) were also normal across the three groups in our study. Similar results have been obtained as far as these two tests of sympathetic components of AFT are concerned in a study by Rossi M et al.<sup>12</sup>

Though the results of SBP to standing was within the normal range in all the three groups, there was a significantly lesser fall in SBP in underweight and overweight groups when compared to normal subjects. The BP response to a change in posture has been found to be complex, when studied in large number of subjects, and were not as characteristic as the responses seen with other tests like either handgrip test or mental arithmetic tasks.<sup>13</sup> Also there is the practical difficulty of measuring the SBP immediately after standing, in order to correctly obtain the results.

**Conclusion** – The AFTs done in our population of females in the age group of 18-25 years was essentially normal, and variations in BMI through the spectrum of underweight, normal weight and overweight did not produce any noticeable abnormalities in the ANS.

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# A Study to Assess the Effect of Intensity of Music on Heart Rate Variability

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## ABSTRACT

**Introduction and Aim:** Music has positive influence on health. In the past few decades, listening to music has transcended frames and has become a modality of treatment in stress management and to enhance recuperation in post-operative period and in many medical conditions. As music influences the autonomic nervous system which in turn determines the health of the cardiovascular system, this study has been designed to evaluate the effect of intensity of music on heart rate variability(HRV).

**Materials and method:** This experimental observational study was carried out on 30 healthy volunteers (15males and 15 females) between the age of 18and 23. The variables of blood pressure(BP), heart rate and lead II ECG were recorded in all participants before and during listening to music. The volunteers listened to the same soft relaxing music at different intensity ranges, 60-70 dB and 71-80dB. Spectral analysis of HRV was done using Kubios software.

**Results:** The assessment of the Frequency domain parameters revealed a significant difference between the groups with significant parasympathetic accentuation when listened to music at low intensity range(60-70 dB range than at 71-80dB range).

**Conclusion:** From the observations of our study it could be inferred that relaxing music when listened at lower intensities is beneficial to confer relaxation and enhance cardiovascular health as inferred from the pronounced parasympathetic parameters.

**Keywords:** Frequency domain indices, Heart rate variability, Intensity of music.

## INTRODUCTION

Down the ages, entwined in civilizations, music has had its strong roots in human history and has been an integral part of man's life. Music's universality could be attributed to its ability to influence the mood, arousal and whereby the cognition.<sup>1</sup> By influencing the mental state, music improves the physical performance and the quality of life.<sup>2</sup> Recently, in the past few decades music

has emerged as a modality of healing and has proven to have positive outcomes on health and recuperation.<sup>3</sup> One of the most prominent effect of music on health is its ability to influence the autonomic nervous system which in turn determines the cardiovascular health.<sup>4</sup> In the recent times, as an effort to assess and affirm the influence of music on healthy modulation of the heart, the heart rate variability is being used as an efficient tool to establish the relation between music and the cardiac activity.<sup>5</sup>

Cardiovascular health is determined by the sympathovagal balance that controls the heart rate and the vascular tone. The normal resting vagal control is overridden by symapathetic accentuation with alertness and activity. As sympathovagal balance could be genetically influenced and the chances of inheriting sympathetic

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pronouncement is proven, which underlies the basis for inherited hypertension and its sequel, maintaining the cardiac regulation towards vagal activity is needed to post-pone the onset of hypertension.<sup>6</sup> This could be done by implementing measures to procrastinate the disease development like life-style modification measures that include listening to beneficial music.

With music being included as a modality of treatment, the health benefits of various features of music have been well researched. It has been found that music with slow tempo with less variation in pitch all through the composition, like the classical music, has healthy outcomes on the cardiac health when compared to fast tempo music.<sup>7</sup> Music in lower pitches are associated with arousal when compared to high pitch composition.<sup>8</sup> It has also been observed that particular music timbre could induce a consistent emotional response.<sup>9</sup> As the present generation is already in the habit of listening to music, researches to analyse the subjective effect of music on individual has made the inference that music imparts positive emotions and improves the feeling of wellbeing.<sup>10</sup>

As various aspects of music have been analysed for its beneficial modulation on health, this study has been designed to assess the effect of intensity of music on heart rate variability as there are no sufficient studies to establish its role in HRV modulation.

## MATERIAL AND METHOD

This cross sectional, observational study was performed in the research lab of Department of Physiology, Sri Venkateshwaraa Medical College Hospital & Research Centre, Puducherry, after obtaining the Institutional Ethical Committee clearance. A total of 30 normotensive volunteers (15 males and 15 females) between the age of 18 and 23 years were recruited from the campus as subjects for this study. These volunteers listened to the same relaxing music at different intensities as two trials

**Trial with 60-70 dB :** volunteers listened music at 60-70 dB

**Trial with 71-80 dB :** volunteers listened music at 71-80 dB

The volunteers were selected based on the following inclusion and exclusion criteria.

**Inclusion criteria:** Volunteers with BP < 120/80 mm Hg (normotensives) and those who were in the normal range of Body mass index (BMI) (18.5 to 22.9 kg/sq. m.) were recruited for the study.

**Exclusion criteria:** Smokers, alcoholics, diabetics and those with history of metabolic, renal and endocrine diseases as well as any acute or recent illness were excluded. Yoga practitioners and those who were on any other medication that affects autonomic nervous system and those who had aversion for music were not included.

## METHODOLOGY

The subjects were informed briefly about the procedure and an informed written consent was obtained from all the subjects. The subjects were requested not to participate in any exercise or heavy physical activity and to avoid having a heavy meal two hours before the test. The subjects were made to come half an hour prior to the commencement of testing procedure, in order to allow the familiarization with the environment and to establish a resting state and the effect of different music intensities on HRV were assessed as follows.

**Recording of resting basal values :** The basal blood pressure was manually measured by using a mercury sphygmomanometer and the Lead II ECG recording was done using PHYSIOPAC PP4 MEDICAID system, Chandigarh. The ECG recording was done for 5 minutes after 20 minutes of rest in supine posture and basal BP was recorded at the end of ECG recording. Heart rate was calculated from ECG obtained. The subjects were requested not to do any gross body movements, conversation and mental activities while recording the ECG.

### Music intervention and HRV recording

The participants listened to the same 5 minutes excerpt of relaxing instrumental piano music which they never listened before, to avoid the influence of music familiarisation influencing the biological variables.<sup>11</sup> The music was listened through headphones. The volunteers listened to the music in the decibel range of 60-70 dB and 71-80 dB with 10 minutes of no music between the trials and HRV was recorded while listening to music. The sequence of music played at different intensities was randomised for each individual. The recording was done in accordance to the recommendation of the Task Force on HRV.<sup>12</sup> The data analysis was then done using the

Kubios HRV analyser. The spectral indices of frequency domain measures of HRV were assessed:

### Frequency domain measures:.

a) Normalized low frequency power (LFnu)- reflects sympathetic activity

b) Normalized high frequency power (HFnu) – reflects parasympathetic activity

c) Ratio of low frequency power to high frequency

power (LF-HF ratio) – reflects sympathovagal balance

d) Total Power (TP)– reflects overall parasympathetic activity

## STATISTICAL ANALYSIS

The data were expressed as mean±SD. To test the significance between basal values and trial values paired ‘t’ test was done (using SPSS version 17). The statistical probability  $p < 0.05$  was considered to be significant.

## RESULT

**Table 1: Comparison of Frequency domain variables of both trials with basal values**

Parameters	Basal values	Trial with 60-70dB	Trial with 71-80dB	P value (Basal vs Trial with 60-70dB)	P value (Basal vs Trail with 71-80 dB)
HR (bpm)	66.51±7.09	58.41±7.87	64.62±6.35	0.0027**	0.0138 *
MAP (mm Hg)	82.09± 4.88	74.21±14.7	78.53±7.01	0.0101*	0.0187*
Lf nu	64.25±5.9	58.65±6.15	61.63±6.01	0.0031**	0.055
HF nu	35.75±5.88	41.35±6.15	38.37±6.007	0.0013**	0.055
LF/HF ratio	1.869±0.453	1.47±0.363	1.669±0.42	0.0011**	0.0463*
TP (m <sup>2</sup> )	906.63±115.72	1211.4±240.6	1005.37±128.59	0.0001***	0.0053**

MAP – mean arterial pressure    HR – heart rate    \*P < 0.05 ; \*\*P < 0.01; \*\*\*P < 0.001

The above table (Table 1) compares the various frequency domain parameters of both trials with basal values .The comparison reveals that the various frequency domain indices are modulated towards vagal enhancement in trial with 60-70dB when compared to trial with 71-80 dB. There is a statistically significant fall in the values of heart rate, mean arterial pressure , LF nu, LF /HF ratio and increase in HF nu and total power with the trial at 60-70 dB.

## DISCUSSION

Music modulates the autonomic activity and the modulation depends on music characteristics. Slow music influences parasympathetic activity, as does the classical music while sympathetic activity increases with heavy metal music.<sup>13,14</sup> Therefore we chose a piece of music that has slow and stable tempo, soft dynamics, consistent, absence of percussive and accented rhythms

and gentle timbre which are the traits of relaxing music which is known to enhance vagal modulation so that the confounding factor of music type on HRV is excluded.<sup>15</sup> As various aspects of music has been analysed for its beneficial effect on health this study has been designed to assess the effect of intensity of music on heart rate variability.

In our study it is observed that the participants’ heart rate and mean arterial pressure (MAP) displayed a fall with the listening of slow relaxing music with significant difference between the trials in heart rate values. Though MAP had no significant difference between the trials, the value was lower in trail with 60-70 dB than with the other intensity range, when compared to the basal value. The reason could be attributed to the perception of the sound of music at that particular intensity range (71-80dB) could have rendered less relaxing than with music at lower intensities. With music administration,



the analysis of frequency domain indices of HRV revealed a significant fall in the sympathetic variables of LF nu in the trial at 60-70dB than with trial at 71-80 dB thus suggesting the role of intensity of music in modulation of autonomic functions. The autonomic modulation towards positive cardiovascular health is suggested by the significant increase in values of HF nu (the marker of vagal activity) and decrease in LF-HF ratio (the reflection of sympathovagal balance) with music listened at low intensities. The ultimate overall vagal activity as reflected by total power is shown to be enhanced with lower intensity range than with the intensity range of 71-80dB thus emphasising the benefit of listening to music at lower range of audible intensity for beneficial autonomic modulation. From our study it could be thus observed that music that is listened for relaxing and stress alleviation could exert its beneficial effect only when listened at low intensities.

The mechanism of vagal modulation by relaxing music could be indirectly inferred from the observation that heavy metal music exposure causes sleep disorders, fatigue, exhaustion and immunologic activity impairment which could be due to its fast beat, timbre or sound intensity.<sup>7</sup> Unlike relaxing music, heavy metal music is listened at higher intensities to boost the mood and increase the arousal.<sup>17</sup> JA do Amaral et al. observed that significant sympathetic enhancement occurred when heavy metal music was listened at 80-90 dB while the same music when listened at lower intensities no significant autonomic modulation was elicited.<sup>16</sup> Observation exists for white noise to cause sympathetic overactivity even at 50dB.<sup>18</sup> Therefore it could be inferred from the above findings that a piece of music when played at a particular intensity appropriate of its kind could elicit the expected outcomes like mood elevation and alertness with fast music and relaxation with slow music.

The probable mechanism of beneficial modulation of health by relaxing music could be due to reward processing dopamine release from ventral tegmental area (VTA) into nucleus accumbens which in turn is connected with hypothalamus that regulates autonomic nervous system.<sup>19</sup> We observed a significant fall in the heart rate in study group which makes us infer that music at lower intensities increases parasympathetic activity which could be attribute to increased calcium/calmodulin-dependent Dopamine synthesis in the brain,

that could cause a reduction in blood pressure and thus influence cardiovascular activity with beneficial modulation.<sup>20</sup> All these observations point to the fact that harmonious composition of sounds that becomes a music shall remain musical only till its qualities are retained within the frames of fair audible range.

## CONCLUSION

In our study we have observed that relaxing music listened at lower intensities of 60-70dB had beneficial modulation on HRV when compared to higher intensities thus making us infer that the relaxing quality of music is also dependant on the intensity that retains musical traits to the sound.

**Conflict of Interest** – NIL

**Source of Funding**- NIL

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# The Effects of Enhanced External Counter Pulsation as a Natural Bypass in Patients of Angina Pectoris and its Relationship with Ejection Fraction and Quality of Life

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## ABSTRACT

**Introduction:** Ischemic heart disease is considered as one of the most common, serious and life threatening diseases. The prevalence of the disease is increasing all over the world and it is predicted to turn into the first cause of death by 2020.<sup>(1)</sup> **Material and Method-** EECP is a mechanical, outpatient and noninvasive procedure in which pneumatic cuffs, similar to those in blood pressure instrument, are tied on both legs of the patient. **Results and Conclusion-** Investigating subscales related to the physical component consisting physical functioning, role limitations due to physical problems, bodily pain, and general health indicates positive effects of EECP on patients' physical status. In this study out of all 60 subjects we randomly selected 10 patients for ejection fraction test out of 10 patients 9 were showing improved EF.

**Keywords-** EECP, QOL, ANGINA PECTORIS, EF, LVEF

## INTRODUCTION

Ischemic heart disease is considered as one of the most common, serious and life threatening diseases. The prevalence of the disease is increasing all over the world and it is predicted to turn into the first cause of death by 2020.<sup>(1)</sup>

Based on the viewpoints of several investigators, EECP is used as a treating method in patients suffering from stable angina, those having symptoms despite undergoing the most appropriate pharmacologic treatments, or those who are not able to endure vascular repairing treatment<sup>(2)</sup>

Enhanced external counter pulsation (EECP) was first introduced in the 1960s as a noninvasive procedure

for the treatment of angina refractory to medical therapy. Since its inception, technological advances have facilitated the procedure and rekindled interest in EECP as a means to treat symptomatic coronary artery disease and heart failure.<sup>(3)</sup>

The principle of EECP is simple: mechanically increase venous return to the heart and decrease cardiac afterload. The procedure itself is similarly straightforward: 3 compressive cuffs are wrapped around each leg and synchronized to inflate during cardiac diastole a retrograde pulse wave is created that increases venous return to the heart, thereby increasing coronary artery perfusion pressure; deflation of the cuffs at the onset of cardiac systole creates negative pressure from the peripheral arterial system that decreases cardiac after load.<sup>(3)</sup>

This method has healing and clinical effects, escalates the perfusion in coronary arteries, opens or forms collaterals (small branches of blood vessels) and increases the level of NO<sub>2</sub> in blood. It therefore enables patients to start their active life again with decreased angina severity.<sup>(3)</sup>

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Moreover, several studies all over the world showed that patients with angina experienced desirable effects of EECP on their quality of life for long periods after the treatment<sup>(4)</sup> The Prospective Evaluation of Enhanced External Counterpulsation in Congestive Heart Failure (PEECH) investigators randomized 187 optimally treated patients with NYHA class II–III HF (70% ischemic) and left-ventricular ejection fractions  $\leq 35\%$  to EECP (35 one-hour sessions over 8 weeks) or to ongoing medical HF therapy alone.

By 6-month follow-up, significantly more EECP-treated patients than controls achieved at least a 60-second increase in exercise time (35% vs. 25%) and an improvement in NYHA class (31% vs. 14%). However, the two groups did not differ significantly in peak oxygen consumption at 6 months or in quality-of-life improvement. More EECP-treated patients than controls discontinued therapy due to adverse events; one EECP recipient experienced a pulmonary embolism during the treatment period<sup>(9)</sup>

The complicated concept of quality of life (QOL) and ageing process has been considered as an important criterion for health outcomes in chronic diseases, such as heart disease, in recent years. The aim of this study was to evaluate the QOL with angina pectoris after treatment with enhanced external counter pulsation (EECP).

## MATERIAL AND METHOD

EECP is a mechanical, outpatient and noninvasive procedure in which pneumatic cuffs, similar to those in blood pressure instrument, are tied on both legs of the patient. During the treatment procedure the patient should lie on a bed in the treatment room. Three electrodes are attached to the patient's chest skin and connected to the electrocardiogram device. This device shows the heart rhythm of a patient during the treatment period. Moreover, blood pressures of patients are controlled during this period. Cuffs are connected to an air resource to be inflated and deflated according to the electrocardiograph. Therefore, they are inflated at the beginning of each diastole and deflated as a systole starts. The inflation starts from the cuffs on the calves, and continues to thighs and finally buttocks. When the cuffs are inflated, blood is pumped from the ends toward the heart. The number of EECP treatment sessions is variable since no specific instruction exists. One widely used regimen in the United States is one session a day,

five days a week, for seven weeks resulting in a total of 35 one-hour sessions<sup>(5)</sup>

### Inclusion Criteria

According to the physician's opinion, patients were selected for EECP and thus entered in this study if they could not undergo other methods, such as coronary artery bypass graft or percutaneous coronary intervention (PCI), or if such methods did not significantly affect their angina pain. In addition, patients with clinical signs of coronary artery disease (CAD), myocardial infarction or ischemia, or congestive heart failure were also included.

### Exclusion Criteria

patients with any severe valvular heart disease, atrial fibrillation, overt congestive heart failure, uncontrolled hypertension (blood pressure  $> 180/100$  while on medications), phlebitis, deep vein thrombosis, bleeding and coagulation problems were not good candidates for EECP and therefore were excluded from the present study. Likewise, pregnant or potentially pregnant women were not included. In addition, patients who did not complete the EECP treatment course or died during the treatment were eliminated from the investigation list.

This quasi-experimental study was conducted on 60 patients with angina pectoris undergoing EECP. Questionnaires were completed through interviews in two stages (before and at the end of EECP treatment). A two-part questionnaire was used to collect data. In the first part, patients' demographic and clinical data including age, sex, weight, height, employment status, the years of enduring coronary artery disease, occupied treatments, used drugs, blood pressure status, blood lipid profile, history of diabetes mellitus, familial history of coronary artery disease, smoking, history of myocardial infarction and left ventricular ejection fraction were recorded. In the second part, the standard 36-item Short Form (SF-36) quality of life questionnaire was utilized to assess quality of life. After obtaining permissions from the patients, the questionnaires were completed before and immediately after the treatment by interviews.

SF-36 is a general standard questionnaire with high validity and reliability. Although it was first used in Sweden, the translated versions are now being utilized in several countries<sup>(6)</sup>

The questionnaire consists of the 36 questions measuring eight health-related concepts. Six questions are related to general health, ten to physical functioning, four to role limitations due to physical problems, two to bodily pain, four to vitality, five to mental health, three to role limitations due to mental problems and two to social functioning. All subscales are summarized in physical and mental scales. For scoring the questionnaire, raw scores of quality of life in each scale (physical or mental) are calculated separately (range from 0 to 100) by adding the scores of the questions in that scale and dividing the result to the number the questions. High scores demonstrate better quality of life.<sup>(7)</sup>

And ejection fraction before and after treatment at MNR medical college between June 2016 to February 2017

The results were analyzed using descriptive statistics (frequency, mean, and standard deviation) and analytical statistics (paired t-test and repeated measures test) in *SPSS*

## RESULTS

Investigating subscales related to the physical component consisting physical functioning, role limitations due to physical problems, bodily pain,

and general health indicates positive effects of EECP on patients' physical status. As previous studies demonstrated<sup>(8)</sup>

The majority of patients were men (59.4%) and aged 56-71 years. In addition, 57.8% had hypertension and 56.3% had hyperlipidemia. A history of myocardial infarction was found in 70.3% of the subjects. Although QOL evaluations showed improvements in all subscales after the treatment. This study showed QOL to improve in patients who undergo EECP ( $p < 0.001$ ). Therefore, EECP is an efficient non-invasive method in treating patients with angina pectoris and in developing their QOL and delaying the process of ageing.

## CONCLUSION

This study showed QOL to improve in patients who undergo EECP. Therefore, EECP is an efficient non-invasive method in treating patients with angina pectoris and in developing their QOL and delaying the process of ageing.

In this study out of all 60 subjects we randomly selected 10 patients for ejection fraction test out of 10 patients 9 were showing improved EF.

**Table 1: AGE AND EF.**

NO.	NAME OF PATIENT	AGE	EF BEFORE TREATMENT	EF AFTER TREATMENT
1	VENKAT	52	40	52
2	AMBAREESHA	58	20	29
3	IRSHAAD	60	30	45
4	MOHIT	45	40	55
5	SURESH	65	30	35
6	PRADEEP	55	29	38
7	GOPICHAND	68	29	29
8	KRISHNA	49	32	48
9	MD.SHEKH	52	36	52
10	BALRAM	58	38	42



**Table 2: Global quality of life scores before and at the end of EECP as measured by SF-36**

SL.NO	QUALITY OF LIFE	BEFORE EECP	AT THE END OF EECP	P
		MEAN (SD)	MEAN (SD)	
1.	Physical functioning	37.04 (28.36)	61.92 ( 28.29)	<0.001
2.	Role limitations due to physical problem	23.64 (32.28)	66.17 (29.81)	<0.001
3.	Body pain	36.64 (20.92)	64.60 ( 16.66)	<0.001
4.	General health	44.65 ( 14.85)	59.58 (11.46)	<0.001
5.	vitality	39.52 (20.12)	61.31 (16.32)	<0.001
6.	Mental health	68.16 (15.19)	66.17 (13.12)	<0.001
7.	Role limitation due to mental problem	36.48 (34.36)	78.04 (32.06)	<0.001
8.	Social functioning	46.72 ( 27.58)	69.68 ( 16.68)	<0.001
9.	Global quality of life	42.05 (27.47)	67.02 (23.46)	<0.001

**Conflict of Interest-** Nil

**Source of Funding-** Self

**Ethical Clearance** -It was given by the institution.

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# Critical Effectiveness of Faculty Development Program for Medical Teachers

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## ABSTRACT

**Background:** Medical Education has undergone major changes over the past decades. There is a dire need of adopting a systematic approach to faculty development to enhance quality education to meet health challenges for 21st Century. Workshops on the Medical education are required for refreshing the knowledge of medical teachers. **Objective:** To assess medical teachers before and after Medical Education training. **Study design:** Prospective questionnaire based study. Participants: A total of 40 Medical teachers from various departments and each was followed after 3 months. **Method:** Each participant filled a pre-session questionnaire before the beginning of training process and a post-session questionnaire immediately after the completion of workshop and three months afterwards. Scores obtained in the pre and post-session questionnaires were compared. Training process during workshop lasted for eight hours per day for three days and participants gave effective feedback at the end of training process. **Result:** Significant improvement in the knowledge of participants was observed immediately and after three months. **Conclusion:** Regular training of the medical teachers is required to keep them updated with the new advances in Medical Education and also it is required to compare their knowledge before and after the session and after a set time period to see the level of improvement and how much they have learned.

**Keywords:** Medical Education, Assessment, Feedback.

## INTRODUCTION

Teaching is a very complex skill and art. It requires eagerness, self-discipline, hard-work, practice and feedback to make sure that you are on the right track. Even those endowed with inherent talent, must undergo training to improve their performance. The concept of training in Medical Education in India started in the late seventies and in 1999, the Medical Council of India insisting every Medical College to have a Medical Education Unit and in 2010, made it mandatory, for every Medical Professional to undergo at least the basic level of training. Teaching facilitates learning and encourages the learners to learn in a better way. The purpose of

teaching is not merely dispensing information, but to develop skills and attitude also <sup>[1]</sup>. Medical school faculty members are being asked to assume new academic duties for which they have received no formal training. These include time-efficient ambulatory care teaching, case-based tutorials, and new computer-based instructional programs. In order to succeed at these new teaching tasks, faculty development is essential. It is a tool for improving the educational vitality of academic institutions through attention to the competencies needed by individual teachers, and to the institutional policies required to promote academic excellence..

In recent years, with the use of new teaching and learning methods, the focus of assessment has been shifted to the use of higher cognitive abilities, communication skills, IT skills and professionalism. Focus is also shifting from competency based education to outcome based education and workplace performance assessment <sup>[2]</sup>. Here comes the role of Basic Medical Education workshops. The current format of Basic Medical Education workshop is in operation since

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nearly four years. In the current scenario of exploding knowledge, certain revisions are required in the format from time to time to make it more useful and acceptable both to the teachers and learners. These revisions are based on the experience gained at previous workshops as well as from feedback given by the participants and faculty members. Teaching programme comprises of all aspects of teaching and learning, both formative and summative assessment and proposed curricular changes like integrated teaching, early clinical exposure, internal assessment and E-learning<sup>[3, 4]</sup>. To fulfil the above perspectives workshop on Basic Medical Education was conducted in Medical Education Unit and the impact of such workshop was assessed by pre and post-session questionnaire assessment as well as feedback analysis from facilitators as well as participants.

## METHODOLOGY

Workshop on Basic Medical Education was conducted in Medical Education Unit from 21-23 July 2016, under the professional guidance and strict supervision of the Medical Education team. A total of 40 participants participated in the training process which were a mix of Professors, Associate Professors, Assistant Professors and Demonstrators of various departments and trained by 17 facilitators including one External Observer in each training process. Random Selection process was used to select participants, preferring those who have not undergone any training process. The workshop was a three days schedule in which at least on an average, eight hours per day were allotted for discussion and skills assessment. Each Participant filled a pre-session questionnaire before the process of training to know how much they are skilled in each aspect of teaching and learning before they have undergone any training. The questionnaire was a score based Performa, where they have to mark either of three (0-1= not at all skilled, 2-4= moderately skilled, 5= highly skilled). All the three domains of learning, i.e.; cognitive, psychomotor and affective were taken care of in the workshop schedule. Out of total effective 24 hours in 3 days schedule, 14 hours were allotted for the discussion, including systems approach, teaching and learning process, group dynamics, taxonomy of learning, educational objectives with individual work and group work, media in the medical education, curricular reforms and implementation and microteaching. About ten hours were allotted for skills assessment like

interactive teaching, teaching in large and small groups, bedside teaching, one-minute preceptor model, student assessment, assessment of knowledge, multiple choice questions (MCQ), oral practical exam (OSCE and OSPE), group work and practical, structured oral viva and a long case and its improvement.

In the end, each participant filled a post-session questionnaire, which was exactly similar as pre-session questionnaire. The responses from the participants were divided into 2 categories: (1) to determine their gain in the knowledge, a questionnaire survey of the participants (score based) at the beginning and end of the workshop and (2) to evaluate their perception about the usefulness of the workshop, a semistructured questionnaire survey of the participants at the end of the workshop. Three-point Likert scale and responses to open-ended questions were used in the second category to document participant's general views. Feedback was taken from each participant and facilitator as well as the external observer. Also each participant filled one more post-session questionnaire after three months period which was mailed to them and received back.

## RESULTS

A total of 40 participants (Table 1) and 17 facilitators participated in the workshop. For pre and post-session evaluation, a 14-point questionnaire having three-score scale (0-1, 2-4 and 5) was used (Table 2). The questionnaire was designed taking into account various modalities of teaching and learning in medical education, including newer methodologies like curricular reforms, problem-based learning, structured oral viva, Objective Structured Clinical Examination (OSCE) and Mini-Clinical Evaluation Exercise (Mini-CEX). The response was taken from the participants in terms of scores that they feel they are skilled in each aspect of teaching and learning (score 0-1= not at all skilled, 2-4= moderately skilled, 5= highly skilled). These scores were then compared with post-session evaluation questionnaire scores. There was a significant improvement in the scores of the participants in the postevaluation questionnaire as compared to pre-evaluation in all the aspects. Some important fields where improvement was seen were types of media and choice in medical education where only 6.66% (score 5=2) participants in the workshop were highly skilled about the use of media like a computer-assisted learning, creating and presenting good PowerPoint slide

presentations as compared to 33.33% (score=10) after the workshop. In teaching and learning methods, in pre-evaluation, only 6.66% participants opted for score 5 as compared to 23.33% in post -evaluation.

Likewise in objective assessment and MCQ, 33.33%, participants opted for score 5 in pre-evaluation as compared to 60.00%, in post-evaluation workshop. In OSCE and OSPE session, change in the score 5 was from 20% to 30%.

Post-evaluation questionnaires filled after three-months showed significant improvements in fields like objective assessment and MCQ, OSCE and OSPE, structured oral viva, long case and Mini Clinical Evaluation Exercise.

**Table 1: Distribution of participants in the workshop**

Participants	Number
Professors	05
Associate Professors	13
Assistant Professors	15
Demonstrators	07

**Table 2: Anonymous Retrospective Pre and Post Evaluation skills Set**

S.NO	Evaluation skills
1	Group Dynamics
2	Systems Approach
3	Learning process& Adult Learning
4	Teaching & Learning methods
5	Types of Media and Choice
6	Microteaching
7	Curricular Reforms
8	Student Assessment
9	Essay & Short Answer Questions
10	Objective Assessment & MCQ
11	OSCE & OSPE
12	Structured Oral Viva
13	Long case & Mini-CEX
14	Effective Feedback

One more programme evaluation questionnaire was filled by each participant ( Table 3) in which it has been found that 93.33% participants replied that the objectives with which the workshop was planned were achieved. 90% replied that the workshop was useful. 60% participants found it to be too tight whereas only 36.66% found it to be optimum. 93.33% participants of the workshop wanted conduction of similar activities frequently. Although some participants found the workshop as lengthy with a tight schedule, most of them found it useful in improving their creativity in the assessment of students.

**Table 3 : Programme evaluation Questionnaire**

S.No	Question	Total	Yes		No		Not Sure	
			No.	%	No.	%	No.	%
1.	Achievement of objectives	40	38	93.33	00	00	02	6.66
2.	Usefulness of workshop	40	37	90.00	00	00	03	10.00
3.	Elicitation of Active Participation	40	38	93.33	00	00	02	6.66
4.	Helpfulness of Learning Resources	40	33	76.66	03	10.00	04	13.33
5.	Helpfulness of Faculty Members	40	37	90.00	01	3.33	02	6.66
6.	Balance between Theory and Practical	40	Too much theory		Too much practical		Optimum	
			No.	%	No.	%	No.	%
			23	43.33	07	23.33	10	33.33

**Cont... Table 3 : Programme evaluation Questionnaire**

7.	Time Management	40	Too Tight		Too Relaxed		Optimum	
			No.	%	No.	%	No.	%
			28	60.00	01	3.33	11	36.66
8.	Organizational aspects	40	Good		Fair		Poor	
			No.	%	No.	%	No.	%
			31	70.00	08	26.66	01	3.33
9.	Organizational arrangements	40	Good		Fair		Poor	
			No.	%	No.	%	No.	%
			33	76.66	06	20.00	01	3.33
10.	Frequency of Similar activities	40	Yes		No		Not Sure	
			No.	%	No.	%	No.	%
			38	93.33	00	00	02	6.66

## DISCUSSION

There was a significant gain in the knowledge of participants as shown by their scores in pre and post-session evaluation questionnaire and evaluation after 3 months. Areas which the participants found most useful in their day to day practice were teaching and learning methods, objective assessment and MCQ, Objective Structured Clinical examination and Objective Structured Practical examination, Microteaching and structured oral viva with Mini Clinical Evaluation Exercise. Several studies have been conducted in India and in neighbouring countries showing the usefulness and effectiveness of Medical Education workshops like in a study conducted at the B.P.Koirala Institute of Health Sciences, Dharan, Nepal to assess the effectiveness of the teacher training workshop, enrolling 26 teachers, it has been found that there was a significant gain in the knowledge of participants ( $p < 0.001$ ) and all the participants (100%) agreed that the training was useful, informative and learned new things<sup>[5,6]</sup>

A teaching programme was offered by the Department of Medical Education at Ankara University School of Medicine for their physical The Journal of Medical Research 103 educators, including 150 professors from different disciplines. 97.5% to 100% participants assessed the course as either good or very good. 82% participants gave optimal rating to the topics covered and time management and 100% recommended that it should be attended by all faculty members<sup>[7,8]</sup>. In a study conducted by Fareed H. Abdulahad, Abubakir M. Saleh and Nazar P. Shabila at Hawler Medical University, Erbil, Iraq to provide a general description of a newly designed teaching course for faculty

members of the medical colleges and assess the course from participants' perspectives, it has been found that a high proportion of the participants rated the different scientific content of most of the sessions as useful. The particularly well-received sessions included teaching methods and learning (96.2%), an ideal lecture (96.2%), motivation to raise the standard of lecture (92.9%), principles of lecturing (92.9%) and regulations and instructions (92.6%)<sup>[9]</sup>

A study was conducted by Shahid Jamal et al in the Department of Medical Education, Army Medical College, Rawalpindi, Pakistan from 2008 to 2010. The objective was to assess the response of the participants of short duration medical education workshops. About 120 participants participated and out of them, 55-70% found hand-outs as useful and 30-45% as very useful. 52-78% participants found the computer presentations and transparencies as below average and the majority of them remarked these activities as very useful and stressed on the continuation of such activities<sup>[10]</sup>.

## CONCLUSION

Faculty development is essential to train medical faculty in essential educational theory and specific teaching skills as well as to encourage a flexible and learner-centred approach to teaching<sup>[11]</sup>. Lot of advances and development in the field of Medical Education are taking place in the today's era, it is essential and required for our Medical teachers to be aware of them. Also they should know how to apply them in their day to day practice of teaching and assessment. This can be possible only with the regular

and frequent conduction of such workshops in the field of Medical Education. These workshops were perceived as an acceptable way of acquiring teaching and learning skills and laid a very positive impact on the minds of participants. Comprehensive faculty development, which is more important today than ever before, empowers faculty members to excel as educators and to create vibrant academic communities that value teaching and learning.

**Conflicts of Interest:** Authors have declared that no competing interests exist for the present study.

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**Ethical Clearance-** Taken from Institutional Ethical committee.

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# Attitude of Faculty Towards Communication Skill Training of Undergraduate Medical Students-A Pilot Study

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## ABSTRACT

**Background** - Doctor patient communication is a core soft skill and forms the very essence of clinical competence. Until recently this core area was given very little importance and students were expected to imbibe the skill on the go during their course. But in the present scenario ,given the fact that patients too are highly informed, it is very essential for clinicians to have good communication skills. Therefore it is only appropriate to incorporate teaching communication skill in the undergraduate medical curriculum.

**Objective-** To assess the attitude of faculty towards communication skill training of undergraduate medical students

**Method-** This is a cross-sectional study involving fifty teaching faculty from the ranks of Lecturer to Professor. The participants were selected by random sampling method. A modified Communication Skill Attitude Scale (CSAS) questionnaire was used to assess the attitude of the participants. Data analysis was done using SPSS version 11.

**Results-** The mean Positive Attitude scores and Negative Attitude scores were calculated. Overall the participants had a statistically significant PAS score which showed that they had a positive attitude towards implementation of communication skill training for medical students. There was no significant difference in the mean PAS and NAS scores between genders, specialty or ranks of the participants.

**Conclusion-** The results of the study indicate that the faculty at Amrita School of Medicine has a very positive attitude to introduction of communication skill training in the undergraduate medical curriculum. Faculty members who participated in the study clearly supported the importance of communication.

**Keyword-** *Communication skill, CSAS questionnaire, PAS score*

## INTRODUCTION

A balanced approach to the patient care experience requires an understanding of patient's illness, their concepts of suffering, coping mechanisms etc. Doctor patient communication is an important skill and

essential to clinical competence<sup>(1)</sup>. Communication is the art of transmitting information ideas and attitudes from one person to another. The General Medicine Council's recommendations on Undergraduate medical education (GMC, 2001) stated that medical graduates should demonstrate the communication skills<sup>(2)</sup>. Many medical schools are now considering methods to integrate communications skill training across the continuum of medical education. The importance of an integrated approach is crucial as research reveals that communication skills cannot only be learned in medical schools but also forgotten if training is not sufficiently targeted and reinforced throughout medical education<sup>(3)</sup>.

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4,5) The consequences of effective teaching strategies are far reaching since good patient doctor communication has been shown to have positive influences on patient recall and understanding, adherence, symptom resolution etc<sup>(6,7)</sup>

Communication skills in medicine was once considered a minor subject are now ranked a core skill<sup>(8)</sup>. Despite this shift in awareness, there has been substantial difficulty in establishing comprehensive communications programs in medical education. Lack of institutional and faculty support has been a major factor<sup>(10)</sup>. The need to integrate communication skill teaching throughout the continuum of undergraduate medical education will require increased faculty involvement<sup>(11)</sup>. There have been several studies looking at student's attitude towards implementation of communication skill teaching in medical curriculum. But very few studies have considered the response of teaching faculty. Hence we took up this study not only to analyze the response of teaching faculty but also to compare the difference in the response among faculties working in different departments. Medical teachers may not be well versed in communication skill teaching and may not have had adequate communication skill training during their own education, indicating the need to consider their attitudes towards communication<sup>(11)</sup>.

Research objective: To assess the attitude of faculty towards communication skill training in undergraduate Medical students.

## MATERIALS AND METHOD

This study was conducted during the period from June to July 2016 after obtaining clearance from Institutional Ethics committee. This was a cross sectional pilot study. Modified CSAS questionnaires were distributed to teaching faculty of various departments of the broad specialties at the Amrita School of Medicine, Kochi. Data was collected from fifty teaching faculty members of different ranks from Professor to Lecturers. The participants were chosen by random sampling method (table of random numbers). Faculty who had undergone Revised Basic Course training in medical education or who had done fellowship in medical education were excluded from the study. The participants were given a personal details questionnaire and modified Communication Skills Attitude Scale (CSAS). The

CSAS scale was modified as it was originally made to assess the attitude of students regarding communication skill training. The questionnaire includes 26 items, 13 of which are written in the form of positive statements and 13 negative statements about communication skills learning. Each item was accompanied by a 5 point Likert scale ranging from 1 (strongly disagree) to 5 (strongly agree). Questions 2, 12, 13, 18 and 23 were omitted. The first and seventeenth questions were made into negative questions. In questions 6 and 7 the word learning was changed into teaching. Questions 11, 12, 20 and 25 were newly framed in the modified version. For testing the reliability of the modified version of the CSAS (originally developed by Rees et al, 2000) the questionnaire was distributed to ten participants and statistically ascertained (Gutman split1/2 coefficient-.930). Brief instructions for completion of this scale of the CSAS are included to ensure that the scale can be self-administered. Analysis was carried out using SPSS VERSION 11. Positive attitude score(PAS) was calculated by adding the scores of items 3,4,6,8,9,11,12,13,15,20,21,23 and 26. The negative attitude score(NAS) was obtained by adding the scores of items 1,2,5,7,10,14,16,17,18,19,22,24 and 25. Both the scales range from 13 to 65 with higher scores indicating stronger positive or negative attitude.

## RESULTS

Out of the 50 participants who responded, 13 were males and 37 females. 16 belonged to clinical specialty and 34 to the non-clinical specialty. The faculty designates included 16 professors, 6 associate professors, 17 assistant professors and 11 lecturers.

There is no statistically significant difference in the mean PAS and NAS scores between male and female participants. Neither was there significant difference in the mean PAS and NAS scores between Professor and Associate Professor, Associate and Assistant Professor(Table 2), Assistant Professor and Lecturer, Professor and Assistant Professor(Table3) and Professor and Lecturer(Table4) of the faculty as shown in tables 2,3 and 4

But overall the participants had a statistically significant mean PAS Scores when compared to the mean NAS scores with a p value of less than <0.001 (p value<0.005 significant) Table 5.

**Table1: Comparison of PAS and NAS scores among Faculty members**

Variables	Group I		P-Value	Group II		P-Value
	Male	Female		Clinical	Non-Clinical	
	Mean±SD	Mean±SD		Mean±SD	Mean±SD	
Sum of PAS	56.61±5.22	53.94±5.71	0.145	56.50±5.20	53.76±5.72	0.112
Sum of NAS	27.30±3.59	25.89±5.63	0.402	27.31±3.80	25.76±5.69	0.330

**Table 2: Comparison of PAS and NAS scores among the faculty members as per their designation**

Variables	Group III		P-Value	Group IV		P-Value
	Professor	Asso.Prof		Asso.Prof	Assist.Prof	
	Mean±SD	Mean±SD		Mean±SD	Mean±SD	
Sum of PAS	54.37±6.73	55.83±6.52	0.653	55.83±6.52	54.82±5.75	0.724
Sum of NAS	26.18±5.10	28.83±4.16	0.271	28.83±4.16	25.52±5.29	0.183

**Table 3: Comparison of PAS and NAS scores among the faculty members as per their designation**

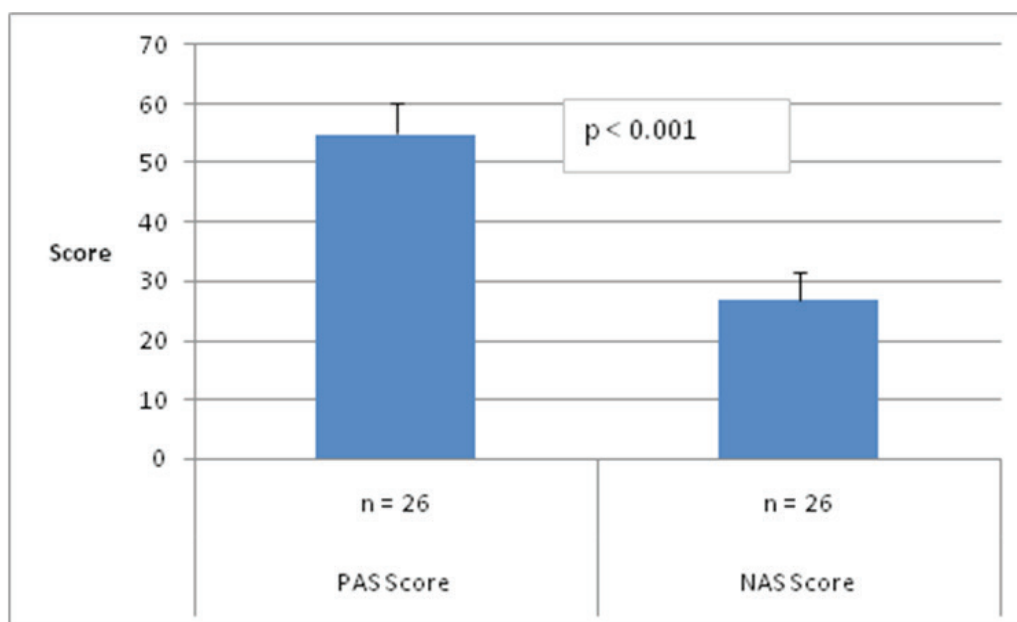
Variables	Group V		P-Value	Group VI		P-Value
	Assist.Prof	Lecturer		Professor	Assist.Prof	
	Mean±SD	Mean±SD		Mean±SD	Mean±SD	
Sum of PAS	54.82±5.75	54.09±3.64	0.711	54.37±6.73	54.82±5.75	0.838
Sum of NAS	25.52±5.29	26.09±5.83	0.794	26.18±5.10	25.52±5.29	0.719

**Table 4: Comparison of PAS and NAS scores among the faculty members as per their designation**

Variables	Group VII		P-Value
	Professor	Lecturer	
	Mean±SD	Mean±SD	
Sum of PAS	54.37±6.73	54.09±3.64	0.900
Sum of NAS	26.18±5.10	26.09±5.83	0.964

**Table 5: Comparison of PAS and NAS scores for the whole group**

Variables	Mean±SD	P-Value
	Sum of PAS	
Sum of NAS	26.88±4.74	<0.001



**Chart 1-Comparison of PAS and NAS score of the study group**

## DISCUSSION

Faculty members who participated in the study clearly supported the importance of communication. A study conducted at Dalhousie University by Donald B.Langille also showed that the faculty scored high scores in the Attitude towards Medical Communication scale, and more highly positive attitudes are found in those who have attended communication workshops<sup>(11)</sup>. We could not find a difference in attitude between males and females. The grade of the participants was also not a factor, indicating that more recently graduated faculty are no more likely to have a higher positive attitude than those who are more experienced. Even though we expected a relationship between the clinical faculty and higher attitude score, this was not found. As a number of studies assessing students' attitude towards communication skill learning has shown positive response from the students' side, it will be easier to implement the communication skill training into the curriculum as both the facilitators and the recipients are in favour of the novel idea. T.Suzuki et al has shown that competency of medical students improved when trained by faculty who had undergone training in communication skill.

Limitations of the study: One of the key limitations of the study was the small sample size. Also, the sample was chosen from a single institute. These were due to time constraints of the study period. This study could assess only the attitude of faculty members in implementing

communication skill training in medical curriculum and not the real impact.

## CONCLUSION

The results of the study indicate that the faculty at Amrita School of Medicine has a very positive attitude to introduction of communication skill training in the undergraduate medical curriculum. The challenge remains for medical teachers to organize communication skill teaching in such a way that students are taken beyond the stage of awkwardness and that they can see that such skills make a difference in everyday medical practice with patients. This training should begin early and continue throughout the undergraduate course.<sup>(13)</sup>

**Acknowledgements:** I thank Dr K.R.Sundaram for the statistical analysis.

**Conflict of Interest-**None

**Source of Funding:** None

**Ethical Clearance:** The study protocol conforms to ethical guidelines of the "World Medical Association Declaration of Helsinki Ethical Principles for Medical Research Involving Human Subjects" adopted by 18<sup>th</sup> WMA General Assembly, Helsinki, Finland, June 1964, as revised in Tokyo 2004

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# Effect of Music on Exercise Performance in Young adults

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## ABSTRACT

**Aim:** To investigate the influence of Music on Exercise performance in young adults

**Objectives:** To establish if there is any effect of music on exercise performance

**Methods:** 50 untrained 1<sup>st</sup> MBBS students between the age group 18-21 years were selected for the study. The study was carried out in Mahavir Institute of Medical Sciences, Vikarabad, Telangana. Student subjects exercised on Treadmill without music and on following day with music. Exercise duration and maximum heart rate achieved during exercise was noted on both the days.

**Results:** Statistical analysis was done using paired t test. There was a significant increase in the exercise duration and maximum heart rate achieved when exercising with music compared to exercising without music.

**Conclusion:** Exercise performance is increased when exercising with music

**Keywords:** Exercise performance, music

## INTRODUCTION

Music can be heard at any major sporting event or in any exercise facility. Music during sporting events or exercise can represent or express the individuality of the participant, motivate the participant, or add excitement to the atmosphere<sup>1</sup>. Research has also found that certain metabolic increases are associated with listening to music<sup>2</sup>.

Research suggests songs with faster tempos and strong rhythms are expected to activate the sympathetic nervous system and enhance performance. In contrast, songs with slower tempos and weaker rhythms are more likely to activate the parasympathetic nervous system and induce relaxation. Many fitness instructors consider the addition of music to exercise similar to an ergogenic aid<sup>3</sup>.

The effect of background music on physical performance remains unclear. Some studies have shown background music increases physical performance in karate and treadmill running, while other studies have found that there were no physiological differences during dart throwing or sit-ups<sup>4,5,6</sup>. While these previous studies have found enhanced performance to be associated with music in general, the specific effects of different genres of music are less understood. Music has been shown to have a greater effect on performance than white noise alone, which leads to the question of how songs of varying tempos and rhythms affect performance<sup>7</sup>.

Different psychophysiological measures have been used to determine whether or not music has any influence, such as heart rate (HR), Borg's Rating of Perceived Exhaustion (RPE) scale, blood lactate, blood pressure (BP), Physical Activity Questionnaire (PAQ), 10-point bipolar Feeling Scale. Terry and colleagues<sup>8</sup> showed participants were inspired to exercise by preferential choices of music after testing a large group of 532 subjects. It was noted that the one commonality being that the music has a strong rhythmical component.

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The Bicycle ergometer and Treadmill are the most common devices used for dynamic exercises. In this study, Treadmill was used for assessing exercise performance.

## MATERIALS AND METHOD

The Study was carried out in Mahavir Institute of Medical Sciences, Vikarabad.

### Study Group:

50 untrained students of 1<sup>ST</sup> MBBS of both the genders between the age group 18-21 years were selected for the study

### Inclusion criteria:

1) No history of Hypertension, Diabetes mellitus and Asthma

2) Normal BMI

3) No history of any illness that limits exercise activity

### Exclusion criteria:

1) Any history of illness or injury limiting exercise activity

All the student subjects were explained about the study and an informed consent was taken

### Exercise testing:

The subjects were tested for exercise between 3pm to 5pm.

Each day 5 students were called for the study. Initially they were made to sit down and relax for 5 min and their resting heart rate was recorded by the pulse Oxy-meter, then they were asked to run on treadmill at self-selected speeds and initial time was noted. They were instructed to stop the exercise whenever they felt tired, fatigued or breathless. The pulse Oxy-meter was placed on their index finger during exercise and their pulse was noted when it reached to its maximum and steady level. When they stopped the exercise, again time was noted. Total duration of the exercise was calculated by subtracting final and the initial timing.

The Next day, same subjects repeated the exercise. During the exercise, self-selected music was played

to them in headphones. Recording of heart rate and duration of exercise was noted as previously.

### Statistical analysis:

Statistical analysis was done using paired t test and Microsoft excel were used.

P value <0.05 was considered as statistical significant.

The results were expressed as Mean  $\pm$  Standard Deviation.

## FINDINGS

**Table:1- Exercise duration without and with music**

Group	N	Exercise Duration (Minutes)	
		Mean	SD
Exercise without Music	50	24.46	8.52
Exercise With Music	50	35.72	12.27

Exercise duration was significantly higher ( $P < 0.05$ ) with music compared to exercising without music

**Table: 2- Maximum Heart rate achieved during exercise without and with music**

Group	N	Maximum Heart rate achieved (beats/min)	
		Mean	SD
Exercise without Music	50	131.63	10.28
Exercise With Music	50	140.09	9.35

Maximum Heart rate achieved was significantly higher when exercising with music compared to when exercising without music

## DISCUSSION

The aim of the present study was to analyze the effect of music on duration of exercise and heart rate.

In our study, duration of exercise increased significantly when music was played. Potteiger et al reported a strong effect of music at moderate exercise



intensities-Fast jazz, slow classical and self-selected music reduced perceptions of exertion relative to a control condition<sup>8</sup>. However, Pujol et al reported that fast music had no effect on performance or fatigue in a maximum intensity cycling test lasting 30 Sec<sup>9</sup>.

A number of studies have tested the effects of stimulative music on self-paced aerobic exercise performance and found that music enhanced work output<sup>10</sup>. It is reported that the intensity of exercise determines the extent to which music can inhibit the processing of other sensory cues<sup>11</sup>.

In our study, heart rate also increased significantly when music was played. Birnbaum et al used fast music, slow music and a no music protocol during steady state exercise and indicated that fast music increased several indices related to heart and lung function<sup>12</sup>.

### CONCLUSION

Exercise performance is better when exercising with music.

**Source of Funding:** Self

**Ethical Clearance:** Taken from Scientific Ethical committee, Mahavir Institute of Medical Sciences, Vikarabad, Telangana.

**Conflict of Interest - Nil**

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# Cardiovascular Response to Submaximal Exercise in Type 2 Diabetics

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## ABSTRACT

**Background:** Diabetes is one of the major causes of premature illness and death worldwide. Limitation in exercise capacity is a strong predictor of cardiovascular dysfunction and all other causes of mortality in diabetics. Since the urban population in India is more prone to Diabetes, as part of this study, an attempt is made to evaluate cardiovascular response to submaximal exercise in type 2 diabetics using Ergospirometer.

Hypothesis tested is, Type2 Diabetes mellitus reduces exercise tolerance.

### Objectives of the Study:

To record and compare the cardiovascular response to submaximal exercise in Type2 diabetics as well as healthy controls, using ergospirometer.

To compare the exercise response with respect to duration of diabetes.

**Methodology:** The diagnosed cases of type 2 diabetes at department of Medicine, Victoria hospital, Bangalore in the age group of 35-50yrs were taken up for the study. Subjects were asked to perform submaximal exercise on MEC PFT system till heart rate reaches 70% of maximal (220-age bpm). Their cardiovascular responses as reflected in the parameters (heart rate, blood pressure and oxygen pulse) were recorded.

**Results and Conclusion:** Results showed that resting systolic ( $p < 0.01$ ) and diastolic blood pressure ( $p < 0.01$ ) is high in diabetics. Abnormal cardiovascular response to submaximal exercise was seen in diabetics with significant rise in systolic BP ( $p < 0.01$ ) and diastolic BP ( $p < 0.01$ ). Exercise intolerance is increasing with the duration of diabetes, which is shown by significant increase in HR ( $p = 0.06$ ), SBP ( $p = 0.04$ ) and reduction in oxygen pulse ( $p < 0.01$ ). Findings of our study prove our hypothesis that there is exercise intolerance in type 2 diabetics even without clinical cardiovascular disease. It also shows that exercise intolerance is progressive with duration of disease. Underlying pathophysiology is multifactorial and involves alterations in ventricular-vascular coupling consisting of cardiac changes and pulmonary changes due to disease which influence integrity of pulmonary connective tissue and microvasculature.

**Keywords:** Type 2 Diabetes, Cardiovascular responses, Ergospirometer, submaximal exercise

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## INTRODUCTION

Diabetes is one of the major causes of premature illness and death worldwide. According to WHO, 346 million people worldwide suffer from diabetes.<sup>(1)</sup> Almost 4 million deaths per year across the globe are attributable to diabetes.<sup>(2)</sup>

The worldwide prevalence of DM has risen dramatically over the past two decades, from an estimated 30 million cases in 1985 to 285 million in 2010. Based on the current trends, International Diabetes Federation projects that 438 million individuals will have diabetes by the year 2030. Although the prevalence of both type 1 and type 2 DM is increasing worldwide, the prevalence of type 2 DM is rising much more rapidly, presumably because of increasing obesity, reduced activity levels as economies become more industrialized, and the aging of the population. It is estimated that in 2030 the greatest number of individuals with diabetes will be aged between 45–64 years<sup>(2)</sup>.

In Asia, the prevalence of diabetes is increasing rapidly and the diabetes phenotype appears to be different from that in the United States and Europe. Asian diabetic features are onset at a younger age and with lower BMI, greater visceral adiposity, and reduced insulin secreting capacity. It was estimated that 50 million Indians were diabetic in 2010, and this number is expected to cross 87 million by 2030<sup>(1)</sup>.

Exercise intolerance is a major complication of type 2 diabetes, which is associated with increased mortality.<sup>(3)</sup> Abnormal hemodynamic responses to exercise have been observed in diabetic subjects. It has also been observed in many previous studies that person with type 2 diabetes mellitus, have reduced maximal oxygen consumption ( $VO_2$  max) compared with non diabetic person even in absence of clinical cardiovascular disease.<sup>(4)</sup>

Studies have shown that limitation in exercise capacity is a strong predictor of cardiovascular dysfunction and all other causes of mortality in diabetic patient. Underlying pathophysiology is multifactorial and involves alterations in ventricular-vascular coupling, consisting of cardiac changes on one hand and compromised distribution at the regional microvasculature on the other.<sup>(5)</sup>

Considering the fact that there is an increasing trend of diabetes in urban population, an attempt is made to evaluate cardiovascular response to submaximal exercise in type 2 diabetics using Ergospirometer and to test the hypothesis that “Type2 Diabetes mellitus reduces exercise tolerance”.

If cardiac limitation is identified early, required intervention and lifestyle modification can be

recommended, which can be of major help to diabetics.

Exercise is a physiological stressful condition which involves enhanced energy expenditure by skeletal muscles. The enhanced oxygen requirement is met by increased activity of oxygen delivery system, consisting of cardiovascular, respiratory overactivity and increased oxygen extraction at tissue level.

During exercise there is an increase in cardiac output and redistribution of blood flow and oxygen delivery to working muscles, without changing cerebral and coronary blood flow. An enhancement of cardiac output is brought about by superior pump performance which is influenced by neurohumoral mechanism<sup>(6)</sup>.

### OBJECTIVES OF THE STUDY

(a) To record and compare cardiovascular response to submaximal exercise in Type2 diabetics and healthy controls using Ergospirometer.

(b) To compare the exercise response according to duration of diabetes.

Cardiopulmonary exercise testing provides a global assessment of integrative exercise responses involving the pulmonary, cardiovascular, haemopoietic and skeletal muscle systems. Indications for cardiopulmonary exercise testing are to evaluate; exercise tolerance, undiagnosed exercise intolerance, patients with cardiac and respiratory diseases.

### MATERIALS AND METHOD

This is a case control study intending to record responses to sub maximal exercise carried out on 40 Type 2 diabetic subjects and 40 normal individuals who were taken as controls. Selection was based on inclusion and exclusion criteria. Study was conducted in Victoria hospital Bangalore.

Group A: Consisted of 40 cases who were diagnosed of type2 diabetes at Victoria hospital, Bangalore.

Group B: Consisted of 40 healthy subjects who were matched with group A in terms of age, sex and BMI.

#### Inclusion Criteria:

1. Age group – 35 to 50yrs.
2. Patient diagnosed of uncomplicated Type 2 diabetes.

### 3. Informed consent

#### Exclusion Criteria:

Subjects with the following conditions are excluded:

1. Hypertension.
2. Severe obesity (BMI >35).
3. Arthritis.
4. Neuromuscular skeletal deformity.
5. Pregnancy.
6. Retinopathy
7. Neuropathies.
8. Nephropathy.
9. History of cardio respiratory illness
10. Peripheral vascular disease

**Treadmill model** – PC ECG12 DE 55 was used for the study. Treadmill was connected to MEC- PFT (Medical Electronic Constructions –Pulmonary Function Test) system data interface version 2012 software; it also had EDS software for interpretation of ECG. ECG was recorded throughout the exercise. It had a connection to air flow meter which analysed inspired and expired air. It also had attachment for oxymeter to check oxygen saturation of blood.

## METHODOLOGY

Institutional ethical committee clearance was obtained before starting the study. Cases were diagnosed type 2 diabetics at Department of Medicine, Victoria hospital, in the age group of 35-50yrs with consideration of inclusion and exclusion criteria.

Subject's complete history was taken to exclude any other medical illness. Subjects were explained the complete procedure and they were familiarized with the instrument. Written informed consent was taken. If subject fulfilled the selection criteria then their general physical examination was carried out. Subject's medical history was checked to rule out retinopathy, peripheral neuropathy and peripheral vascular disease. Urine Microalbuminuria was checked to rule out nephropathy. Twelve lead ECG was connected which was read throughout the procedure and heart rate was calculated by RR interval. Face mask was connected from which inspired and expired air was analyzed. Oxymeter was connected to check the oxygen saturation throughout the procedure. Before starting the exercise resting cardiac

parameter (Heart rate, Systolic BP, diastolic BP and oxygen pulse) was noted. Subjects were then asked to perform submaximal exercise on MEC PFT system. Subjects performed steady state aerobic exercise to reach 70% of their maximal heart rate. Maximal heart rate was calculated by 220 minus age of subject. The exercise protocol used was

A warm up at 3 km/hr speed for 2min

Followed by 5 km/hr with 0% elevation till 70% of maximal heart rate is reached.

This speed was selected to mimic the daily normal walking speed of the subjects. Their cardiovascular response as reflected in the parameters (heart rate, oxygen pulse and blood pressure)<sup>(7)</sup> were obtained. Immediately after the exercise, blood pressure was recorded to check the BP response to exercise. Same exercise was repeated with controls whose Age, sex and BMI matched with cases. Later, results were compared between cases and controls.

## RESULTS AND STATISTICAL ANALYSIS

Descriptive and inferential statistical analysis has been carried out in the present study. Results are presented as Mean  $\pm$  SD. Significance is shown by

+ Suggestive significance (P value: 0.05 < P < 0.10)

\* Moderately significant (P value: 0.01 < P < 0.05)

\*\* Strongly significant (P value: P < 0.01)

**Table 1: Age distribution of case and controls (in years) P=0.96**

	Case		Control	
	No	%	No	%
35-40	20	50	19	47.5
41-45	5	12.5	6	15
46-50	15	37.5	15	37.5
Total	40	100	40	100
Mean $\pm$ SD	42.45 $\pm$ 5.39		42.35 $\pm$ 5.2	

**Table 2: Cardiovascular parameters between cases and controls at rest.**

	Case	Control	P value
Heart Rate b/min	76.45 ±5.1	75±7.1	0.31
Systolic BP mm Hg	124.55±7.9	116.4±5.4	<0.01**
Diastolic BP mm Hg	80.1±6.2	75.6±5.4	<0.01**
O2 Pulse ml/beat	5.2±3.7	3.9±1.2	0.06 <sup>+</sup>

**Table 3: Cardiovascular parameters attained after submaximal exercise between cases and control**

	Case	Control	P value
Heart Rate b/min	124.3±3.8	124.3±3.6	0.96
Systolic BP mm Hg	150± 16.3	137.2±10.2	<0.01**
Diastolic BP mm Hg	80.3±7.5	76.3±6.08	<0.01**
O2 Pulse ml/beat	7.3± 4.4	8.4±2.3	0.18

**Table 4: Table shows subdivision of subjects according to their duration of diabetes in cases**

Duration in yrs	No of cases	%
<1yr	10	25
1-5yrs	20	50
>5yrs	10	25

**Table 5: Cardiovascular response to submaximal exercise in sub groups divided according to duration of diabetes**

	<1yr	1-5yr	>5yr	P value
H R b/min	124.46±3.5	123.06±3.4	126.56±4.1	0.06 <sup>+</sup>
Sys BP mm Hg	141±13.7	150±14.1	159±19.2	0.04*
Dias BP mm hg	80.4±6.8	80.2±5.7	80.4±6.8	0.99
O2pulse ml/beat	11.67±4.3	11.67±4.3	2.52±0.9	<0.01**

Table 1 shows age distribution of the cases and controls matched with p value 0.96.

Gender distribution of two groups studied were matched with p=1.0. All the anthropometric parameters were matched between two groups with insignificant p value.

It is seen in Table 2 that there is significant high resting systolic (p=<0.01) and diastolic BP (p=<0.01) in diabetics compared to non diabetics.

Table 3 show cardiac responses after submaximal exercise. It is evident that there is significant rise in systolic (p=<0.01) and diastolic BP (p=<0.01).

Table 4 shows sub division of diabetics according to their duration of diabetes.

Table 5 shows cardiac response of diabetics with respect to their duration of diabetes. Which shows significant increase in HR (p=0.06), SBP (p=0.04) and reduction in oxygen pulse (p=<0.01), corresponding to their duration of diabetes.

## DISCUSSION

High resting heart rate and blood pressure seen in diabetics compared to controls may be due to cardiac autonomic neuropathy which is commonly seen in diabetics even without evidence of ischemic heart disease<sup>(8)</sup>

Our findings of significant rise in systolic and diastolic BP in diabetics is in agreement with the currently understood role of the autonomic nervous system in controlling the cardiovascular response to exercise. Cardiac autonomic neuropathy which is commonly seen in diabetics even without evidence of ischemic heart disease could be the reason for abnormal increase in systolic and diastolic BP.<sup>(8)</sup>

The early increase in muscle blood flow may be attenuated in type 2 diabetes, and there is evidence for macro and microvascular dysfunction in type 2 diabetes that could explain impaired microvascular blood flow responses during exercise. In diabetics there could also be impaired control or mal-distribution of muscle blood flow<sup>(9)</sup> These findings of the present study appear to support the importance of impaired skeletal muscle oxygen delivery as a determinant of the submaximal exercise impairment in type 2 diabetes<sup>(7)</sup>

In our study we have seen that the response of heart rate and systolic blood pressure increasing significantly



and oxygen pulse is significantly decreasing with duration of disease. It may be due to advancing cardiac neuropathy and microvascular damage and ischemic changes of myocardium with the duration of diabetes.

It is well known that reduced functional capacity is associated with an increased risk in terms of cardiovascular mortality, both in healthy subjects and coronary heart disease patients. <sup>(10)</sup>

Physical inactivity has been identified as a major determinant of type 2 diabetes, and increased activity has been shown to improve insulin sensitivity and glycemic control among non diabetic individuals, as well as those with impaired glucose tolerance or overt type 2 diabetes. <sup>(11)</sup>

Diet and exercise form the foundation of a healthy lifestyle. These are especially important for people living with diabetes mellitus; as they are the most practical non-pharmacological means by which patients may significantly improve their blood glucose levels. Exercise increases insulin sensitivity (both short and long term), lowers blood sugar levels, reduces body fat and improves cardiovascular function. Because of this, exercise offers enormous benefit to patients with diabetes. <sup>(12)</sup>

Considering all the advantages of exercise in diabetics and by the finding of our study that diabetics have exercise intolerance, it is advisable for life style changes which include proper diet with regular aerobic exercise.

## CONCLUSION

A case control study was conducted at Victoria hospital Bangalore. This study intended to record cardiovascular responses to submaximal exercise in type 2 diabetic subjects aged 35-50 years and compare the values with age and BMI matched normal controls and to check the hypothesis that diabetics have reduced exercise tolerance compared to non diabetic subjects even in absence of clinical cardiovascular disease.

Findings of our study prove our hypothesis that there is exercise intolerance in type 2 diabetics even without cardiovascular disease. It also shows that exercise intolerance is progressive with duration of disease. It is advisable for life style changes which include proper diet with regular aerobic exercise. This will be of major

help to the diabetics by reducing the progression of the disease as such and by reducing other causes of mortality.

**Conflict of Interest:** Nil

**Source of Funding:** Self

**Ethical Clearance:** Institutional ethical committee

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# Relationship between Glycated Haemoglobin [HbA1c] Levels and Blood Haemoglobin Levels -A Cross-Sectional Study

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## ABSTRACT

**Introduction:** HbA1c is a reliable biomarker and an indicator of insulin resistance for testing individuals for diabetes and prediabetes. The normal range for the HbA1c level is between 4% and 5.6%. Change in the total haemoglobin concentration is generally not included during the assay of the haemoglobin. The aim of the study is to study the effect of anemia on HbA1c levels in individuals with controlled diabetes.

**Material and Method:** It is a cross-sectional study with a sample size of 76 individuals with controlled diabetics. Haemoglobin assay, HbA1c assay, Random blood sugar and peripheral smear study was done in all subjects. The subjects are divided into three groups based in haemoglobin levels: Group I (>14 g/dl), Group II (9-14 g/dl), Group III (<9 g/dl).

**Results:** There is a significant statistical difference between haemoglobin levels and RBS in group I, II and III, ( $p = 0.000$ ). There is no significant difference between HbA1c between the groups. It is found that in Group II there is significant negative correlation between the haemoglobin and RBS ( $r = -0.350$ ,  $p = 0.05$ ) as well as between haemoglobin and HbA1c. ( $r = -0.412$ ,  $p = 0.021$ ).

**Conclusion:** It is concluded that HbA1c levels increase in diabetics with anemia compared to diabetics without anemia. This variation questions the sovereignty of using HbA1c levels, - as a yardstick to assess blood glucose levels among anaemic.

**Keywords:** HbA1c, haemoglobin, anaemia, diabetes

## INTRODUCTION

HbA1c is an important indicator of long-term glycemic control with the ability to reflect the cumulative glycemic history of the preceding two to three months. HbA1c not only provides a reliable measure of chronic hyperglycemia but also correlates well with the risk of long-term diabetes complications. The American Diabetes Association has recommended glycated hemoglobin (HbA1c) as a possible substitute to fasting blood glucose for diagnosis of diabetes<sup>1</sup>. Using

the HbA1c as a biomarker for monitoring the levels of glucose among diabetic patients was first proposed by Koenig et al in 1976. Not being bound by the time of the day on the part of the patient, the HbA1c is a very convenient test to administer and evaluate<sup>2</sup>. HbA1c is a reliable biomarker and an excellent indicator of insulin resistance for testing individuals for diabetes and prediabetes.

Normal adult hemoglobin consists predominantly of HbA ( $\alpha_2\beta_2$ ), HbA2 ( $\alpha_2\delta_2$ ), and HbF ( $\alpha_2\gamma_2$ ) in the composition of 97%, 2.5%, and 0.5%, respectively. About 6% of total HbA is termed HbA1, which in turn is made up of HbA1a1, HbA1a2, HbA1b, and HbA1c fractions, defined by their electrophoretic and chromatographic properties. HbA1c is the most abundant

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of these fractions and in health comprises approximately 5% of the total HbA fraction<sup>4</sup>.

Hemoglobin and the blood glucose interact to form aldimine in a reversible reaction. This is a nonenzymatic process that occurs continuously in vivo. The major sites of hemoglobin glycosylation, in the order of prevalence, are  $\beta$ -Val-1,  $\beta$ -Lys-66, and  $\alpha$ -Lys-61. HbA1c is the most abundant of these fractions and in health comprises approximately 5% of the total HbA fraction<sup>5</sup>. The formation of the glycated hemoglobin is a normal part of the physiologic function cycle. However, as the average plasma glucose increases, so does the amount of glycated hemoglobin in the plasma. Glycosylation of hemoglobin and increased glucose levels tends to affect RBC properties, lowering the RBC flexibility and increasing their aggregation tendency, leading to increased blood viscosity<sup>6</sup>.

The normal range for the HbA1c level is between 4% and 5.6%. HbA1c levels between 5.7% and 6.4% is considered prediabetic and levels of 6.5% or higher is seen in patients with diabetes<sup>7</sup>. According to the American Diabetes Association Guidelines published in 2011, HbA1c levels should be maintained below 7% in all diabetic patients in order to prevent the development of microvascular complications<sup>8</sup>. The HbA1c is recommended to be performed at least twice a year in diabetes patients with stable blood glucose levels.

HbA1c levels are also affected by the presence of variant hemoglobins, hemolytic anemias, nutritional anemias, uremia, pregnancy, and acute blood loss and in other conditions that shortens erythrocyte survival or decreases mean erythrocyte age. Hence in these conditions HbA1c measurement is not appropriate for diagnosis of diabetes<sup>9,10</sup>.

HbA1c levels being one of the most important indicators in the diagnosis and prognosis of diabetic patients: it is also important to note the fact that the haemoglobin levels in the patients can affect such HbA1c measurement. Any increase or decreases in the haemoglobin level can later the diabetic indicator. Hence in this study the effect of serum haemoglobin levels on the HbA1c is studied. The aim of the study is to study the effect of anemia on HbA1c levels in individuals with controlled diabetes. The objective of the study is to find the variation in HbA1c levels between normal and anemic individuals with diabetes and also to find the

relationship between HbA1c and RBC morphology in such individuals.

## MATERIALS AND METHOD

The study was conducted in Madha Medical College - General Hospital, Kovur, Chennai during October 2015 to June 2016. It was a cross sectional study with a sample size of 76 individuals with controlled diabetics. Institutional ethical committee approval was taken. Voluntary adults in the age group of 30-65 years with controlled diabetics (RBS < 126mg/dl) were included in the study. Patients with detected abnormal haemoglobin levels, uncontrolled diabetes (RBS > 126mg/dl), chronic kidney disease, recent blood transfusion and pregnant women were excluded from the study.

The sample size was estimated based on study reported by Christy 2014, considering the HbA1c and haemoglobin levels in diabetes patients. In the present study expecting similar results with 80% power, 95% confidence interval and effect size of 0.28, the study requires minimum of 76 subjects.

Study procedure was explained to the patients and informed written consent was obtained. General history was taken and physical examination was done on the subjects. After the sterile precaution, 5 ml was venous blood was collected and stored. The estimation of haemoglobin, HbA1c was done by standard methods. Random blood sugar was estimated and peripheral smear was done to look at the morphology of the RBCs.

Estimation of haemoglobin was done by Drabkin's method (cyanmethemoglobin). Blood is diluted in Drabkin's solution which consists of potassium cyanide and potassium ferricyanide. Potassium ferricyanide converts Hb to methemoglobin. Further potassium cyanide converts methemoglobin to cyanmethemoglobin (HiCN). The absorbance of the solution is measured in a spectrophotometer at a 540nm wavelength. Calculation of haemoglobin concentration was done using a standard curve.

About 2.5 ml of the blood sample were transferred to tubes containing EDTA for estimation of HbA1c. It was detected using Fast Ion-Exchange Resin Separation High performance liquid chromatography (HPLC) Method. Random blood sugar was detected by using accu-check glucometer which used rapid glucose oxidase method.

Thin peripheral smear was made and it was stained using the Leishamann stain. The smears were observed under microscope and the morphology of RBC were noted.

The subjects were divided into three groups based in haemoglobin levels. The group I consists of normal individual with haemoglobin more than 14 g/dl. The group II consists of patient with mild to moderately anemic (9-14 g/dl). The group III consists of patients with severe anemia (<9 g/dl).

The descriptive analysis of the participants were done and represented as mean and standard deviation. The mean haemoglobin, HbA1c were presented and RBS in the all the three groups were compared using ANOVA. Pearson Correlation test was done between

the parameters in the groups. All statistical analysis was done using Microsoft Excel 2010 and SPSS software (version 18, 2016).

## FINDINGS

This is a cross sectional study consists of Diabetic patients in the age group of 40 years to 60 years. The mean age was  $53.4 \pm 4.55$  years. The baseline parameters of the study participants in the three groups were included in the study. The group I consists of normal individual with haemoglobin more than 14 g/dl. The group II consists of patient with mild to moderately anaemia (9-14 g/dl). The group III consists of patients with severe anaemia (<9 g/dl). The details are depicted in the table 1.

**Table 1: Description of base line parameters in study participants of all the three groups**

Parameters	Group I	Group II	Group III
Total number (n)	11	33	32
Age (in years)	$50.2 \pm 3.4$	$55.1 \pm 4.33$	$53.23 \pm 5.89$
Mean Height (in cms)	$164.2 \pm 5.8$	$161.7 \pm 6.34$	$157.4 \pm 4.24$
Mean Weight (in Kgs)	$70.2 \pm 8.4$	$65.4 \pm 7.2$	$63.8 \pm 12.4$
BMI (in Kg/m <sup>2</sup> )	$26.11 \pm 4.76$	$24.62 \pm 5.6$	$25.21 \pm 8.7$

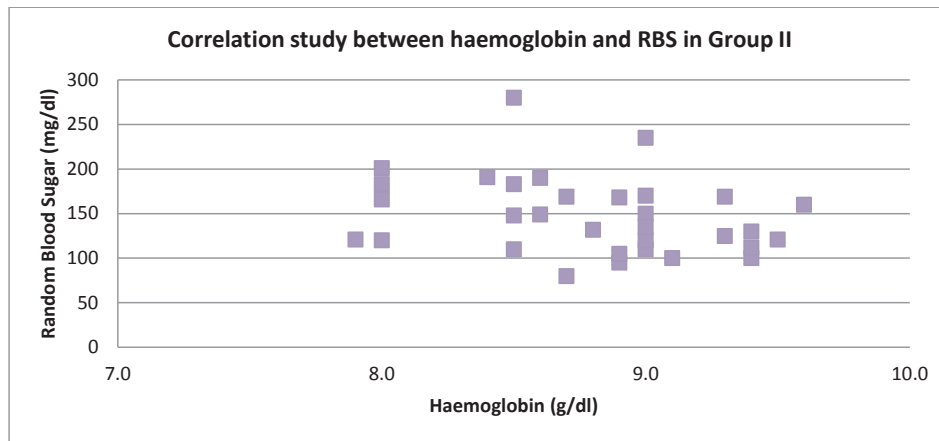
The study parameters compared using one way analysis of variance (ANOVA). The details are depicted in table 2. There is a significant statistical difference between haemoglobin levels in group I, II and III, ( $p = 0.000$ ). There is significant statistical difference between RBS in group I, II and III, ( $p = 0.027$ ) with RBS more in the group III when compared to group I and II. There is no significant difference between HbA1c between the groups. Comparison of HbA1c between the three groups is depicted in the graph.

**Table 2: Comparison of study parameters between three groups**

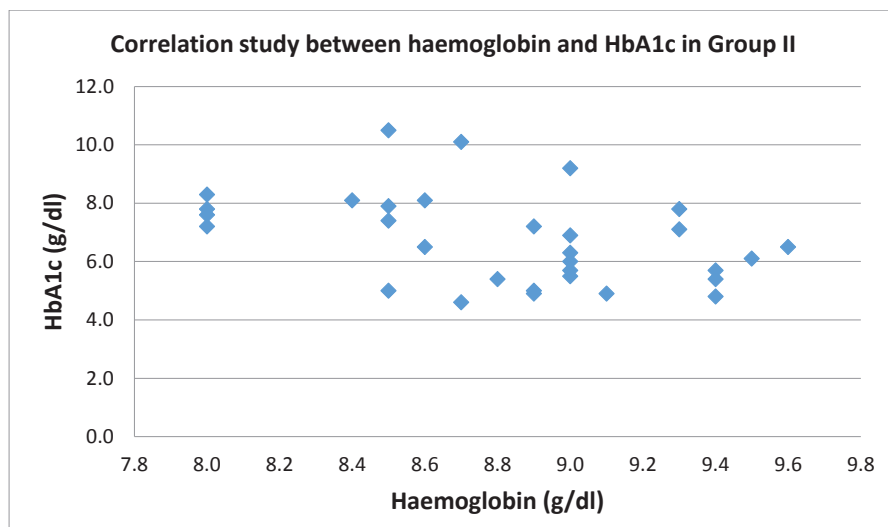
Parameters	Group I	Group II	Group III	P value
Haemoglobin (in g/dl)	$14.86 \pm 0.29$	$9.35 \pm 0.17$	$7.65 \pm 0.31$	0.000*
HbA1c (in g/dl)	$5.51 \pm 0.13$	$6.24 \pm 0.76$	$7.16 \pm 0.26$	0.187
RBS (in mg/dl)	$108.82 \pm 9.79$	$111.36 \pm 7.75$	$116.31 \pm 9.22$	0.027*

\*p value less than 0.05 is considered statistically significant

A Pearson correlation study was done between the haemoglobin levels and HbA1c, RBS levels in group II. It is found that there is significant negative correlation between the haemoglobin and RBS ( $r = -0.350$ ,  $p = 0.05$ ). There is significant negative correlation between haemoglobin and HbA1c. ( $r = -0.412$ ,  $p = 0.021$ ). Graph 1 and Graph 2 depicts the correlation studies in group II.

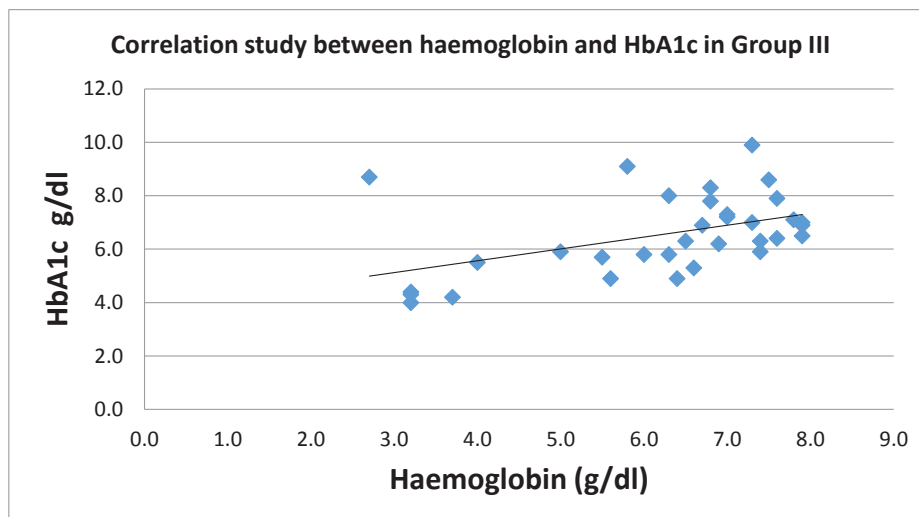


Graph 1: Correlation study between haemoglobin and RBS in Group II.

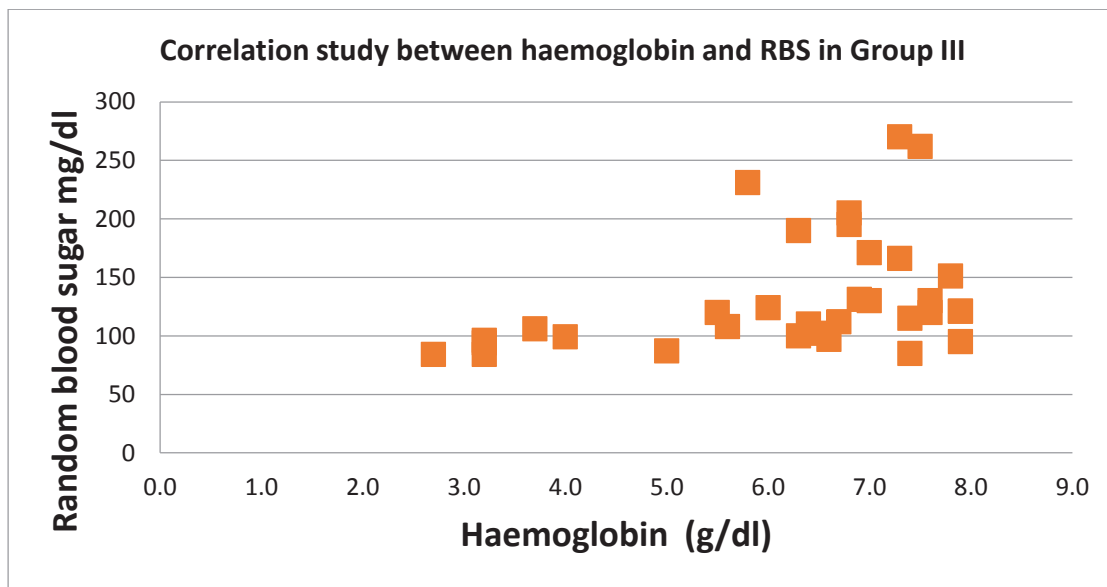


Graph 2: Correlation study between haemoglobin and HbA1c in Group II.

Pearson correlation studies were applied between haemoglobin, HbA1c and RBS in group III patients. It is found that there is significant positive correlation between the haemoglobin and RBS ( $r= 0.469$ ,  $p= 0.006$ ). There is significant positive correlation between haemoglobin and HbA1c. ( $r= 0.388$ ,  $p=0.026$ ). Graph 3 and Graph 4 depicts the correlation studies in group III.



Graph 3: Correlation study between haemoglobin and HbA1c in Group III.



Graph 4: Correlation study between haemoglobin and RBS in Group III.

During the peripheral smear observation, there was it found that only 8 slides had abnormality not related to haemoglobin. Abnormality in the form of thrombocytopenia (2), malaria parasite positive (1), and leucocytopenia (5) is seen.

### CONCLUSION

The study compares the hemoglobin level and its effect on HbA1c levels in diabetic patients. In general all diabetic patients there is exist a positive correlation between blood sugar and HbA1c levels. But the change in hemoglobin concentration has its effects on glycosylation of hemoglobin.

All the subjects were patients with diabetes with good control in blood sugar levels but still their HbA1c levels were different with a wide variation. There is increase in HbA1c levels with decreased in hemoglobin level. It is observed in this study in participants with severe anemia there is increase in HbA1c with significant correlation between hemoglobin levels and HbA1c levels. This indicates significant raise of HbA1c levels with anemia irrespective of RBS levels. This is in accordance with the study conducted by Christy A L et al., 2014 and Tarim O et al., 1999<sup>11,12</sup>.

The reason for this is well explained by understanding the mechanism by glycosylation occurs in hemoglobin. Hb glycation is an irreversible process and it increases with increase in RBC age. In anemia, decrease in RBC production and compensatory increase in average age of

RBCs leads to increase in HbA1c levels (Sluiter et al., 1980)<sup>13</sup>.

After the relationship between glycemic control and the HbA1c concentration was demonstrated, many tests have been developed to determine the HbA1c concentration. The test results are standardized to the International Federation of Clinical Chemistry (IFCC) Reference Measurement Procedure (RMP) in harmony with the efforts of the National Glycohemoglobin Standardization Program (NGSP)<sup>14</sup>. Consideration of Haemoglobin levels during and before measurement of HbA1C becomes important. In another study by Kim et al it is reported that iron deficiency anemia is common among women and is associated with shifts in A1C distribution from <5.5 to > or =5.5% and Iron deficiency has been reported to elevate A1C levels apart from glycemia<sup>15</sup>.

In this study HbA1c is correlated positively with blood sugar levels. In a similar study reported that levels of HbA1c are strongly correlated with FPG and American Diabetic Association has recently recommended HbA1c with a cut-point 6.5% for diagnosing diabetes as an alternative to fasting plasma glucose (FPG 100 mg/dl) based criteria<sup>17</sup>. However the studies fail to consider the standardization of Hemoglobin levels before reporting the HbA1c.

In another study by Zendjabil 2015, it is suggested that the HbA1c concentration is expressed in percent and a number that is between 4 and 6% and HbA1c cannot



be used in some cases and fructosamine assay to be considered instead<sup>18</sup>.

It is concluded that HbA1c levels increase in diabetics with microcytic hypochromic anemia compared to diabetics without anemia. This variation questions the sovereignty of using HbA1c levels, - as a yardstick to assess blood glucose levels among anaemics. The better alternate method would be fructosamine test. The limitation of study is macrocytic anemia was not considered and only RBS was taken into account.

**Conflict of Interest:** None

**Source of Funding:** Self

**Ethical Clearance:** Obtained

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# Corelation of Stress with Hematological Parameters and Serum Cortisol Levels before and During Examination in 1<sup>st</sup> MBBS Students

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## ABSTRACT

Examinations conducted by the college as well as university in medical field of education create changes in the haematological parameters and biochemical parameters like serum cortisol levels. The students are under constant stress since the time of admission and this exaggerates during the time of examination. These changes need to be examined.

**Aims and objectives:** 1. To demonstrate the effect of examination on haematological parameters and serum cortisol levels in 1<sup>st</sup> year medical students.

2. To prove that stress alters haematological parameters and cortisol levels.

3. To prove that serum cortisol level is a good indicator for determination of stress.

**Materials and Method:** About 30 1<sup>st</sup> year medical students between the age group of 18-19 yrs were selected for the study carried out for 10 months at KBNIMS Gulbarga, Karnataka. Haematological parameters [Hb%, RBC count, WBC count, Platelet count, neutrophil, lymphocyte, monocyte] and serum cortisol levels were estimated. 2 samples of blood were obtained. 1<sup>st</sup> sample of about 5ml was collected at the time of admission & 2<sup>nd</sup> sample 6 months during their 2<sup>nd</sup> internal assessment viva -voce examination. Blood samples for haematological parameters were analysed using Celtac  $\alpha$  Automated Hematology analyser from Nihon Kohden and for serum cortisol levels were analysed using Fully automated bidirectionally Interfaced Chemi Luminescent Immunoassay.

**Results:** When blood samples were compared, it was found that those samples taken during the exams showed a significant increase in Haemoglobin%, lymphocyte & monocyte count and a significant decrease in platelet, WBC and neutrophil count. A significant increase in serum cortisol levels were also noted.

**Conclusion:** It is concluded that examinations conducted in medical profession produce changes in haematological parameters and serum cortisol levels suggesting stress alters these parameters. If proper care and support from the faculty along with the parents is provided, it will boost their immune system and enable the students to cope up adequately with exam stress.

**Keywords:** Examination, stress, haematological parameters and serum cortisol.

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## INTRODUCTION

What is stress? Does it cause changes in haematological parameters and hormone levels like cortisol?

Stress is nothing but a force applied to the body which produces strain and internal changes. This force

may be a physical, chemical, infective agent aggressing an organism resulting in a response with an attempt to restore the previous condition. Stress is often linked to professional courses particularly medical. It is proved that academic stress in medical colleges will affect the psychology of the student which in turn is reflected by changes in the haematological parameters and hormones. Examinations are so stressful to produce changes in haematological parameters and blood pressure <sup>1</sup>. Academic examinations are mediated as one of the psychological stressors while achievement in examination is associated to future professional life of students <sup>2</sup>. Stress generated by academic examination shows significant changes in haematological parameters <sup>3</sup>. Anxiety induced stress is common and this exaggerates at the time of examination. This altered state of mind results in variation in biochemical parameters like serum cortisol levels. It is well documented that stress whether in physical or psychological form derails normal functioning process of organ system which is mainly reflected in the form of altered autonomic nervous system<sup>4</sup>. The role of increased autonomic nervous system activity with hypothalamic-pituitary-adrenal axis during stress is termed due to adaptive mechanisms

<sup>5,6</sup>. The present study attempts to correlate the effect of examination stress on haematological and biochemical parameters like Serum Cortisol levels.

## MATERIALS AND METHOD

About 30 healthy 1<sup>st</sup> year medical students between the age group of 18-19 yrs were selected for the study carried out at KBNIMS Gulbarga Karnataka. The study was for a period of 10 months. Hematological parameters like Hb%, RBC count, WBC count, Platelet count, neutrophil, lymphocyte & monocyte were estimated along with serum cortisol levels after taking informed consent. Under aseptic precautions 2 samples of 5ml of blood were obtained. 1<sup>st</sup> sample was collected at the time of admission & 2<sup>nd</sup> sample was collected 6 months during their 2<sup>rd</sup> internal assessment viva -voce examination. Blood samples for haematological parameters were analysed using Celtac  $\alpha$  Automated Hematology analyser from Nihon Kohden and for serum cortisol levels were analysed using Fully automated bidirectionally Interfaced Chemi Luminescent Immunoassay. Statistical analysis was done by Student “t” test using SPSS 20.0 version software. A p value of < 0.05 was considered to be statistically significant.

## RESULTS

The haematological parameters before and during exam were compared and results are presented in Table 1 and 2

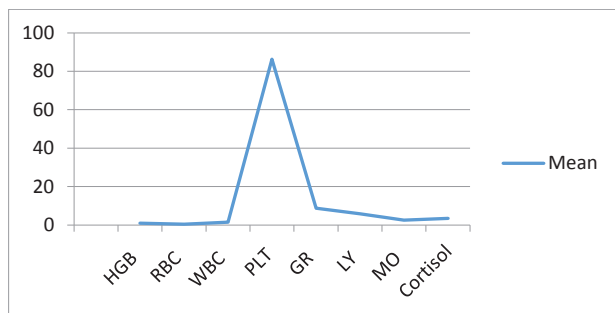
**Table No. 1: Values of haematological parameters before and during exam**

Parameters	Before Exam			During Exam		
	Mean	Std. Deviation	Std. Error Mean	Mean	Std. Deviation	Std. Error Mean
HGB g/dl	13.620	1.7460	0.3188	14.557	1.4107	0.2576
RBC 10 <sup>6</sup> / $\mu$ L	5.17	1.112	0.203	4.7477	0.48022	0.08768
WBC 10 <sup>3</sup> / $\mu$ L	8.340	1.7651	0.3223	6.893	1.2145	0.2217
PLT 10 <sup>3</sup> / $\mu$ L	357.27	117.497	21.452	271.03	42.822	7.818
Neutrophil 10 <sup>3</sup> / $\mu$ L	65.477	6.5626	1.1982	56.787	7.9782	1.4566
Lymphocyte 10 <sup>3</sup> / $\mu$ L	31.020	5.7973	1.0584	36.830	7.1624	1.3077
Monocyte 10 <sup>3</sup> / $\mu$ L	3.503	1.4696	0.2683	5.987	1.4102	0.2575

**Table 2: Comparison of haematological parameters before & during exam**

Parameters	Mean	Std. deviation	Std. Error Mean	95% Confidence interval of the difference		t-value	P-value
				Lower	Upper		
HGB g/dl	0.9367	1.2560	0.2293	-1.4057	-0.4677	4.085	0.000 **
RBC 10 <sup>6</sup> /μL	0.4246	1.21296	0.22146	-0.02826	0.87759	1.918	0.065
WBC 10 <sup>3</sup> /μL	1.4467	1.2803	0.2337	0.9686	1.9247	6.189	0.000 **
PLT 10 <sup>3</sup> / μL	86.233	123.460	22.541	40.133	132.334	3.826	0.001 **
Neutrophil 10 <sup>3</sup> / μL	8.6900	8.7964	1.6060	5.4054	11.9746	5.411	0.000 **
Lymphocyte 10 <sup>3</sup> / μL	5.8100	7.8279	1.4292	-8.7330	-2.8870	4.065	0.000 **
Monocyte 10 <sup>3</sup> / μL	2.4833	1.8291	0.3339	-3.1663	-1.8003	7.436	0.000 **

\*p < 0.05 is significant and \*\*p < 0.001 is highly significant and p > 0.05 is insignificant.



**Figure 1: Mean values of haematological parameters and Serum Cortisol levels**

**Table 3: Serum cortisol levels before and during exam**

Paired Samples Statistics				
	Mean	N	Std. Deviation	Std. Error Mean
Cortisol_ Before_ Exam	7.2620	30	2.46881	0.45074
Cortisol_ During_ Exam	10.6553	30	3.22017	0.58792

**Table 4: Comparison of serum cortisol levels before and during exam**

Paired Samples Test									
	Paired Differences					t	df	p-value	
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference					
				Lower	Upper				
Cortisol	3.39333	3.22963	0.58965	2.18737	4.59930	-5.755	29	0.000	

The values obtained in **Table 1 and 2** and **Figure 1** show increase in haemoglobin, lymphocyte and Monocyte counts and decrease in WBC, Platelet and Neutrophil counts during the exam. The difference is statistically significant. No significant changes were observed in RBC count.

The values obtained in **Table 3 and 4** and **Figure 1** show increase in Serum Cortisol levels during the exam. The difference is statistically significant.

## DISCUSSION

The present study determined the haematological parameters and serum cortisol levels before and during the examination. Examinations in Medical students are so stressful to produce changes in haematological parameters<sup>1</sup>. There is increase in Haemoglobin count during the examination similar to the study done by Maes M, Vander Planken M, Van Gastel A, Et al. There is increase in lymphocyte count in our study suggesting acute stress induces not only lymphocytosis but also a greater increase in CD8(+) CD62L(-) T cells compared to CD8(+) CD62(+) T cells. A brief exposure to stress increases the number of circulating CD8 suppressor/cytotoxic T cells<sup>7</sup>. The increase in Monocyte count suggests the effects of stress hormones on the induction of migration and recruitment of immune cells. Stress hormones such as catecholamines (the end products of the sympathetic nervous system) are able to modify the circulation and cell trafficking of virtually all immune cells, such as lymphocytes, polymorphonuclear cells, monocytes, dendritic cells and NK cell. A recent study by Nicole Powell *et al.* published in the October 8, 2013 issue of *PNAS*, suggests that stress hormones may contribute to pro-inflammation through selective expansion and/or recruitment of a specific subpopulation of immature, proinflammatory monocytes. In the 1970s and 1980s stress was often considered immunosuppressive. Recent evidence, however, indicates that psychological stress influences immunity in a less monochromatic way, but at multiple levels and can be in either direction, in terms of its pro- or anti-inflammatory effects. Thus, taken as a whole, the study may identify an additional mechanism by which adverse social environments and stress can enhance the risk of inflammation-related diseases in susceptible individuals. Previous research indicates that in the bone marrow, catecholamines originate mostly from the sympathetic nerve fibers, and myelopoiesis is negatively affected by  $\alpha$ 1-adrenoreceptors expressed on bone marrow cells<sup>8</sup>. Monocytes cause inflammation as part of the stress response. The monocytes flock to the brain regions that send out stress signals: the amygdala and hippocampus, which are involved in processing feelings of fear, and the prefrontal cortex, which is supposed to regulate the fear regions. Once

there, monocytes change the way the genes of brain cells behave. When the fear regions of the brain become overactive, the result is anxiety.

Short time stress such as an exam can enhance neutrophil functions. Chronic psychological stress suppresses neutrophil functions. Stress can be a positive or negative event. There is no way to predict how an individual's immune system will respond to stress.

For some the immune system is suppressed. The body releases several chemicals during times of stress and this includes catecholamines. According to an article by Hannah Koenker of the University of Illinois, catecholamines can suppress the activity of certain white blood cells, leaving the immune system weakened. This change appeared to be most drastic in individuals who had strong reactions to stress such as an elevation in heart rate and blood pressure. Humans do not perform as well under stress as they do in a relaxed state. Creating an adequate amount of platelets is difficult when the body is under mental or physical stress. Stress and also lack of sleep drops the platelet count. There are two kinds of stress. Acute stress is a normal part of everyday life and helps our stress response system stay on the ball. Problems arise when we are repeatedly exposed to the same stressor out of many different stressors for an extended period of time. When this happens, we can fall prey to the effects of chronic stress.

In our study there is significant decrease in the WBC count, Neutrophil count and platelets suggesting that, the students are under constant stress till they are admitted in the professional course superadded by the acute stress of examination. Our study also showed significant increase in Serum Cortisol level during the exam when compared to before the exam. It is well documented that stress whether in physical or psychological form derails normal functioning process of organ system which is reflected in the form of altered Autonomic nervous system<sup>4</sup>. The response of stress varies from individual to individual based on their capacity and emotional state. The role of increased autonomic nervous system activity with hypothalamo-pituitary-adrenal axis during stress is termed due to adaptive mechanisms<sup>5,6</sup> and lead to excess production of cortisol as observed in our study similar to the study demonstrated by Miller and Callaghan *et al*<sup>9</sup>. Hence suggesting academic stress at the level of 1<sup>st</sup> year medicine can lead to pathological state in students if proper counselling and care is not



provided to students. Similar results were obtained by Goyal S et al<sup>10</sup>. suggested chronic stress to be associated with increased cortisol levels. In our study, no significant change in RBC count was observed.

### CONCLUSION

Haemological parameters and Serum Cortisol levels are good indicators for determination of stress as demonstrated in our study. There are different types of stress — acute stress, episodic acute stress, and chronic stress — each with its own characteristics, symptoms, duration and treatment approaches. The amount of stress increases more when the students appear for the internals and university exams compared to the routine tests like part completion tests. However exams emphasize the ability to understand, organise and recall information. In this competitive era students need to adapt themselves so as to cope up with any kind of stress effectively. High social support appears to attenuate the magnitude of changes in immune cells suggesting a role for social support in protecting against immune decrements during the times of stress<sup>11</sup>. If proper care and support from the faculty along with the parents is provided, it will boost their immune system and enable the students to cope up adequately with exam stress.

**Source of Funding :** Self

**Conflict of Interest :** Nil

**Ethical Clearance:** Obtained from Institutional Ethics Committee.KBNIMS Kalaburagi, Karnataka.

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# Thyroid Function and Gender Influence in Patients of Undialyzed Chronic Renal Failures

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## ABSTRACT

**Background** – Chronic Renal Failure(CRF)patients on dialysis have shown alteration in their thyroid status, however conflicting results do not allow to have conclusive identification of the impact. **Aim** – The study was to evaluate the thyroid hormone profile and gender influence in undialyzed CRF patients and to compare with that of healthy individuals. **Method** – The study group was consisted of 80 volunteers including 40 undialyzed CRF patients and 40 age matched healthy volunteers. Thyroid hormonal profile like Total Triiodothyronine (TT3), Total Thyroxine (TT4),FreeTriiodothyronine (FT3),Free Thyroxine (FT4),Thyroid Stimulating Hormone (TSH) and Renal function parameters like serum creatinine were estimated by standard automated methods and compared with healthy control groups.

**Results** – The serum levels ofTT4,TT3 and FT4 showed significant decreases and TSH increased significantly in undialyzed CRF patients compared to the healthy controls. Interestingly obvious differences were observed in between genders within the undialyzed CRF patients. **Conclusion** – On the basis of observation reported here, it can be summarized that renal insufficiency may lead to subclinical hypothyroidism in un dialyzed CRF patients. Nevertheless, the statistical tests used here did not find significant influences of gender in the current study

**Keywords**– *Undialyzedchronic Renal Failure, TotalTriiodothyronine, FreeTriiodothyronine, Total Thyroxine, FreeThyroxine, Thyroid Stimulating Hormone.*

## INTRODUCTION

Kidneys play a significant role in metabolism, degradation and excretion of several hormones including that of thyroid gland. Thyroid hormones are necessary for growth and development of kidney<sup>(1)</sup>. So impairment in the kidney function may lead to disturbed thyroid physiology<sup>(2)</sup>.Chronic Renal Failure (CRF) is well recognized as worldwide public health problem causing considerable morbidity and mortality<sup>(3)</sup>. CRF patients frequently have signs and symptoms suggestive of thyroid dysfunction and vice versa<sup>(1)</sup>.Hyperthyroidism, hypothyroidism and euthyroid state have all been reported by various workers. A high proportion of CRF patients who are on hemodialysis therapy develop goiter and thyroid dysfunction<sup>(4,5)</sup>. However, literature describing in vitro thyroid function tests in undialyzed CRF patients is somewhat controversial. There is agreement that plasma thyroid binding globulin concentrations are usually

normal<sup>(6,7)</sup>, but both normal<sup>(8)</sup> and low normal serum total T4 levels have been reported<sup>(9)</sup>. Serum free T4 concentrations have been reported as normal<sup>(10)</sup>as well as low<sup>(11)</sup>. Normal serum T3 levels have been reported<sup>(12)</sup>, while others have found T3 levels to be subnormal in varying proportions<sup>(13,14)</sup>. Different treatments used in the management of patients with kidney and thyroid diseases may be accompanied by changes or adverse effects that affect thyroid and kidney function respectively<sup>(15)</sup>. Knowledge of alterations of thyroid hormone metabolism in CRF patients is required to accurately diagnose and treat concurrent hypothyroidism and hyperthyroidism. Furthermore thyroid diseases including goiter, hypothyroidism, thyroid nodules and thyroid cancer may occur more frequently in CRF patients and may be underdiagnosed due to limited clinical awareness<sup>(16)</sup>.With this background, we consider biochemical screening through thyroid hormone quantification to assess thyroid

function is of paramount importance in undialyzed CRF patients. Although thyroid status is profoundly influenced by renal function, this has not been studied in detail<sup>(17,18,19)</sup>. The current study was aimed to evaluate the Thyroid hormone levels in undialyzed CRF patients. Whether gender is influencing undialyzed CRF patients or not is unclear. Therefore, inclusion of study of gender influence in thyroid hormone profile in undialyzed CRF patients is also considered worthwhile.

## MATERIALS AND METHOD

The study was conducted on 40 undialyzed CKD patients as evidenced by serum creatinine level above 2.0mg/dl who visited the Nephrology Department, NRI Medical College during June 2013 to May 2015. Forty healthy controls (having serum creatinine level within normal range) have been included in the study. Informed consent was taken from all subjects involved in the study and the study was approved by the Institutional Ethics Committee.

None of the study groups are having preexisting health problems or complicating CKD. The undialyzed CKD patients were on renal diet (50gms protein and 5gms salt/day). The healthy controls were not on any kind of prescribed medication or dietary restrictions. The blood samples were collected and serum was stored to carry out the biochemical tests.

Serum levels of creatinine were assayed as mentioned by Padmanabhanpreethi et al<sup>(20)</sup>. Serum levels of TSH, T<sub>3</sub>, T<sub>4</sub>, FT<sub>3</sub>, FT<sub>4</sub> were assayed as mentioned by Fernandez-Ulloa M et al<sup>(21)</sup>.

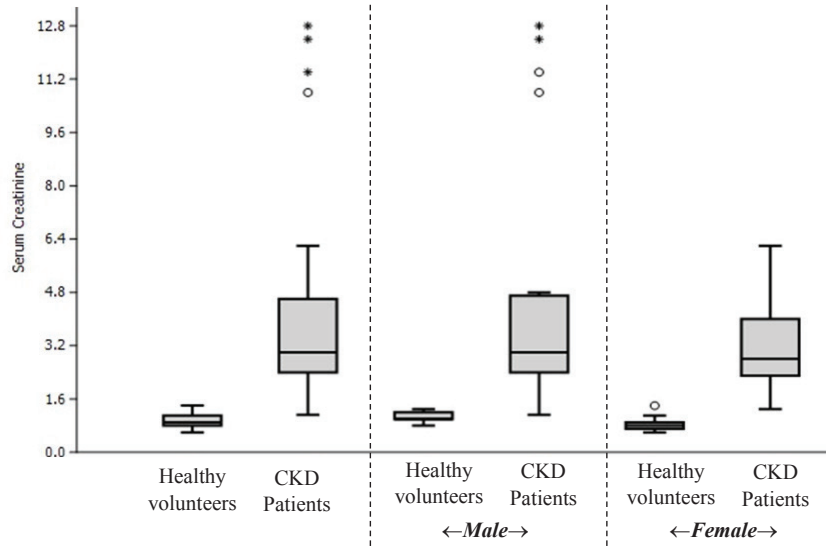
Significance of difference between the groups are calculated by student's t- test with probability of < 0.05.

## RESULTS

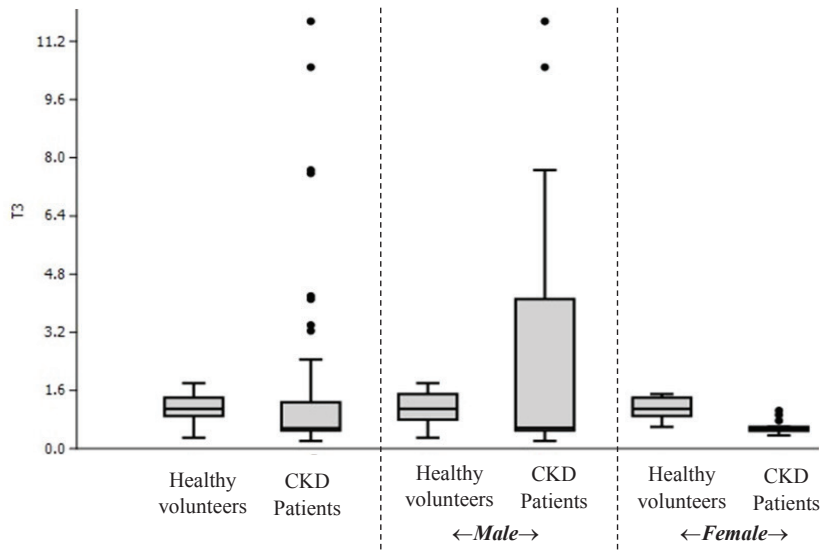
Median values of serum creatinine in healthy

volunteers and undialyzed CKD patients are presented in figure -1. The serum creatinine level of CKD patients were noted to be increase by 2.7-3.3 folds found to be statistically significant in comparison to that of the healthy volunteers. Figure -2 shows that median values of serum T<sub>3</sub> levels of undialyzed CRF patients and were noted to decrease (49%) significantly in comparison to that of the healthy volunteers. However, when compared separately for both males and females, both male and female undialyzed CRF patients show significantly decrease (48%) and (49%) serum T<sub>3</sub> level compared to healthy counterparts. From figure -3 median serum FT<sub>3</sub> level of undialyzed CRF patients were noted to decrease (1.6%) insignificant in comparison to that of healthy volunteers. However when compared separately for males and females, male undialyzed CRF patients showed insignificant decrease (4.7%) whereas female undialyzed CRF patients showed insignificant increase (1.9%) in serum FT<sub>3</sub> level compared to their healthy counterparts. From figure - 4, median serum levels of T<sub>4</sub> in undialyzed CRF patients were noted to decrease (20%) significantly in comparison to that of healthy volunteers. when compared separately for male and female, both male and female CRF patients show significantly decrease (21%) and (22%) compared to their healthy volunteers. Figure - 5 shows median serum FT<sub>4</sub> level of undialyzed CRF patients were noted to decrease (19%) significantly in comparison to healthy volunteers. When compared separately for males and females, both male and female undialyzed CKD patients show significantly decrease (24%) and (18%) serum FT<sub>4</sub> level compared to their healthy volunteers.

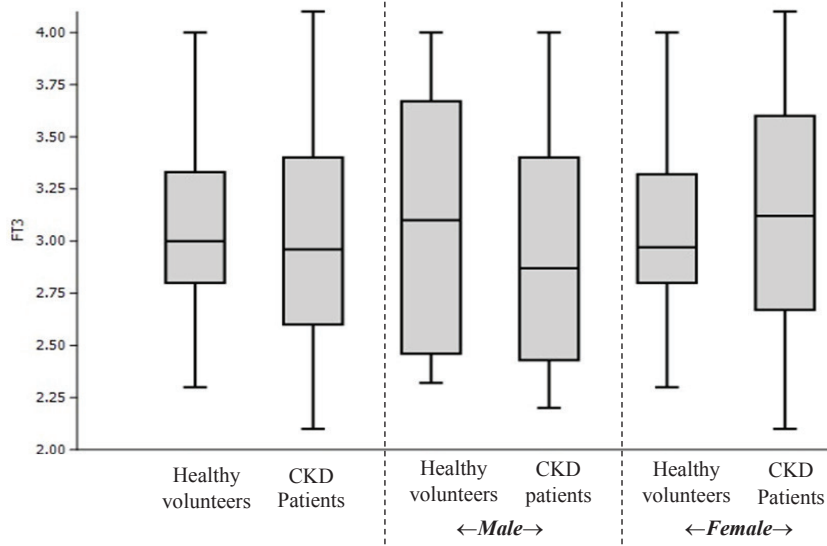
From figure-6 median serum TSH level of undialyzed CKD patients were noted to increase 2.5 fold significantly in comparison to that of the healthy volunteers. However when compared separately for males and females, male patients showed relatively lesser increment (2fold) than the female patients (3 fold).



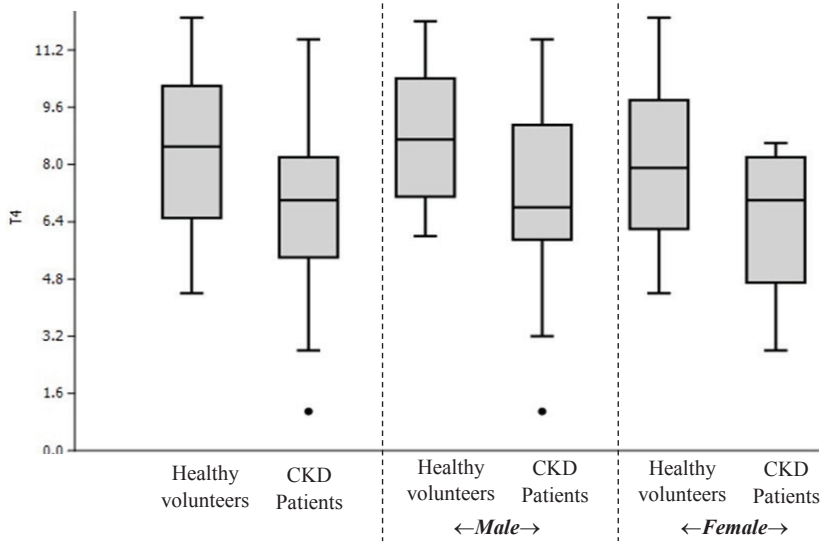
**Figure- 1.** Box and whisker plot of collected data of serum levels of creatinine (mg / dL) of included patients and volunteers indicating median value (as cross line inside the box), 1<sup>st</sup> to 3<sup>rd</sup> quartile of values (as rectangular box), maximum and minimum (range) values (as line outside the box with cap) and outliers (as dark dots), if any.



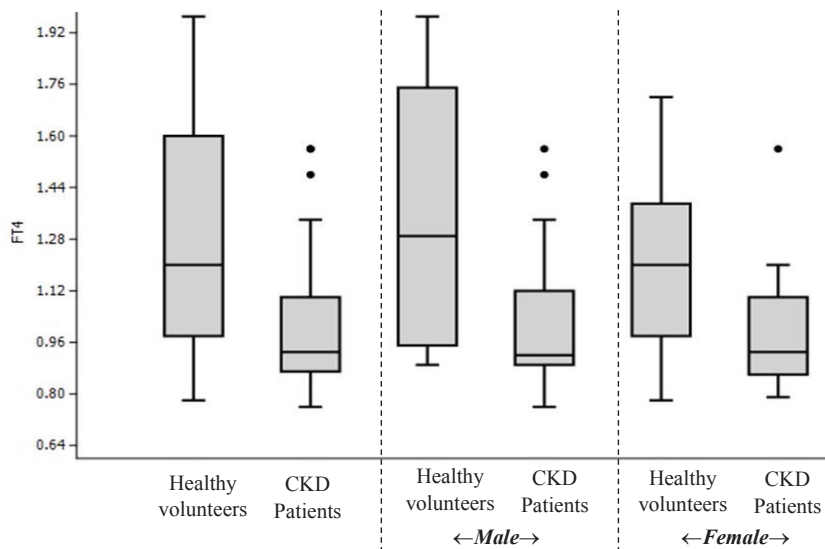
**Figure - 2.** Box and whisker plot of collected data of serum levels of Triiodothyronine (T3; ng / mL) of included patients and volunteers indicating median value (as cross line inside the box), 1<sup>st</sup> to 3<sup>rd</sup> quartile of values (as rectangular box), maximum and minimum (range) values (as line outside the box with cap) and outliers (as dark dots), if any.



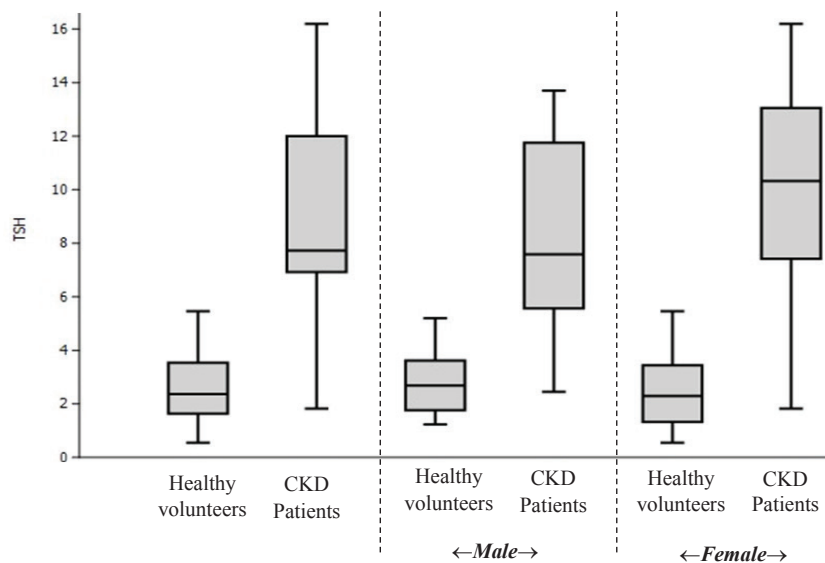
**Figure - 3.** Box and whisker plot of collected data of serum levels of free Triiodothyronine (FT3; pg / mL) of included patients and volunteers indicating median value (as cross line inside the box), 1<sup>st</sup> to 3<sup>rd</sup> quartile of values (as rectangular box), maximum and minimum (range) values (as line outside the box with cap) and outliers (as dark dots), if any.



**Figure -4.** Box and whisker plot of collected data of serum levels of Tetraiodothyronine (T4; µg / mL) of included patients and volunteers indicating median value (as cross line inside the box), 1<sup>st</sup> to 3<sup>rd</sup> quartile of values (as rectangular box), maximum and minimum (range) values (as line outside the box with cap) and outliers (as dark dots), if any.



**Figure-5.** Box and whisker plot of collected data of serum levels of free Tetraiodothyronine (FT4;  $\mu\text{g} / \text{dL}$ ) of included patients and volunteers indicating median value (as cross line inside the box), 1<sup>st</sup> to 3<sup>rd</sup> quartile of values (as rectangular box), maximum and minimum (range) values (as line outside the box with cap) and outliers (as dark dots), if any.



**Figure - 6.** Box and whisker plot of collected data of serum levels of Thyroid stimulating hormone (TSH;  $\mu\text{IU} / \text{mL}$ ) of included patients and volunteers indicating median value (as cross line inside the box), 1<sup>st</sup> to 3<sup>rd</sup> quartile of values (as rectangular box), maximum and minimum (range) values (as line outside the box with cap) and outliers (as dark dots), if any.

## DISCUSSION

In recent years, incidence of chronic kidney disease is on rise<sup>(22)</sup>. Number of studies was directed at assessing the magnitude of the problem and factors complicating CKD. A host of metabolic and endocrine functions normally undertaken by the kidneys are impaired in CKD patients. Many hormones including parathyroid hormone, insulin, glucagon, prolactin etc. suffer abnormal regulation<sup>(15)</sup>. Taking cues from these established facts, several investigators studied hormonal profiles of various sorts in relation to declining renal function. Of particular interest are, reports of compromised thyroid function in CKD patients<sup>(8,5)</sup>. Studies on thyroid profile on undialyzed CKD patients are few but with conflicting results.

The present study focused on unsettled issue of thyroid function status in undialyzed CKD patients along with scrutiny for any gender based pattern compared with healthy volunteers. Two interesting facts were apparent with respect to gender, in CKD patients available for this study. 60% (n=24) of the CKD patients happen to be males while 40% (n=16) are females. This observation in the study points to greater proclivity of males to CKD. Secondly, severity was also greater in males; the mean serum creatinine being of 4.55mg/dl in males while it was 3.13mg/dl in females.

A decreased Total Triiodothyronine (TT<sub>3</sub>) level in patients with CKD was thus found to be statistically significant. The decrease in serum TT<sub>3</sub> was more or less same in both male and female patients. However, Free Triiodothyronine (FT<sub>3</sub>) level did not alter much in CKD patients compared to healthy volunteers. It was also found that the levels of both Total Thyroxine (TT<sub>4</sub>) and Free Thyroxine (FT<sub>4</sub>) showed a significant decline in undialyzed CKD patients compared with healthy volunteers. Similar observations of decline in TT<sub>3</sub>, TT<sub>4</sub> and FT<sub>4</sub> except for FT<sub>3</sub> were reported in majority of the CKD patients investigated by Prajapati et al<sup>(15)</sup> and Silverberg et al<sup>(10)</sup>. However Ramirez et al<sup>(12)</sup> observed normal T<sub>4</sub>, FT<sub>4</sub> and T<sub>3</sub> levels in CKD patients. Even though, Czernichow et al<sup>(9)</sup> and Hershman et al<sup>(11)</sup> reported normal levels of TT<sub>4</sub> and FT<sub>4</sub> in CKD patients, the values were on the lower side. These instances testify inconsistency of changes reported in thyroid levels in CKD patients by many other investigators. Possibly, differences in the study design, number of subjects, methods of estimation, geographical and many others

variables may account for inconsistent results among these studies. Yet the biochemical evidence of disturbed thyroid hormone profile in all the above studies including the present one, despite clinical euthyroid state, was a notable feature. Available literature did not offer any cause or explanation for the observed thyroid hormone abnormalities in chronic renal disease. Decreased TT<sub>4</sub> and FT<sub>4</sub> levels observed in the present study may point alterations in hormone production, distribution or excretion as suggested by Prajapati et al<sup>(15)</sup>.

According to them, all levels of the Hypothalamo-pituitary-Thyroid axis may be involved. A wide range of selenium dependent enzymes, referred to as selenoproteins involved in different functions including formation of thyroid hormones, are synthesized in the kidney. These include enzymes like Glutathione peroxidases, Iodothyronine deiodinases, Thyroxine reductase, etc. which are involved in thyroid metabolism. Some of these enzymes are said to be extensively expressed in the kidney, primarily in the basolateral plasma membrane. It is postulated by Iglesias et al<sup>(21)</sup> that decreased formation of these selenoproteins in chronic renal impairment may indirectly affect thyroid hormone synthesis. Other possible explanation of decreased thyroid hormones was based on inhibition of organification of iodine due to direct suppression of synthesis in the gland by iodide itself via Wolf-Chaikoff effect<sup>(24)</sup>. In 1983, Robertson et al reported increase in serum iodine levels due to decreased glomerular filtration rate (GFR) with renal impairment<sup>(25)</sup>. According to Marou et al, excess availability of iodine is controlled in the thyroid gland by inhibiting organification of iodine<sup>(24)</sup>. They also proposed that iodopeptides are formed that temporarily inhibit mRNA formation and protein synthesis causing reduction in thyroid peroxidase and therefore resulting in reduced thyroglobulin iodination.

As regards Thyroid Stimulating Hormone (TSH) level in CKD patients, again opinion is divided. According to Rajagopalan et al<sup>(1)</sup> and Pandey and Devi<sup>(26)</sup> there is not much significant increase in serum TSH levels in CKD patients. However, increase in TSH level in CKD patients have been reported by Mooradian and Morley<sup>(27)</sup> as well as Basu and Mohapatra<sup>(28)</sup>. Thus, there is no uniform agreement pertaining to levels of TSH in CKD patients. In the present study, the mean TSH level in CKD patients was increase statistically significant in CKD patients. There was only negligible margin of



difference between males and females. Increased TSH levels in the context of low circulating  $TT_4$ ,  $FT_4$  and  $T_3$  observed can be the result of long loop negative feedback effect on Hypothalamo-Pituitary axis as is well known<sup>(29)</sup>.

To summarize, the serum levels of  $TT_4$ ,  $FT_4$  and  $TT_3$  showed a significant decrease and TSH showed a significant increase in undialyzed CKD patients compared to healthy controls. But the major limitations to arrive at a definitive conclusion relating declining thyroid function with the increasing creatinine levels in CKD needs sufficiently large sample for each grade of creatinine level. Considering the kidneys active role in metabolism, excretion and regulation of hormonal balance, compromise thyroid function is likely. However, this aspect may open up new avenues in the management of chronic renal disease with promise of slowing the deterioration and morbidity.

### CONCLUSION

The present study clearly demonstrates subclinical hypothyroidism in undialyzed CRF patients. There is no earlier report of gender variation in Thyroid hormone profile in undialyzed CRF patients. However the present investigation shows that gender is significantly influencing the thyroid hormone profile in undialyzed CRF patients. Nevertheless, the statistical tests used here did not find significant influences of gender in the current study. Even though, the number of patients studied in this study are sufficient as per the statistical requirements, with more number of patients and stricter inclusion criteria may provide a better understanding of the thyroid profile in undialyzed CRF patients.

**Conflict of Interest:** Nil

**Source of Funding:** Self

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# Impact of Chronic Alcoholism on Temporal Cognition and Coordination of Motor Activity

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## ABSTRACT

**Introduction:** Alcohol abuse constitutes a worldwide social and health problem which is associated with a variety of neurocognitive changes. The precise neurophysiological basis of these changes is not yet fully understood. In chronic alcoholics specific deficits are often observed in their perceptual capabilities, motor control, problem solving abilities and memory function. This study was aimed to compare the critical flicker fusion frequency (CFFF) and finger tapping test to diagnose neurocognitive function loss in chronic alcoholics.

**Methodology:** A total of 91 subjects, 48 chronic alcoholic subjects and 43 non alcoholic subjects aged between 25 to 50 years were included in this study. All participants underwent the CFF test using Nethra CFFF device (Mavom labs pvt.ltd) built on python free source software and finger tapping test by counting device through Audacity (Cross-Platform Sound Editor) to record inter twitch interval. The testing procedures were repeated three times to ensure that the subject was sufficiently familiar with it before CFF & finger tapping test measurements were initiated.

**Results:** The present data reports CFFF and inter twitch interval in chronic alcoholics and healthy age matched controls (non alcoholics). The Mean CFFF in chronic alcoholics is  $35.58 \pm 0.52$  Hz which is significantly lesser than non-alcoholics i.e,  $39.46 \pm 0.39$  Hz (P value < 0.001). Inter twitch interval (ITI) determined from finger tapping test in both groups. The mean ITI in chronic alcoholics is  $311 \pm 7.73$  ms, which is also significantly slower than control group  $244 \pm 5.07$  ms (P value < 0.001).

**Conclusion:** The results of the current study conclude that, chronic alcoholism decreases the temporal cognition and fine motor skills.

**Keywords:** Critical Flicker Fusion Frequency, Inter twitch interval, Cognition, Alcoholism.

## INTRODUCTION

Alcoholism is a broad term for drinking of any alcohol that leads to intellectual and physical health problems<sup>1</sup>. Chronic alcoholism refers to the persistent, progressive, excessive and compulsive

drinking of alcohol leading to psychological and physical dependence or addiction. The predominant feature of alcoholism is consumption of large quantities of alcohol despite knowledge of the adverse effects of such behavior<sup>2</sup>. The physical dependency caused by alcohol can lead to an affected individual having a very strong urge to drink alcohol. These characteristics play a role in decreasing an alcoholic's ability to stop drinking<sup>3</sup>. Alcohol consumption acutely stimulates dopamine release from the major terminal area of the mesolimbic Dopaminergic system, Nucleus Accumbens. Enhanced dopamine transmission in the Nucleus Accumbens plays a critical role in the positive

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rewarding aspects of drugs abuses and the initiation of addictive process. Chronic administration is associated with functional alterations of this important part of the brain reward system. Global dysregulation of the dopaminergic system caused by chronic alcohol consumption produces drug dependence reinforcement and is most likely involved in the development of drug addiction<sup>4</sup>. Excessive and prolonged alcohol use may lead to permanent structural and functional damage to the brain<sup>5</sup>. Recently researchers have focused on alcohol related issues across the world. In chronic alcoholics specific deficits are often observed in their perceptual capabilities, motor control, problem solving abilities and memory function. It is still unclear that cognitive impairment or dementia of chronic alcoholism is due to direct neurotoxicity of ethanol or portrayal of additional underlying pathology or multifactorial<sup>6</sup>. The correlation between intensity and period of alcohol consumption and occurrence of cognitive loss is not well established. This is due to different types and strengths of liquor available across the countries, varying definitions of leisure drinking and pathological drinking, different cultural beliefs and different definitions of standard drink<sup>6</sup>. Diversity in quantity, duration of drinking and difficulties in obtaining an accurate self report of alcohol use may complicate attempts of correlating alcoholism to impairment of cognition.

## METHODOLOGY

In this case control study, a total of 91 subjects were recruited, out of which 48 chronic alcoholics and 43 non alcoholics were subjected to tests that determine cognitive impairment and loss of coordination of motor activity. These tests are Critical Flicker Fusion Frequency (CFFF) and Finger Tapping Test (FTT). Institutional ethical committee has approved this study. The entire procedure was explained and written informed consent form was obtained from each participant. Participants were advised to abstain from alcohol 24 hours before collecting data.

**Critical Flicker Fusion Frequency (CFFF):** The time resolving ability of eye is determined by measuring the critical flicker fusion frequency. CFFF was measured for all study participants by Nethra CFF device (Mavom labs pvt.ltd) built on python free source software and the participants were sufficiently familiarized with it. Patient watched a Red Light Emitting Diode (LED) 5mm in diameter that was pulsed with square wave and

duty cycle was 50%. The light frequency at which it was perceived to be non-flickering was considered as fusion frequency. The frequency of light was decreased gradually until the light was perceived as flickering. Participants were instructed to press a button when flicker was perceived<sup>8</sup>.

**Finger Tapping Test (FTT):** It is a psychomotor test that examines motor function, motor speed and coordination. Finger tapping test was done by a hardware device through Audacity (Cross-Platform Sound Editor) to calculate Inter Twitch Interval (ITI). The Subjects palm was made immobile and flat on the board, with fingers extended, and the index finger placed on the button. Subjects tapped their index finger on the button as quickly as possible within a 10-s time interval. The number of counts were recorded and analyzed by open source Audacity software (Cross-Platform Sound Editor). Inter twitch interval (ITI) in milli seconds was analyzed offline<sup>9</sup>.

**Statistical analysis:** Statistical analysis was carried out using graph pad prism & data was represented as mean and SD. Normality of data was tested using Kolmogorov-Smirnov test. A *p* value of > 0.05 indicated normal Gaussian distribution. The data sets were skewed and Mann-Whitney test was performed.

**Results:** The present data reports the above tests were compared between the cases (Alcoholics) and healthy age matched controls (non alcoholic subjects). Values are expressed as mean  $\pm$  SD in the tables.

**Table 1: Showing CFFF and ITI measurements in alcoholic and non-alcoholic participants.**

Cognition & Coordination parameters	Chronic Alcoholics Mean $\pm$ SD	Non Alcoholics Mean $\pm$ SD	<i>p</i> - value
Inter Twitch Interval (ms)	311 $\pm$ 7.73	244 $\pm$ 5.07	<0.001**
Critical Flicker Fusion Frequency (Hz)	35.58 $\pm$ 0.52	39.46 $\pm$ 0.39	<0.001**

## DISCUSSION

Cognition is the mental process of acquiring

knowledge by the experiences, thoughts and senses<sup>11</sup>. Cognition is a large domain which includes memory, judgment, attention, comprehension and computation. Human cognition uses the existing knowledge and creates an advanced comprehension for a goal directed behavior. One of the causes for neurocognitive deficit is chronic alcoholism which leads to structural and functional damage in some of the cortical areas especially in pre frontal and orbito-frontal cortex<sup>12</sup>. The behavioral changes observed in these cases are probably related to deficits in the gabaergic and serotonergic activities which influence the decision-making process, behavior of repeated alcohol consumption and process of substance dependence<sup>13</sup>. Chronic alcoholism leads to a state of confusion and diminished attention level in their perceptual abilities. The chronic effects of alcoholism are impaired memory, learning, abstraction, visuo-spacial analysis, psychomotor speed processing, information processing speed and cognitive efficiency. Alcohol-dependent subjects committed more errors in the tasks and took more time to complete the task. Brain alterations stemming from the chronic consumption of alcohol may reach very advanced stages of mental deterioration such as in the case of alcohol-induced persistent dementia<sup>14</sup>.

A motor skill is a learned ability to cause a predetermined movement outcome with maximum certainty. The theme of motor skills is to optimize the ability to perform the skill at the rate of success, precision, and to reduce the energy consumption required for performance. Continuous practice of a specific motor skill will result in a much more improved performance. Motor learning is a relatively permanent skill as the capability to respond appropriately is acquired and retained<sup>15</sup>.

In this study, we expected that participants with a history of alcoholism would have impaired cognition and disparities in fine motor skills. We found that alcoholics performed worse than non-alcoholics. Similar results of executive functions and working memory deficits in alcoholism was reported by Parson OA (1998). The alterations found in alcohol-dependent subjects seem to represent diffuse brain damage and although they improve substantially during withdrawal, some deficits remain even years after the last alcohol ingestion.<sup>11</sup>

## CONCLUSION

The current study conclude that, chronic alcoholism decreases the temporal perception of cognition as defined by decreased CFFF and fine motor skills by slower ITI of finger tapping test.

**Limitations:** The current study probed into two domains of cognition that is temporal perception and motor coordination in alcoholics. The effect of alcoholism on other cognitive domains has not been tested. Recent studies on acute alcoholism reported improved perception. This study includes only chronic alcoholics and quantity could not be recorded.

**Conflict of Interest:** None declared

**Funding:** Self-funding

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# Blood Picture in Petrol Pump Workers

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## ABSTRACT

**Background:** Benzene is blended with petrol as an antiknock agent. As petrol evaporates during refilling vehicles, The air around petrol station contains higher level of Benzene. Absorption of Benzene by humans can occur through inhalation, ingestion and directly through skin. Due to long working hours, Petrol station workers are inhaling high volume of Benzene. They are also exposed to more pollutants emitted by the vehicles.

It is generally known, that hematopoietic tissues are sensitive targets of benzene toxicity. Benzene being genotoxic any chronic low level exposure correlates with the development of variety of blood disorders in humans, which range from, reduction in concentration of peripheral blood cells to Aplastic anemia, Pancytopenia, Lymphomas, acute Myelogenous leukemia and its variants. It can also result in increased blood cell count, chromosomal aberrations in lymphocytes.

Considering all the previous studies which have varied results in hematological parameters, we took up the study with below stated objective.

**Objectives of study:** To find any aberration in the complete blood cell count and to check for any morphological change in blood cells of petrol pump workers.

**Materials and Method:** Study group consisted of forty petrol pump workers within Bangalore metropolitan area, with high level of air pollutants .They were further divided into four groups according to their work experience correlating to duration of benzene exposure. They were also classified as smokers and non smokers. Written informed consent was taken. Thorough general physical examination was done. Subjects with complaints of infection and under any medications were excluded. 3ml intravenous blood was drawn in a EDTA coated tube and was analyzed by Beckman Coulter automated counter. Peripheral smear was stained with Leshman stain and observed at pathology lab of Victoria hospital, Bangalore.

**Results and Conclusion:** Reports obtained showed 43% subjects to be normal, 44% with Eosinophilia, 7% with Lymphocytic predominance, 3% with Anaemia and 3% with Neutrophillia.

Statistical analysis showed significant p value (<0.05) for lymphocyte count with duration of exposure.

Remaining blood cell count did not show any significant change with duration of exposure or in relation to smoking habit. The results obtained from present study indicated there is some amount of toxic effect of benzene and air pollutants on petrol pump workers. Improved detection and prevention technologies are needed to check the early changes and to improve the health conditions of the workers.

**Keywords:** Petrol Pump Worker, Benzene, Leucocytosis, Genotoxic

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Abbreviation: EDTA-Ethylene di amine tetra acetic acid

## INTRODUCTION

Benzene also called benzol is a colorless liquid with sweet odor. Small amount of benzene is found in

air, water and soil. Benzene is emitted from industrial as well as natural sources. Benzene is also an important industrial chemical and a component of petrol. The major source of human exposure to benzene are smoking tobacco, exhaust from motor vehicles and industrial emission. Usually humans are exposed to small amount of benzene; people living in cities are generally exposed to higher level of benzene than those in rural areas. <sup>(1)</sup>

Benzene is also blended with petrol as an antiknock agent. As benzene is very volatile, it can easily escape during vehicle refueling, the resulting vapor is 3 times heavier than air. <sup>(2)</sup>This increases benzene level in the air around petrol pumps. Air around petrol pump usually contains 100-250 ppb of benzene and also studies have detected high level of benzene in petrol pump workers. <sup>(3)</sup>

According to Bahrami AR et al studies, concentration of benzene in breathing zone of petrol station workers was 3 times higher than the threshold level (0.5ppm) recommended by American Conference of Governmental Industrial Hygienists (ACGIH). <sup>(4)</sup>

In India, the Central Pollution Control Board has established  $5\mu\text{gm}/\text{m}^3$  as an annual average ambient air quality standard concentration for benzene. However, studies conducted by Majumbar et al in Kolkata, Sehgal et al in Delhi, Singla et al in Agra, recognized high concentration of benzene, toluene and xylene at Petrol pump stations far exceeding those laid down by Indian National Ambient of Air Quality standards. <sup>(5)</sup>

Absorption of Benzene can occur through inhalation, ingestion and directly through skin. <sup>(2)</sup> Due to long working hours, Petrol station workers are inhaling high volume of Benzene. They are also exposed to more pollutants emitted by the vehicles at their working place.

Exposure to petrol vapors is identified by International agency for research of cancer (IARC) as possibly carcinogenic to humans, mainly on basis of well established carcinogenicity of Benzene component. <sup>(2)</sup>

It is generally known that hematopoietic tissues are sensitive targets of benzene toxicity. Benzene being genotoxic any chronic low level exposure correlates with the development of variety of blood disorders in humans, which range from, reduction in concentration of peripheral blood cells to Aplastic anemia, Pancytopenia, Lymphomas, Acute myelogenous leukemia and its variants. It can also result in increased blood cell count,

chromosomal aberrations in lymphocytes.

Considering all the previous studies which have varied results in hematological parameters, we took up the study with below stated objective.

## OBJECTIVES OF STUDY

To find any aberration in the complete blood cell count and to check for any morphological change in blood cells of petrol pump workers.

### Inclusion criteria

Male

Petrol pump worker

Minimum 1 yr of experience

Minimum 8 hrs work/day

Written informed consent

### Exclusion criteria

Any present infection

Any H/O renal disease

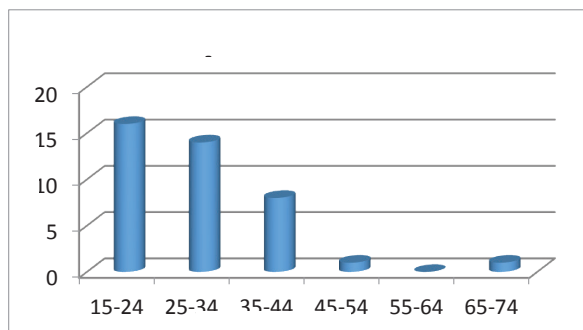
Any immune suppressant medications

## METHODOLOGY

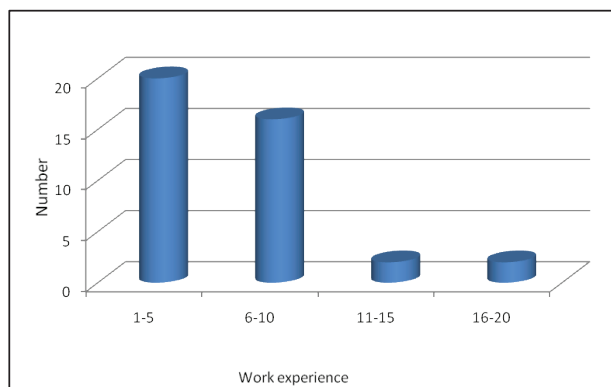
Study group consisted of forty petrol pump workers with in Bangalore metropolitan area, with high level of air pollutants .They were further divided into four groups according to their work experience which shows duration of exposure. They were also classified as smokers and non smokers. Written informed consent was taken. Thorough general physical examination was done. Subjects with complaints of infection and under any medications were excluded. 3ml intravenous blood was drawn in an EDTA coated tube and was analyzed by Beckman Coultee automated counter. Peripheral smear was stained with Lieshman stain and observed at pathology lab of Victoria hospital, Bangalore.

## RESULTS AND STATISTICAL ANALYSIS

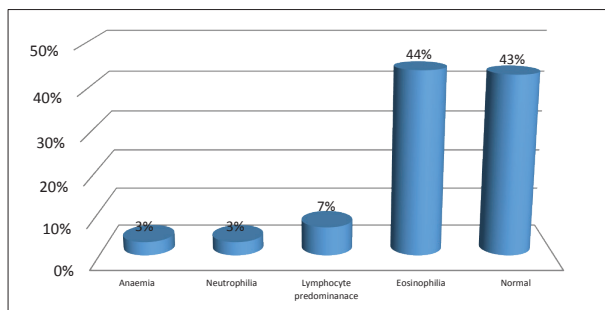
The difference in measured parameters and the normal values were evaluated using ANOVA. Statistical measures such as Mean, Standard deviation, percentages were implicated to describe and analyze the data. Statistically significant values were determined at  $P < 0.05$ . All data obtained was presented as mean  $\pm$ SD.



Graph 1: Showing age distribution of the subjects (P=0.6)



Graph 2: Work experience distribution of the study population



Graph 3: Report of the study sample

Table 1: Description of hematological parameters

PARAMETERS	TOTAL(mean ± SD)	REFERENCE <sup>(6)</sup>
WBC	8.5±2.1	7±3 (10 <sup>9</sup> /l)
RBC	5.02±0.5	5.0±0.5 (10 <sup>12</sup> /l)
HB	14.99±1.45	15±2 (g/dl)
HCT	0.43±0.38	0.45±0.05 (l/l)
MCV	83.7±14.3	92±9 (fl)
MCH	30.6±3.7	29.5±2.5 (pg)
MCHC	34.6±1.3	330±15 (g/dl)
PLT	236±51.2	150-400 (10 <sup>9</sup> /l)
NEUTROPHIL #	4.9±1.6	2-7 (10 <sup>9</sup> /l)
NEUTROPHIL %	57.8±9.4	40-80 %
LYMPHOCYTE #	2.7±0.8	1-3 (10 <sup>9</sup> /l)
LYMPHOCYTE %	32.5±8.5	20-40 %
MONOCYTE #	0.32±0.7	0.2-1 (10 <sup>9</sup> /l)
MONOCYTE %	2.7±2	2-10 %
EOSINOPHIL #	0.6±0.6	0.02-0.5 (10 <sup>9</sup> /l)
EOSINOPHIL %	6.8±6.7	1-6 %
BASOPHIL #	0	0.02-0.1 (10 <sup>9</sup> /l)
BASOPHIL %	0	< 2 %

Table 2 : Association between duration of exposure with relation to the hematological parameters

Variable	Exposure				P value
	1-5 years	6-10 years	11-15 years	> 15 years	
WBC	8.95 ± 2.6	7.89 ± 1.2	6.95 ± 1.1	9.85 ± 2.2	0.25 (NS)
RBC	5.08 ± 0.6	5.06 ± 0.4	4.65 ± 0.4	4.47 ± 0.6	0.2 (NS)
HB	15.32 ± 1.4	14.86 ± 1.4	12.9 ± 1.4	14.7 ± 1.8	0.1 (NS)
HCT	43.51 ± 4.2	43.4 ± 3.2	37.3 ± 0.7	42.35 ± 2.8	0.1 (NS)
MCV	85.96 ± 7.9	85.75 ± 4.7	80.15 ± 2.2	87.25 ± 4.6	0.6 (NS)
MCH	31.03 ± 4.1	29.77 ± 2.5	27.7 ± 0.7	36.55 ± 5.4	0.055 (NQS)
MCHC	34.8 ± 1.2	34.3 ± 1.4	34.6 ± 0.14	35.5 ± 1.4	0.5 (NS)
PLT	238.5 ± 43.5	233.9 ± 60.6	279 ± 19.8	183.5 ± 37.47	0.31 (NS)
NEUTROPHIL #	5.04 ± 1.9	4.66 ± 0.93	4.65 ± 1.20	6 ± 2.82	0.7 (NS)

**Cont.... Table 2 : Association between duration of exposure with relation to the hematological parameters**

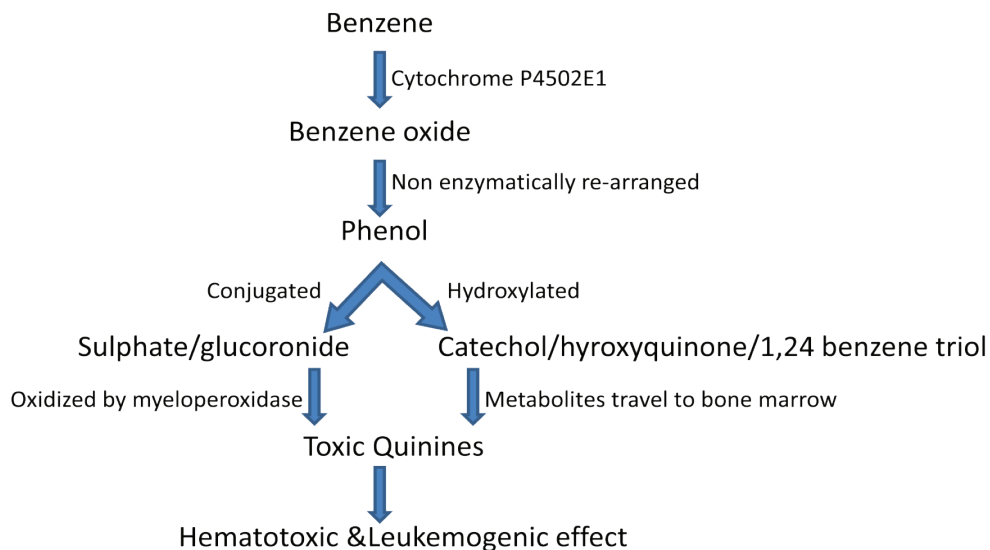
NEUTROPHIL %	55.65 ± 10.6	59.2 ± 6.8	67 ± 7.1	58.5 ± 16.3	0.4 (NS)
LYMPHOCYTE #	2.92 ± 0.75	2.46 ± 0.6	1.65 ± 0.2	3.3 ± 0.6	0.03 (S)
LYMPHOCYTE %	34.15 ± 9.3	31.37 ± 7	23.5 ± 0.7	35 ± 14.1	0.33 (NS)
MONOCYTE #	0.25 ± 0.2	0.44 ± 1.1	0.13 ± 0.1	0.2 ± 0	0.8 (NS)
MONOCYTE %	3.05 ± 2.3	2.5 ± 1.8	2 ± 1.4	2 ± 0	0.7 (NS)
EOSINOPHIL #	0.7 ± 0.7	0.5 ± 0.6	0.45 ± 0.5	0.4 ± 0.1	0.8 (NS)
EOSINOPHIL %	7.15 ± 6.7	6.6 ± 7.3	7.5 ± 7.8	4.5 ± 2.1	0.9 (NS)
BASOPHIL #	0	0	0	0	
BASOPHIL %	0	0	0	0	

Graph 3 shows 44% of subjects having Eosinophilia, 43% subjects were normal, 7% with Lymphocytic predominance, 3% with Anaemia and 3% with Neutrophilia. Table 2 shows statistically significant increase in Lymphocyte count with significant p value, it also correlates with increase in duration of exposure to benzene in the workplace. Table 1 and 2 shows all other blood counts which are insignificantly raised but not correlating with duration of exposure or their smoking status.

No morphological changes seen on peripheral smear examination.

## DISCUSSION

The precise mechanism by which Benzene induces carcinogenic effect in blood cells is unclear, but it is proved that conversion of Benzene to its metabolites is the reason behind its adverse effect.



Sensitivity to benzene toxicity may result from series of polymorphisms in enzymes which modulate the production of toxic Benzene metabolites; among all the enzymes involved in production of benzene metabolites, polymorphism of CYP2E1 is a critical one. There is a direct correlation of high activity of CYP2E1 with increased Benzene metabolites which in turn increases the impact on individual. <sup>(7)</sup>

## CONCLUSION

Results obtained from present study shows that there is some amount of toxic effect of Benzene and air pollutants on petrol pump workers. Complete blood count may be useful in detection of early hematological changes among petrol pump workers who are exposed to benzene vapors.

Improved detection and prevention technologies are needed to check the early changes noted in the study and to improve the health conditions of the workers.

**Conflict of Interest:** Nil

**Source of Funding:** Self

**Ethical Clearance:** Institutional ethical committee

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# Iron Deficiency Anemia in Adolescent School Girls from Low Socio-economic Background and their Cognitive Achievement

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## ABSTRACT

**Introduction:** Iron Deficiency (ID) is the most common cause of nutritional anemia and the prevalence of Iron Deficiency Anemia (IDA) in India is highest in the world. Adolescent females from low socio-economic background are more at risk of developing IDA due to greater growth demands, nutritional deficiencies, infections with parasites and due to menstrual blood loss. IDA can lead to many hematological disturbances, decreased physical work capacity and impaired cognitive functions. **Material and Method:** This study was done on adolescent female school girls of 12-15 years age from a government school to find out the prevalence of IDA, to assess the relation between ID and cognitive functions and to know whether treatment with oral iron supplementations for 3 months will reduce the number of anemic students and improve their cognitive functions. Their Complete Blood Picture and Serum Ferritin levels were determined. The scholastic performance assessment and the cognitive functions were tested. The participants were divided into 2 groups:

**Group I:** Anemic (Hb < 12 gm %) and ID (serum ferritin < 12 µg/L) students.

**Group II:** Non-anemic (Hb > 12 gm %) and non-iron deficient (serum ferritin > 12 µg/L). **Results:** In our study, prevalence of IDA was found to be 67 %. In the cognitive tests, the scores of attention and concentration, verbal retention for similar parts, verbal retention for dissimilar parts, recognition were significantly less in group I compared to group II. There was no significant difference in the scores of recent memory, remote memory between the two groups. **Conclusion:** The results of this study indicate that ID definitely and independently affects the cognitive functions. Supplementation with iron for 3 months in these students led to reduction in the number of anemic students by 50% and rapid improvement in the scholastic performance and cognitive functions.

**Keywords:** Iron deficiency anemia, adolescent females, low socio-economic level, serum Ferritin levels, cognitive functions,

## INTRODUCTION

Anemia is a common disorder in which Red blood cells are reduced in number or deficient in Hemoglobin leading to reduced oxygen carrying capacity of blood<sup>1</sup>.

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Iron Deficiency (ID) is the most common cause of nutritional anemia in India. Iron Deficiency Anemia (IDA) is a condition where one has inadequate amounts of iron to meet body demands due to diet deficient in iron or poor bio-availability of iron in food especially during periods of rapid growth or due to chronic blood loss. People at risk are young female adolescent children particularly in developing countries from poor socio-economic status who form a vulnerable group due to greater growth demands, nutritional deficiencies, infections due to parasitic infestations<sup>2</sup>, and exacerbated a few years later due to menstrual blood loss<sup>3</sup>. In India,



prevalence of IDA ranges between 60-70% among the adolescent girls living in rural areas<sup>4</sup>.

IDA causes easy fatigability, reduced resistance to infection, impaired physical growth and can affect cognitive performance<sup>5</sup>. The reduced physical work capacity also leads to poor academic performance is due to less availability of oxygen to the tissues due to less hemoglobin leading to reduced cardiac output<sup>6</sup>. Among the cognitive impairments caused by ID, those referring to attention span, intelligence, sensory perception functions are mainly cited as well as those related to emotions and behavior<sup>7</sup>. Experimental studies show that ID is capable of causing cognitive impairments in animals and humans with brain mitochondria damage as a basis for these alterations.<sup>8</sup>

However, little is known about the impact of IDA on cognitive functions in adolescent girls.

It is suggested that early detection, treatment with iron therapy and proper educational intervention, the functional changes get corrected within few days significantly even before there is significant rise in hemoglobin and reduces morbidity from IDA and will restore proper health helping them in better and efficient academic performance.

### **OBJECTIVES OF THIS STUDY**

- To determine prevalence of anemia in adolescent female school children.
- To assess the effect of iron deficiency on cognitive functions in anemic iron deficient adolescent girls.
- To know whether treating the anemic, ID girls with iron and folic acid supplements for 3 months will bring about a reduction in anemic patients by 50% and improvement in cognitive functions.

### **MATERIALS AND METHOD**

This study was conducted on 100 female adolescent school children of age group 12-15 years studying in a government High school in a rural area in Hyderabad. Ethical committee clearance and Written consent was obtained from the Head of the school and parents of the students to carry out the study. A pre-validated standard questionnaire was distributed to the students to be

filled under their parent's guidance. The questionnaire addressed detailed dietary history, socio-economic status, family size and details about menstrual history. Venous blood samples were collected and their Complete Blood Picture using automated cell counter. Serum Ferritin levels were measured using ELISA method.

The scholastic performance was assessed from mathematics score of these students. For measuring the cognitive functions, one multicomponent test was done for testing memory, attention and concentration which consisted of 10 subtests: 1. Recent memory 2. Remote memory 3. Mental balance 4. Attention and concentration 5. Delayed recall 6. Immediate memory 7. Verbal retention for similar pairs 8. Verbal attention for dissimilar pairs 9. Visual retention 10. Recognition.

The data obtained was statistically analyzed using t-test to compare means using SPSS software. Odds ratio and 95% confidence limits were calculated using multiple logistic regression analysis. All variables with p values <0.05 were considered as statistically significant.

The participants after screening were divided into 2 groups:

**Group I:** Students who were anemic (Hb < 12 gm%) and iron deficient (serum ferritin < 12 µg/L)

**Group II:** Students who were non-anemic (Hb > 12 gm %) and non-iron deficient ( serum ferritin > 12 µg/L)

Students who were not willing were excluded from the study. Among group I students, the severity of anemia was graded as mild (Hb 10-12 gm/dl), moderate (Hb 8-10 gm/dl) and severe anemia (Hb < 8 gm/dl).

Counselling and health education to group I students and their parents was done to motivate them in taking food rich in dietary Iron, maintain personal hygiene and to explain the essential role of iron supplementations in treatment of Anemia. Problems resulting from ID in the forthcoming life was also explained to them. The schools were visited fortnightly for supply of oral iron supplements for 3 months to group I students. At the end of 3 months, Complete Blood Picture with blood cell indices, and Serum Ferritin levels were determined using venous blood samples and the cognitive tests were done once again and the data statistically analyzed and compared with the previous one.

## RESULTS

Out of 100 female adolescent students in the age group of 12-15 years that participated in the screening test, 67 female students were detected with Anemia with hemoglobin <11.5 gm/dl with serum ferritin levels < 12 µg/L, who were considered to be group I. So the prevalence of IDA was 67%. The mean hemoglobin in group I was 10.67 gm/dL and group II students was 12.46 gm/dl and the mean Serum Ferritin levels in group I were 8.62µg/L and group II were 22.27µg/L ( table 1).

The mean age group of study participants was 13±2 years. Their complete blood picture showed microcytic, hypochromic picture in group I.

Among Group I, which included 67 students, many (57%) of the students were Vegetarian and 43 % Non-vegetarians. There was a significant association between anemia and vegetarian diet( $p<0.05$ ).

A Major portion(61%) of the students in Group I were from low socio-economic status, 28% from middle socio-economic status and 10% from high socio-economic status showing a significant association between the incidence of anemia and low socio-economic status ( $p<0.05$ )(table 2).

Among group I, Majority of the students(64%) were suffering from mild anemia (Hb levels between 10 and 12 gm% ) and 32% of them had moderate anemia( Hb levels between 8 and 10 gm % )and only 4% of them were having severe anemia (Hb levels below 8 gm %) (table 3).

42 % of the study students had dysmenorrhea with heavy periods. These findings suggest that there is a significant association between anemia and heavy flow during periods( $p<0.05$ ).

Students with IDA had greater than twice the risk of scoring below average in mathematics than the normal subjects. The difference in mathematics score was highly significant( $p<0.05$ ) between group I and group II students (table 4).

The cognitive scores of multicomponent test was assessed and the scores of attention and concentration, verbal retention for similar parts, verbal retention for dissimilar parts, recognition were significantly less in group I compared to group II. There was no significant difference in the scores of recent

memory, remote memory between the two groups(table 5).

After 3 months of taking oral iron supplements and iron rich diet, the study revealed a decrease in the incidence of anemia in the subjects of group I by 52% which shows the success of the program(table 6). There was improvement in the performance of students in mathematics and the scores improved. There was improvement in the scores of attention, verbal communication.

**TABLE 1: Number of students in different Groups, Mean serum ferritin levels and Hemoglobin levels**

Groups	No. of students	Mean serum ferritin(µg/L)	Mean Hb levels(gm/dl)
Group I	67	8.62	10.67
Group II	33	22.27	12.46

**Table 2: Prevalence of Anemia depending on socio-economic status among the subjects(n=67)**

Socio-economic status	No. of students	Percentage(%)
Low socio-economic group	41	61
Middle socio-economic group	19	29
Upper socio-economic group	7	11

**Table 3: Severity of anemia among subjects(n=67)**

Severity of anemia	No. of students	Percentage(%)
Mild	43	64
Moderate	21	32
Severe	3	4

**Table 4: The mean mathematical score of the students in different groups**

Groups	Mean Mathematics score
Group I (n= 67)	41.76± 6.36
Group II (n=33)	73.15± 5.92

**Table 5: The scores of multicomponent test for cognition in Group I and Group II**

Test	Group I Anemic, iron deficient N= 67 Mean ± SD	Group II N= 33 Non-anemic ,Non- iron deficient Mean± SD
Recent memory	4.37 ±0.13	4.59± 0.22
Remote memory	5.76± 0.53	5.60±.72
Mental balance	1.67 ±0.56	3.05± 0.74
Attention and concentration	11.28 ±3.76	17.2± 2.14
Delayed recall	8.36± 2.21	9.12 ±1.46
Immediate recall	1.86 ±0.74	1.94 ±0.73
Verbal retention for similar pairs	4.2 ±0.68	4.93± 0.42
Verbal retention for dissimilar pairs	4.3± 0.74	4.38±0.59
<b>Visual retention</b>	2.36 ±1.28	2.95± 1.36
Recognition	7.68 ±1.64	9.25 ±2.34

**Table 6: Result of Interventional study on incidence of anemia(n=67)**

Groups	No. of students	Percentage(%)
Anemic	16	24
Non-anemic	51	76

## DISCUSSION

The main aim of this study was to determine the prevalence of anemia in adolescent females in rural areas which was found to be 67%. Seshadri et al<sup>9</sup> reported a similar prevalence of 60% and 63% respectively. Anemia in this age group is identified as an important health problem by Demayer.<sup>10</sup>

In our study, among anemic adolescents, the

proportion of mild anemia (64%) was high, followed by moderate anemia (32%) and severe anemia (4%). This finding is in parallel to studies done by Saratha A et al in 2010 in Pondicherry<sup>11</sup> and Gupta A et al 2012 in Simla<sup>12</sup>. These results indicate that there is increased prevalence of mild anemia is due to increased nutritional deficiency in these areas. The blood picture of the study participants showed microcytic hypochromic anemia. There is scientific evidence that physical and physiological changes occurring in adolescent girls place a great demand on their nutritional requirements and make them more vulnerable to nutritional deficiencies due to ID and blood loss during menstruation.<sup>12</sup>

Further, majority of the students (61%) of group I were from low socio-economic status and 29% of students belonged to middle socio-economic group and only 11% of students belonged to high socio-economic group. These results indicate that the prevalence of anemia is more common in the low socio-economic status of the study group. Lower Socio-economic status plays a leading role in determining the nutritional status of the students and higher prevalence of anemia in them due to lack of money, lack of knowledge and poor availability of iron in their diet. These results are comparable to the results of the study done by Rawat et al<sup>13</sup> on adolescent girls in rural area of Meerut.

Verbal learning, attention span and concentration are most important among the cognitive functions for academic performance. In our study, anemic iron deficient adolescent girl students scored less marks in mathematics compared to non-anemic students. These results are in accordance with the study results of Prestonjee<sup>14</sup> where iron deficient adolescents both anemic and non-anemic had lower mathematical scores compared to adolescents with normal iron stores.

The scores of multicomponent test of verbal learning, attention, and mental balance was less in group I compared to group II. There was no difference in scores of recent memory and remote memory, delayed and immediate recall between group I and group II students. These findings are in accordance with the findings of study done by Bruner et al<sup>15</sup> where baseline cognitive functions were assessed on non-anemic iron deficient adolescent girls suggesting that iron deficiency even in absence of anemia can cause decrease in at least some aspects of cognitive functions. Lower performance in cognitive tests in group I students is strongly related to

lower hemoglobin levels leading to decreased delivery of iron to the host tissues including the central nervous system. ID is associated with many metabolic processes that impact brain functioning like mitochondrial electron transport, neurotransmitter synthesis and degradation, protein synthesis and organogenesis. There is evidence that ID during growth spurt alters metabolism and neurotransmission, myelination and gene and protein profiles<sup>16</sup>. Cognition is seen to be adversely affected in anemic students of both lower and high socio-economic groups.

After 3 months of treatment with oral iron supplements, there was significant improvement in scores of mathematics, and also improvement in the scores of other cognitive tests like attention, verbal learning and concentration in group I students. This is because some cognitive functions become better with iron supplementation. This is in accordance with the study done by Sen and Kanani<sup>17</sup>, present evidence that iron and folic acid supplementation in children between 9 and 13 years leads to modest but significant improvement in the various cognitive tests.

### CONCLUSION

According to our study, IDA is prevalent more among adolescent females in rural areas of low socio-economic group (67%). Most of the students had mild anemia and were vegetarians. The anemic students had low scholastic performance in the form of low scores in mathematics and low scores in cognitive tests like verbal learning, mental balance, attention and concentration when compared to the non-anemic students of the same age group. This indicates that ID definitely and independently affects the cognitive functions. Supplementation with iron for 3 months in these students led to reduction in the number of anemic students by 50% and rapid improvement in the scholastic performance and cognitive functions.

There is definitely a need for initiation of such programs for supplementation of iron and folic acid to anemic and iron deficient school going adolescent females for prevention of disturbances in hematological and cognitive functions.

**Conflict of Interest:** Nil

**Source of Funding:** Self

**Ethical Clearance:** Taken and consent taken from the participants and their parents for participation in the study.

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# Study of Biochemical Parameters in Primary Hypertension

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## ABSTRACT

Primary hypertension is increasingly common and is associated with significant morbidity. Studies suggest that salt may increase blood pressure not simply by effects on extracellular volume but rather as a consequence of hyperosmolarity. Dietary NaCl intake is related to hypertension prevalence and the age related increase in blood pressure may be augmented by a high NaCl intake. Low dietary calcium and potassium may also contribute to the risk of hypertension.<sup>1</sup>

**Aims and Objectives:** The aim of the present study is to compare and analyse the relation between blood pressure and biochemical parameters in hypertensives and normotensives in Kalaburagi so that it will be a useful predictor of cerebrovascular diseases and coronary artery disease through regular investigations.

**Materials and methods:** The study is undertaken in 50 hypertensives taken as subjects and 50 normotensives taken as control during the academic year 2015-2016 KBNIMS, Kalaburagi with the age range being 40- 60 yrs. Ethical committee clearance was taken and consent obtained from all the study subjects. Blood pressure was recorded in the sitting posture in the right arm using mercury sphygmomanometer using both palpatory and auscultatory method. Three readings are taken and the average of the three recordings are obtained. Serum electrolytes and serum calcium are estimated using reagent assay kits from transasia Biomedicals Ltd semiautomated analyser.

**Results and conclusion:** In the present study, the mean values of Serum Sodium, Serum Chloride and Serum Uric acid are higher with more significance ( $p < 0.01$ ) while the mean values of Serum Potassium and Serum Calcium are found to be lower in hypertensives with a higher significance. ( $p < 0.01$ ). Thus, it can be concluded that in patients of primary hypertension, significant changes are seen in serum electrolytes, serum calcium and serum uric acid which can be used for early detection of hypertensive prone individuals.

**Keywords :** Hypertension, Coronary artery disease.

## INTRODUCTION

Hypertension is reported to be the fourth contributor to premature death in developed countries and seventh in developing countries. Recent reports indicate that nearly 1 billion adults had hypertension in 2000 and this is predicted to increase to 1.56 billion by 2025. Earlier reports also suggest that the prevalence of hypertension

is rapidly increasing in developing countries and is one of the leading causes of death and disability in developing countries<sup>2</sup>.

## AIMS AND OBJECTIVES

The aim of the present study is to compare and analyse the relation between blood pressure and biochemical parameters in hypertensives and normotensives in Kalaburagi so that it will be a useful predictor of cerebrovascular diseases and coronary artery disease through regular investigations. Identification of these high risk patients may allow an earlier introduction of antihypertensive treatment and correction of the risk factors to prevent the progression or to induce the

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regression of silent vascular damage before a clinical event develops by instructing change in the lifestyle or prescribing medications or both.

**Materials and Methods:** The study is undertaken in 50 hypertensives taken as subjects and 50 normotensives taken as control during the academic year 2015-2016 KBNIMS, Kalaburagi with the age range being 40- 60 yrs. Ethical committee clearance was taken and consent obtained from all the study subjects. Blood pressure was recorded in the sitting posture in the right arm using mercury sphygmomanometer (Diamond deluxe BP apparatus , Pune, India) using both palpatory and auscultatory method. Three readings are taken and the average of the three recordings are obtained. Blood samples are collected after an overnight fasting from antecubital vein by making the subject to sit comfortably

on a chair. About 4 ml of blood is collected in vacutainers through disposable syringe under aseptic precautions and allowed to stand for 30 min at room temperature to allow complete clotting and clot retraction. Samples are centrifuged at 2000 g for 15 mins and readings are taken using the Semi automated analyser. [Erba Mannheim]. Serum electrolytes and serum calcium are estimated using reagent assay kits from transasia Biomedicals Ltd.

**Result and Discussion:** The data is analysed and all values are expressed as Mean  $\pm$  standard deviation. Statistical significance of differences between control and study groups are evaluated by student “ t ” test . A p- value of  $< 0.05$  is considered to be statistically significant and p- value of  $< 0.01$  is considered to be highly significant.

**The mean biochemical values are presented in the Table 1**

Parameter	Normotensive (MEAN $\pm$ SD )	Hypertensive ( MEAN $\pm$ SD )	P value	Significance
Sodium	138.44 $\pm$ 6.39	142.94 $\pm$ 5.65	$< 0.01$	HS
Potassium	4.53 $\pm$ 0.49	4.10 $\pm$ 0.64	$< 0.01$	HS
Chloride	97.28 $\pm$ 3.74	100.08 $\pm$ 3.92	$< 0.01$	HS
Calcium	9.41 $\pm$ 0.94	8.85 $\pm$ 0.54	$< 0.01$	HS
Uric acid	4.87 $\pm$ 1.00	5.60 $\pm$ 1.05	$< 0.01$	HS

HS- Highly significant

The mean value of Serum Sodium in hypertensive group is found to be 142.94 mg/dl (SD  $\pm$  5.65), while in normotensive controls it is found to be 138.44 mg/dl (SD  $\pm$  6.39).

The mean value of Serum Potassium in hypertensive group is found to be 4.10 mg/dl (SD  $\pm$  0.64), while in normotensive controls it is found to be 4.53 mg/dl (SD  $\pm$  0.49).

The mean value of Serum Chloride in hypertensive group is found to be 100.08 mg/dl (SD  $\pm$  3.92), while in normotensive controls it is found to be 97.28 mg/dl (SD  $\pm$  3.74).

The mean value of Serum Calcium in hypertensive group is found to be 8.85 mg/dl (SD  $\pm$  0.54), while in normotensive controls it is found to be 9.41 mg/dl (SD  $\pm$  0.94).

The mean value of Serum Uric acid in hypertensive group is found to be 5.60 mg/dl (SD  $\pm$  1.05), while in normotensive controls it is found to be 4.87 mg/dl (SD  $\pm$  1.00).

The mean values of Serum Sodium, Serum chloride and Serum Uric acid are higher in hypertensive group with a higher significance.

The mean values of Serum Potassium and Serum Calcium are lower in hypertensive group with a higher significance.

## DISCUSSION

In the present study, the mean values of Serum Sodium and Serum Chloride ( **Figure-1 & 2**) are found to be significantly higher in hypertensive group.

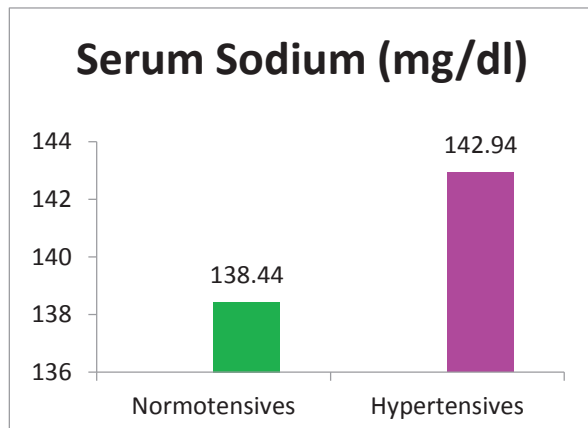


Figure-1

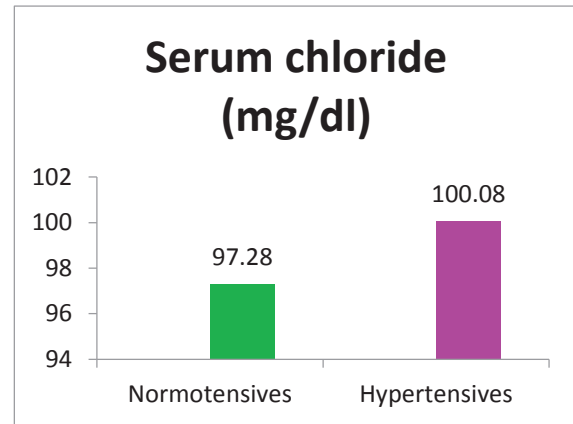


Figure-2

These findings are similar to the findings reported by Christopher J. Bulpitt<sup>3</sup>, A.F. Lever et al<sup>4</sup>, Esa Soppiu<sup>5</sup>, Eduardo Pimenta<sup>6</sup>, Hans Oberleithner et al<sup>7</sup> and Al-Muhana et al<sup>8</sup>.

Hans Oberleithner et al studied on how a small increase in plasma sodium concentration may contribute to the pressor response of dietary salt. According to the authors, there is evidence that plasma sodium concentration may be a determining factor in the development of hypertension. Sodium and chloride are positively related. Sodium accumulates in the extracellular space either when the kidneys cannot adequately adjust salt excretion to salt uptake and/or the concentration of aldosterone is raised. Salt can be stored, osmotically inactive, in the skin and other extracellular compartments, but the sodium storage capacity of such compartments is limited, particularly in hypertensive animals receiving mineralocorticoids. Thus, a limited ability to store sodium, a raised concentration of aldosterone, or a renal inadequacy to excrete sodium effectively may lead to an increase in plasma sodium concentration. A rise in plasma sodium has indeed been observed in hypertensive individuals. Such elevation of plasma sodium concentration is small (only a few mmol) presumably because a rise in plasma osmolality is accompanied by water retention. The results demonstrate that small acute changes in plasma sodium concentration have a significant effect on the stiffness and deformability of endothelial cells.

The endothelium is a metabolically active “organ” able to release a number of vasodilator factors, including nitric oxide. Thus, mechanical stress causes nitric oxide synthase activation, leading to nitric oxide release and

vasodilation. For example, large rhythmic deformations of the highly elastic endothelial cells *in vivo*, which are induced by shear stress, stimulate nitric oxide metabolism. In a study, by mimicking a shear stress situation *in vitro*, it was observed that high extracellular sodium concentration in conjunction with aldosterone stiffens endothelial cells and reduces nitric oxide release from the vascular endothelium<sup>9</sup>.

The present study suggests that such small changes in plasma sodium could have a large impact on endothelial function. Already a change of plasma sodium concentration of 5mmol, which still is considered to be in the physiological range, causes a change in endothelial stiffness by  $\approx 10\%$ . It is possible therefore that maintaining plasma sodium concentration and aldosterone concentrations in the low physiological range helps to keep arterial blood pressure normal and prevents vascular and end organ damage<sup>7</sup>.

## POTASSIUM

In the present study, the mean values of Serum potassium are found to be significantly lower in hypertensive group ( **Figure- 3**).

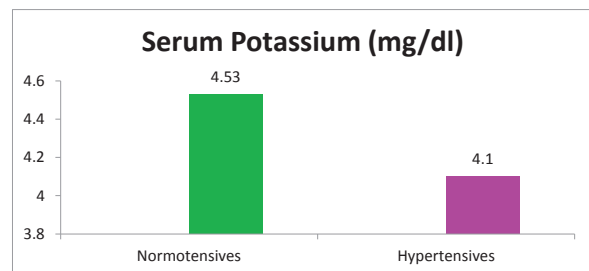


Figure-3

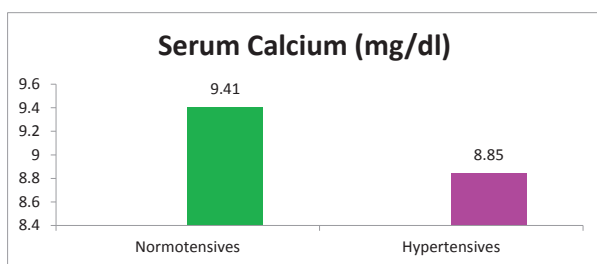
Based on the present study results, it can be concluded that serum potassium level is positively correlated with increase in blood pressure in primary hypertensives.

Christopher J. Bulpitt studied on sodium excess or potassium lack as a cause of hypertension. Summarised a very low sodium intake may prevent hypertension, a very high intake may directly cause hypertension <sup>3</sup>.

A F Lever carried out study of arterial pressure and body content of electrolytes in patients with essential hypertension and normal controls and concluded that two mechanisms probably operate in essential hypertension. In the early stages of the disease blood pressure is raised by an abnormal process related more closely to potassium than to sodium<sup>4</sup>.

John G F Cleland et al observed hypertensive subjects have similar mean values for body element composition as normal subjects. Potassium, a largely intracellular electrolyte, and sodium, much of which is present in bone and so not freely exchangeable with the extracellular pool, were poorly related to serum concentrations <sup>10</sup>.

**CALCIUM:** In the present study, the mean levels of Serum calcium are found to be significantly lower in the hypertensive group ( **Figure- 4**).



**Figure-4**

The present study shows that the levels of serum calcium are inversely related to blood pressure and decreased serum levels of calcium are seen in primary hypertensives.

These findings are similar to the findings reported by K. Sudhakar et al <sup>11</sup>, Paul Elliot <sup>12</sup> and Al- Muhana et al <sup>8</sup>.

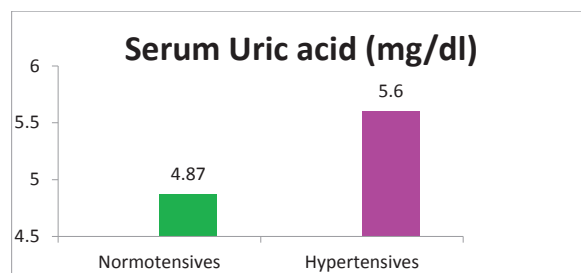
Alterations in intracellular calcium are thought to be involved in the common pathway mediating the secretion and action of many hormones, including the

pressor action of catecholamines and angiotensin II. Intracellular calcium may be involved in regulation of blood pressure. Calcium regulating hormones like 1, 25 dihydroxy vitamin D, levels of plasma renin activity, circulating ionised calcium contribute to the pathophysiology of essential hypertension <sup>11</sup>.

Reichel et al also reported reduced calcium in males with elevated diastolic blood pressure <sup>13</sup>.

However Kosch et al did not find any change in serum calcium levels in hypertensives <sup>14</sup>.

**Serum uric acid:** In the present study, the mean values of Serum uric acid are found to be significantly higher in the hypertensive group ( **Figure- 5**).



**Figure- 5**

The present study shows positive relation between serum uric acid and blood pressure in primary hypertensives.

Breckenridge estimated the incidence of hyperuricaemia in patients, and attempted to find the mechanism of the raised serum uric acid, concluded the cause of hyperuricaemia appears to be a defect in renal tubular handling <sup>15</sup>.

This association suggests that serum uric acid may be considered a marker for the presence of an adverse cardiovascular risk factor profile and that improvement of this profile by whatever means may also influence serum uric acid concentration <sup>16</sup>.

Elevated uric acid also predicts and is commonly present in early hypertension, and pilot studies have suggested that lowering uric acid may lower blood pressure in new onset hypertensive subjects <sup>17</sup>.

## SUMMARY AND CONCLUSION

In the present study, the variables found significant can be suggested as predictors of hypertension. The

results are consistent with others in that age and baseline systolic blood pressure are the strongest determinants of hypertension incidence.

The sodium and chloride are found to be significantly higher in the hypertensive group. Sodium is a risk factor for hypertension. Sodium stiffens vascular endothelium and reduces nitric oxide release. Excess sodium intake induces hypertension by increasing fluid volume and preload, thereby increasing cardiac output. Sodium excess may increase blood pressure in multiple other ways as well; affects vascular reactivity and renal function.

Potassium is found to be decreased in hypertensives. Potassium softens vascular endothelium and increases nitric oxide release. It is generally accepted that high sodium intake raises the arterial blood pressure whereas high potassium intake has the opposite effect. An increase of extracellular potassium concentration significantly diminishes the stiffness of endothelial cells and improves the release of NO.

Calcium is one of the risk factor for hypertension and cardiovascular diseases. Calcium regulating hormones like 1,25 dihydroxy vitamin D, levels of plasma renin activity, circulating ionised calcium contribute to the pathophysiology of essential hypertension. The free intracellular calcium concentration determines the tension in vascular smooth muscle cells, thereby resulting in peripheral vascular resistance. Calcium has a direct effect on peripheral vascular tone. Alterations in intracellular calcium are thought to be involved in the common pathway mediating the secretion and action of many hormones, including the pressor action of catecholamines and angiotensin II. Intracellular calcium may be involved in regulation of blood pressure.

Uric acid is increased in hypertensives. Increase in uric acid levels can be mainly due to impaired renal functioning. Chronic elevation in uric acid can inhibit endothelial function, activate the renin-angiotensin system, and cause blood pressure elevation in animals. Lowering uric acid not only prevents these hemodynamic and histological changes but also prevents most of the features of the metabolic syndrome, including the development of hypertension, hyperinsulinemia, hypertriglyceridemia and obesity.

From the above study, it can be concluded that in patients of primary hypertension, significant changes are seen in serum electrolytes, serum calcium and serum uric acid which can be used for early detection of hypertensive prone individuals.

**Source of Funding:** Self

**Conflict of Interest:** Nil

**Ethical Clearance:** obtained from institutional ethics committee of KBNIMS, Kalaburgi, Karnataka

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# Stress Levels and Heart Rate Variability (HRV) in Police Personnel

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## ABSTRACT

**Background:** Stress is inherent in uniform services. Police officers experience frequent and ongoing stressors in their work. **Objectives:** The objective of this study was to correlate between stress levels and heart rate variability in police personnel. **Method:** This was a cross-sectional study involving 150 police personnel of same cadre and age group. The study was conducted to measure different aspects of stress using various validated questionnaire such as stress indicators questionnaire, Perceived Stress Scale (PSS) and Operational Police Stress Questionnaire (PSQ-Op). The correlation between Heart Rate Variability [High Frequency (HF), Low Frequency (LF), and HF to LF ratio] and stress levels was observed by measuring the HRV and then comparing it with the stress levels that were calculated by the results of questionnaire. **Results:** The study's result showed that the majority (more than 50%) of subjects were under high stress and all the subjects were under high stress due to their occupation that is police job. Subjects with less stress showed more of parasympathetic activity that is HF was high whereas subjects with more stress showed more of sympathetic activity that is increased value of LF. **Conclusion:** The physical, environmental, occupational, personality traits are related to the level of stress in Police Personnel.

**Keywords:** Police Personnel, Stress, Heart Rate Variability.

## INTRODUCTION/BACKGROUND

Stress can be described as a circumstance that disturbs, or likely to disturb, the normal physiological or psychological functioning of a person. Walter Cannon demonstrated that stimulation of Autonomic Nervous System (ANS), particularly the sympathetic system, readied the organism for, fight and flight response characterize by hypertension, tachycardia and increased cardiac output. This was useful in animal that could flight or flee, but in person who could do neither by virtue of being civilized, the ensuing stress resulted in disease.<sup>1</sup> Police officers play a very significant role for maintaining law and order in the society despite all the shortcomings and limitations in the department especially with regard to the infrastructure facilities, manpower and periodic training.

Possible Sources of Psychological Stress in Police work itself: role conflict (e.g. apprehension of criminals while needing to maintain their rights), irregular work schedule, danger inherent in the profession, inability to resolve and close many problems in the community,

witnessing human suffering, seriousness of the consequences of one's actions, critical incidents such as shootings or mass disasters, public perceptions that may be inaccurate.

There have been several studies conducted on officers in different parts of the world and some in India, which reveal that the present working conditions and several other factors have led to increased level of stress among officers. A study by Rakesh Kumar Singh in 2007 conducted on Central Reserve Police Force (CRPF) found that the situation becomes worse because of environmental factors like poor living conditions, odd and prolonged duty hours, toxic leadership and negative image in society and media.<sup>2</sup> Another study conducted by Sibnath Deb and others in 2005 on West Bengal police officers found that powerlessness, poor peer relations, low status, strenuous working conditions, unprofitability and political pressures are main causes of stress.<sup>3</sup>

The above studies show that there is substantial awareness of stress and its related problems affecting



police personnel. But very few studies have done to show the actual effect of the stress of physiological functions.

HRV is the degree of variation of heart rate during the day under the balanced influence of sympathetic and parasympathetic component of the cardiac autonomic nervous system. It expresses the total amount of variation of both instantaneous heart rate and RR intervals. HRV also indicate the extent of neuronal damage to autonomic nervous system. HRV has been shown to be associated with high predictive value in many diseases where a disturbance in the autonomic activity is likely, for example in conditions like cardiovascular diseases, metabolic diseases like diabetes mellitus, neurological diseases like Parkinson's disease, gastrointestinal diseases like irritable bowel syndrome, chronic respiratory diseases like asthma, certain infections, neoplasia, surgeries like vagotomy and prognosis of disease.<sup>4,6</sup> In HRV analysis, low-frequency (LF) power depict sympathetic activity whereas total power (TP), high-frequency (HF) power reflect parasympathetic activity, and ratio of LF to HF power (LF/HF ratio) represents sympathovagal balance.<sup>7</sup>

## OBJECTIVES

The objective is to find out the cause, level of stress and its effect on both mental and physical health of police personnel. And to compare between stress levels and heart rate variability in police personnel.

## MATERIALS AND METHODOLOGY

The study was approved by the Institutional Research Ethical Committee (Human) of Navodaya Medical College, Raichur, Karnataka.

**Study design:** Cross sectional comparative analytical study.

**Study sample:** A sample of 150 apparently healthy male police personals aged between 20-40 years with job experience of 5-10 years from police stations of Raichur was recruited as subjects. The sample was taken from cadre of constable. Smokers, alcoholics and subjects with any systemic diseases were excluded from the study.

Age, sex and anthropometrically matched 50 males were recruited as controls.

## Method of collection of data:

Standard data collection procedures were followed in this study. At first, permission from the Raichur Police commissioner was taken. Then, this permission letter was shown to the Officer in Charge (OC) of the police stations. For taking consent police personnel was briefed about the general purpose of the study and requested to cooperate with the researcher. The OC was also informed that the investigation is purely academic and their responses to the questionnaire would be kept confidential.

## Questionnaires

The questionnaires were broadly classified into 3 groups to assess different stress indicators, Perceived Stress and stress levels. Following are the set of questionnaire that were distributed among the participants

### 1. Stress Indicators Questionnaire<sup>8</sup>

This questionnaire indicates the most affected aspect of life by stress. This questionnaire is in form of scale grading from 1-5 from almost always to never. The score will be totalled. It has sub parts which includes a range of score depicting the stress levels from very low, medium, high, very high, and danger.

#### Types

- Physical indicators consisting of 12 questions,
- Sleep indicators consisting of 5 questions,
- Behavioural indicators consisting of 17 questions
- Emotional indicators consisting of 20 questions
- Personal habits consisting of 9 questions.

### 2. Stress Assessment by Perceived stress scale<sup>9</sup>

The Perceived Stress Scale (PSS) is a classic stress assessment instrument. This tool, while originally developed in 1983, remains a popular choice for helping us understand how different situations affect our feelings and our perceived stress. This question in this scale asks about feelings, thoughts during last month. In each case, it is asked to indicate how often the participant felt or thought certain way. Although some questions are similar, there are differences between them.

PSS consists of 10 questions, each asked to answer in a scale ranging from 0-4 from never, almost never, sometimes, fairly often to very often respectively. The scores were added and a range of 0 to 40 indicates higher perceived stress.

### 3. The Operational Police Stress Questionnaire (PSQ-Op)<sup>10</sup>

The Operational Police Stress Questionnaire is been developed exclusively for police to assess occupational stress by evaluating their response to 20 questions, which has to be answered according to the scale given ranging from 1-7- from no stress at all to a lot of stress. The score is added and the participants score is compared to the range to assess the stress levels.

#### Heart Rate Variability

By using power lab 8/30 series with dual bio amplifier (AD instruments, Australia, model no ML870) ECG was recorded for 5 min in supine position. HRV analysis was done by low frequency(LF) power, high frequency (HF) power and LF to HF ratio.

#### Statistical analysis and methods

Data was collected by using a structure proforma. Data entered in MS excelsheet and analysed by using SPSS 19.0 version IBM USA. Quantitative data was expressed in terms of Mean and Standard deviation.

The stress level responses were expressed as percentage. All the subjects were divided in to 3 groups depending on the stress levels as low stress, moderate stress and high stress groups.

HRV was compared between controls and low stress, moderate stress and high stress groups.

Comparison of mean and SD between all groups was done by using One way ANOVA test. A 'p' value of <0.05 was considered as statistically significant.

## FINDINGS

### Stress Indicators Questionnaire

#### 1. Physical indicator:-

According to the scale of measuring the physical indicators, in the total sample of 150 subjects(in percentage 100%), 28% of subjects were found to have

above high effect of stress on physical health. 36% were found to have medium effect and around 36% were found to have low effects. [Table 1].

#### 2. Sleep indicators:-

55.6% of subjects were found to have above high effect of stress on sleep health. 21.3% were found to have medium effect and around 23.1% were found to have low effects. [Figure 1].

#### 3. Behavioural indicator:-

31.91% of subjects were found to have above high effect of stress on behaviour. 22.95% were found to have medium effect and around 45.4% were found to have low effects. [Figure 1].

#### 4. Emotional indicator:-

39.35% of subjects were found to have above high effect of stress on emotional factors. 21.3% were found to have medium effect and around 39.35% were found to have low effects. [Figure 1].

#### 5. Personal indicator:-

42.5% of subjects were found to have above high effect of stress on personal life. 22.95% were found to have medium effect and around 34.54% were found to have low effects. [Figure 1].

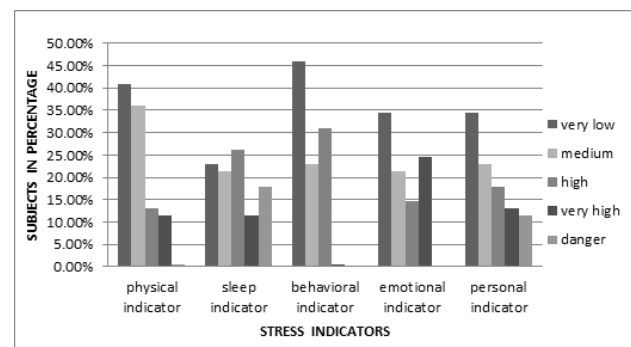


Figure – 1

### Stress Assessment by Perceived stress scale

According to the scale of measuring the individual stress levels by perceived stress scale, 24.59% of subjects were found to have above high level of stress. 37.7% were found to have medium levels and around 37.71% were found to have low levels. [Figure 2].

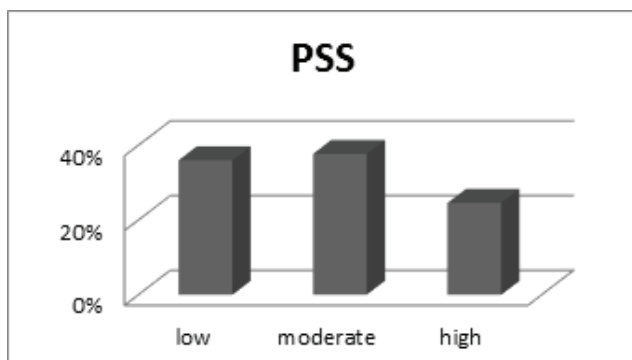


Figure – 2

**The Operational Police Stress Questionnaire (PSQ-Op)**

According to the scale of measuring the occupational stress in police personnel by using PSQ-Op , in the total sample of 60 subjects (in percentage 100%), 100% of subjects were found to have above high stress levels due to their work. So therefore 100% showed high levels of stress where as 0% showed low and moderate level of stress due to their occupation demands. [Figure 3].

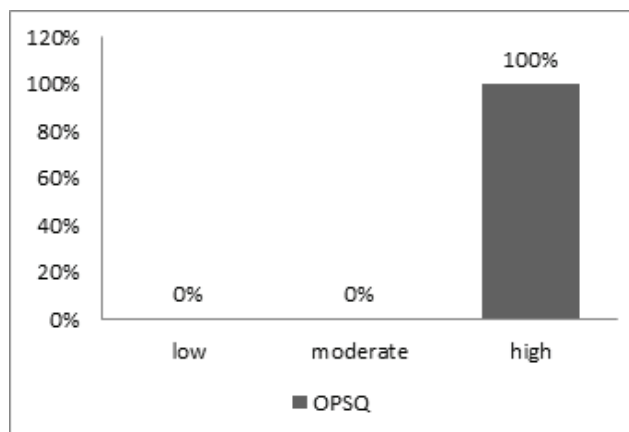


Figure – 3

**Heart Rate Variability**

There was a statistically significant difference in HRV when controls are compared with groups of different levels of stress. On the basis of assessment of stress based on the survey done by above questionnaires, subjects with less stress showed more of parasympathetic activity that is HF was high whereas subjects with more stress showed more of sympathetic activity that is increased value of LF.

**Table – 1: Comparison of HRV between Controls and Low stress group**

	Group	Number	Mean ± SD	P Value	Inference
<b>LF(low frequency)in ms<sup>2</sup></b>	Control	50	653.48±11.20	.0001	Highly significant
	Low Stress	54	771.28±13.82		
<b>HF(High Frequency) in ms<sup>2</sup></b>	Control	50	564.29±17.54	.0001	Highly significant
	Low Stress	54	476.96±21.49		
<b>LF/HF</b>	Control	50	1.15± .04	.0001	Highly significant
	Low Stress	54	1.61± .08		

**Table – 2: Comparison of HRV between Controls and Moderate stress group**

	Group	Number	Mean ± SD	P Value	Inference
<b>LF(low frequency) in ms<sup>2</sup></b>	Control	50	653.48±11.20	.0001	Highly significant
	Moderate Stress	49	818.40±23.31		
<b>HF(High Frequency) in ms<sup>2</sup></b>	Control	50	564.29±17.54	.0001	Highly significant
	Moderate Stress	49	448.08±16.62		
<b>LF/HF</b>	Control	50	1.15± .04	.0001	Highly significant
	Moderate Stress	49	1.82±.07		

**Table -3: Comparison of HRV between Controls and High stress group**

	Group	Number	Mean $\pm$ SD	P Value	Inference
LF(low frequency) in ms <sup>2</sup>	Control	50	653.48 $\pm$ 11.20	.0001	Highly significant
	High Stress	47	963.65 $\pm$ 7.70		
HF(High Frequency) in ms <sup>2</sup>	Control	50	564.29 $\pm$ 17.54	.0001	Highly significant
	High Stress	47	371.57 $\pm$ 17.81		
LF/HF	Control	50	1.15 $\pm$ .04	.0001	Highly significant
	High Stress	47	2.59 $\pm$ .12		

## DISCUSSION

In this study, more than 50% of the police personnel in the sample were found to be suffering from psychological stress, which is consistent with the stress reported by Rao *et al.* in 28.8% of CISF personnel.<sup>11</sup> Other studies such as done by Geetha *et al.* in Bangalore police personnel found high stress levels in 60% of population.<sup>12</sup> Deb *et al.* also found high stress in 79.4% of traffic constables in Kolkata.<sup>13</sup> The higher levels of stress reported by these studies as compared to the present study could be because of socio-demographic differences.

In this study, the effects of stress are seen in all aspects of life in police personnel. The study shows majority of the subjects were having physical effects of stress, in which specifically severe or chronic lower back ache (73%) and muscle spasms in neck and in shoulders (64%) were observed. Majority of the subjects (77%) had trouble falling. On observing behavioral indicator it showed majority of subjects had behavior issues specially due to working late (100%) and going to work even when sick (100%). On emotional level majority of the subjects showed disturbances especially as they end up worrying a lot (73%) and difficulty in or hiding their emotions from family and friends (90%). The lack of interest on personal aspect was observed, lack of ambition (89%) and spending less time on oneself (96%) was major factors.

In this study the observations taken under the operational police stress questionnaire is one of the important aspects to depict the level as well as the cause of stress in the subjects. The observations pointed that the main cause is the demands of their occupation. In this study it was observed that in all subjects (100%) the shift work, working late alone, over-time demand, work

related activities on day offs, not enough time to spend with friends and family, no time to stay physically fit, fatigue, occupation related health issues, limitations to social life and the feeling of always being on work were the major causes of high stress levels. These observations proved the increased stress level in subjects is due to their work that is being in the job of police and the job demands end up adding up stress. This can be resolved by distributing work and counseling, creating a healthy work environment also by providing adequate time to relax and spend time with family and recreation.

Finally when comparing HRV with the stress levels calculated in subjects, the study showed increased sympathetic activity with increased stress. This can be predisposing factor in the development of cardiovascular morbidity and mortality in the near future. HRV can be used as a screening tool for early diagnosis of stress related cardiovascular morbidity.

## CONCLUSION

The main conclusion can be drawn from this study is that all the physical, environmental, occupational, personality traits are related to the level of stress. In these subjects counseling to achieve healthy life is required by educating them about advantages of exercises, following a time pattern, eating habits, anger management, personal involvements should be undertaken. It is necessary for few amendments and changes to provide them with a healthy life and work environment. Is it fair to blame their insensitive behavior without knowing the actual cause and the amount of stress they face?

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# Vitamin B12 levels in Type 2 Diabetes Mellitus Patients on Metformin Use

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## ABSTRACT

Prevalence of type 2 diabetes mellitus (T2DM) is increasing globally. Metformin is commonly used anti-diabetic drug to treat type 2 diabetes mellitus patients. Metformin usage is associated with deficiency of vitamin B12. Studies have reported conflicting results on association between metformin usage and vitamin B12 deficiency. Hence, the present study is undertaken to study the vitamin B12 levels in T2DM patients taking metformin.

**Materials and methods:** total of 140 subjects were included in this study. Among them 70 were normal controls, their HbA1c: 5.5 to 6.5 % (group I) and 70 T2DM subjects their HbA1c: >7.5 % (group II). T2DM patients were on metformin and have been on the drug for a minimum of 5 years. Age of the study subjects was 40 to 60 years. 5 mL random venous blood sample is collected from all subjects in vacutainers, 2 mL in plain tube and 3 mL in EDTA tube. The serum sample was used for the estimation of random sugar (GOD-POD method) by using ERBA chemistry analyzer, vitamin B12 by ELISA method, using mini VIDAS and EDTA sample is used for estimation of HbA1c by using BIORAD-D10. Data were expressed as mean  $\pm$ SD. P value <0.05 is considered as statistically significant.

**Results:**In the present study, random blood sugar and HbA1c levels were significantly increased in T2DM subjects compared with controls. Vitamin B12 levels were significantly decreased in T2DM subjects compared with controls. HbA1c is inversely correlated with Vitamin B12.

**Conclusion:** It may be suggested that determination of vitamin B12 level in T2DM patients on metformin use might help in identifying patients that would benefit from B12 supplementation.

**Keywords:** Diabetes mellitus, vitamin B12 deficiency, micro and macro vascular complications.

## INTRODUCTION

The prevalence of Diabetes mellitus (DM) is increasing globally and is one of the leading cause of morbidity and mortality. [1] Globally, DM is the 6<sup>th</sup>

leading cause of death. In 2014, 422 million people were diagnosed with DM as compared to 108 million in 1980. [2] In India, the prevalence of diabetes mellitus is increasing. In 2000, India topped the highest number of diabetic subjects globally (31.7 millions). It is predicted that by 2030, DM may affect up to 79.4 millions in India. [3,4]

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Type II diabetes mellitus (T2DM), occurs due to insulin resistance and relative insulin deficiency or both. Common complications of diabetes mellitus are diabetic neuropathy, nephropathy, retinopathy, coronary artery



disease (CAD), cerebrovascular disease, etc. [4]

Metformin is commonly used anti-diabetic drug in type 2 diabetes mellitus patients. [5] The side effects due to metformin usage are mild, these include gastrointestinal symptoms, such as abdominal distress, soft stools and diarrhea and these adverse effects disappear after cessation of the drug. [6,7] It is well tolerated in most of T2DM patients, most of the patients continue to take it for years and characterized by excellent improvement in cardiovascular morbidity and mortality associated with T2DM. [8-10]

Vitamin B12 is also known as cobalamin. It is a water-soluble vitamin and plays an important role in hemopoetic, neuro-cognitive and vascular systems, involved in synthesis of DNA, metabolism of fatty acids and energy production. Vitamin B12 exerts its physiological effects by facilitating methylation of homocysteine to methionine, which is later activated to S-adenosyl methionine (SAM) that donates its methyl group to methyl acceptors. B12 is also involved in the conversion of methyl malonyl CoA to succinyl CoA. [8] Studies have shown that, prolonged usage of metformin causes B12 deficiency. It may affect the calcium dependent absorption of B12. [11, 12]

Vitamin B12 deficiency in the general population of India varies from 12% to 67%. [13] Prolonged usage of metformin, associated vitamin B12 deficiency, may cause peripheral neuropathy in T2DM patients. Neuropathy occurs due to B12 deficiency affects around 30% diabetics. [14] Studies have showed the association between prolonged metformin usage and B12 deficiency with conflicting results. [15] Hence, the present study is undertaken to study the vitamin B12 levels in T2DM patients taking metformin.

## MATERIALS AND METHOD

A total of 140 subjects of both male and female were included in this cross-sectional study. Among them 70 were normal controls, their HbA1c: 5.5 to 6.5 % (group I) and 70 T2DM subjects their HbA1c: >7.5 % (group II). The study was conducted in Department

of Biochemistry in collaboration with Department of Endocrinology, JNU Institute of Medical Sciences & Research Center, Jaipur. The study was approved by Institutional Ethical Committee and written informed consent from study participants. T2DM patients were on metformin and have been on the drug for a minimum of 5 years. Age of the study subjects was 40 to 60 years.

### Exclusion criteria:

Patients with hypertension, autoimmune disorders, cerebrovascular disease, gastrectomy, small bowel resection, liver disease, chronic kidney disease, thyroid disease, patients with recent intake of oral or intramuscular vitamin B12 supplement were excluded from this study. Clinical and physical examination was done for all the study subjects. 5 mL random venous blood sample is collected from all subjects in vacutainers, under aseptic conditions, 2 mL in plain tube and 3 mL in EDTA tube. The blood samples were centrifuged at 3000 rpm for 10 minutes to obtain serum. The serum sample was used for the estimation of random sugar (GOD-POD method) by using ERBA chemistry analyzer, vitamin B12 by ELISA method, using mini VIDAS and EDTA sample is used for estimation of HbA1c by using BIORAD-D10.

### Statistical Analysis:

Data were expressed as mean  $\pm$ SD. P value <0.05 is considered as statistically significant. Spearman's correlation coefficient for correlation of vitamin B12 and HbA1c. Distribution of vitamin B12 levels in 3 groups were expressed in percentages. Statistical analysis was done by using SPSS 20.0, Stata 8.0.

## RESULTS

Distribution of vitamin B12 levels in study groups were presented in table 1. Random blood sugar and HbA1c levels were significantly increased in T2DM (group II) compared with controls (group I). Vitamin B12 levels were significantly decreased in T2DM subjects compared to controls (Table 2). HbA1c is inversely correlated with Vitamin B12 (Table 3).

**Table 1: Distribution of vitamin B12 levels in study groups.**

Vitamin B12 levels (pg/mL)	Group I, Controls (n=70)		Group II, T2DM (n=70)	
	Number	Percentage	Number	Percentage
Deficiency (<200 pg/mL)	10	14.2	30	42.8
Intermediate (200-300 pg/mL)	14	20	29	41.4
Normal (>300 pg/mL)	46	65.7	11	15.7

**Table 2: Comparison of study parameters between controls and T2DM patients**

Parameters	Group I Controls (n=70) Mean±SD	Group II T2DM (n=70) Mean±SD	P value
RBS (mg/dL)	108.3±18.0	222.1±91.4	0.000*
HbA1c (%)	6.15±0.41	8.59±1.3	0.000*
Vitamin B12 (pg/mL)	496.48±282.75	248.92±110.70	0.000*

\*Significant

**Table 3: Spearman's rho correlation between HbA1c and Vitamin B12**

Parameters	Correlation Coefficient (r)
HbA1c	- 0.133**

\*\*Correlation is significant at the 0.01 level (2-tailed).

## DISCUSSION

The present study is undertaken to study the vitamin B12 levels in T2DM patients taking metformin. This study showed that there is a significant decrease in vitamin B12 levels in type 2 diabetes mellitus patients compared to controls and there is an inverse correlation between vitamin B12 levels and Glycated hemoglobin (HbA1c).

T2DM is one of the important health problems globally. It leads to macrovascular and microvascular complications. Metformin is an anti-diabetic drug. Serum vitamin B12 deficiency is one of the complications associated with metformin usage. In the present study, serum vitamin B12 levels were significantly reduced in T2DM patients who were taking metformin compared to controls. [16]

Metformin-induced B12 deficiency has been ascribed to the binding of hydrophobic tail of biguanide to hydrocarbon core of membranes. Positively charged

biguanide group gives a positive charge to the membrane and can displace divalent cations like calcium. The B12 uptake into ileal cells is dependent on calcium and thus impaired by metformin. [17] Indian diet is reported to contain low levels of calcium; this could be another factor causing B12 deficiency. [18] The risk of vitamin B12 deficiency associated with metformin use is greatly influenced by increasing age, dose and duration of metformin use. [19,20] Reduced B12 absorption and levels following metformin use, starts as early as 4<sup>th</sup> month. Clinically overt features of B12 deficiency manifest by 5-10 years. [21,22]

The exact mechanism of Vitamin B12 deficiency is controversial. The proposed mechanisms to explain metformin induced vitamin B12 deficiency among patients with T2DM include: alterations in small bowel motility which stimulates bacterial over growth and consequential vitamin B12 deficiency, competitive inhibition or inactivation of vitamin B12 absorption, alterations in intrinsic factor (IF) levels and interaction

with the cubulin endocytic receptor, the increased consumption of Vitamin B12 by bacteria. It can alter the level of intrinsic factor which could affect vitamin B12 absorption adversely. Metformin has also been shown to inhibit the calcium dependent absorption of the vitamin B12-IF complex at the terminal ileum. This inhibitory effect is reversed with calcium supplementation. [23 - 25]

#### **Limitations:**

Study was done in random blood sample. Duration of usage of metformin was 5 years. Large prospective studies are recommended.

#### **CONCLUSION**

In our study, metformin use was associated with significantly reduced levels of vitamin B12. Therefore, it may be concluded that determination of B12 levels in T2DM patients on metformin use might help in identifying patients that would benefit from B12 supplementation.

**Acknowledgement:** Nil

**Conflict of Interest:** Nil

**Funding:** Nil

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# Serum Iron in Obese and Non-obese Children

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## ABSTRACT

**Aim:** The study is aimed to investigate serum Iron levels in obese children

**Objectives:** To establish if there is any influence of obesity on serum iron levels in children

**Method:** 20 children between the age group 7-12 years of both the genders whose BMI was equal to or greater than 95th percentile were classified as obese based on the 2000 CDC Growth Charts<sup>7</sup>. 5ml of venous blood was collected under aseptic conditions and serum iron assessed using the quantitative colorimetric determination of iron and compared with serum iron levels of non-obese children of the same age group.

**Results:** Statistical analysis was done using unpaired t test and there was a significant decrease in serum Iron levels in obese children when compared to non-obese children.

**Conclusion:** Serum iron is lower in obese children and they are at risk of developing iron deficiency anemia.

**Keywords:** serum Iron, obesity, CDC Growth charts, unpaired t test.

## INTRODUCTION

A putative connection between obesity and hypoferrremia first suggested more than half a century ago. In 1961, Wenzel et al. measured lower levels of serum iron in obese subjects compared with non-obese subjects<sup>1</sup>. Data from NHANES III were examined for an association between Iron deficiency and weight. Obesity was a risk factor for Iron deficiency anemia in both boys and girls, but rates were approximately three times higher in girls<sup>2</sup>. Obesity is a global health issue and in recent years its prevalence has increased dramatically<sup>3</sup>.

Obese children and adolescents are not only prone to develop complications such as high blood pressure, dyslipidemia and type 2 diabetes mellitus, but also tend to consume a diet high in calories and low in nutritional value and are thus at increased risk of micronutrient deficiency especially iron deficiency<sup>4</sup>. However, the latest research

suggests that obesity-associated low-grade inflammation and the iron-regulatory protein hepcidin play a principal role in the regulation of endogenous iron homeostasis<sup>5</sup>. The negative consequences of iron deficiency anaemia on cognitive and physical development of children, and work productivity of adults are of major concern<sup>6</sup>.

The present study is aimed to examine serum iron status in obese and non-obese children.

**Material and Method:** The present study was carried out in Mahavir Institute of Medical sciences

**Study group:** 20 children between the age group 7-12 years of both the genders whose BMI was equal to or greater than 95th percentile were classified as obese based on the 2000 CDC Growth Charts<sup>7</sup>

**Control group:** 20 children between the age group 7-12 years of both the genders whose BMI was less than 95<sup>th</sup> percentile were classified as non-obese based on the 2000 CDC growth charts<sup>7</sup>.

**Inclusion criteria:** 1. Age between 7-12 years

**Exclusion criteria:** 1. History of any fever or any acute illness

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## 2.children taking any iron supplements

Informed written consent was obtained from the parents and A volume of 5 ml venous blood was drawn under aseptic conditions

Serum Iron was assessed using the quantitative colorimetric determination of iron.

### Statistical analysis:

Statistical analysis was done using unpaired t test

P value < 0.05 was considered as statistically significant

The results are expressed as mean  $\pm$  standard deviation.

## FINDINGS:

**Table.1: serum Iron( $\mu\text{g}/\text{dl}$ ) values in study and control group**

Group	N	Mean	Standard deviation	p-value
Study group	20	48	11.8	0.0003*
Control group	20	62	10.2	

Serum iron was significantly lower in study group

## DISCUSSION

In our study,serum iron was significantly less in obese children compared to non-obese children.As technology develops children and adolescents spend more time in sedentary leisure activities such as watching television, playing video games, and using computers. On the other hand, foods with high energy content and low iron levels lead to increasing obesity and iron deficiency in children and adolescents<sup>8</sup>.

Some studies have shown that overweight children have higher circulating hepcidin concentrations and lower iron status in comparison with children who are not overweight<sup>5</sup>. Comparable results were documented in Iranian and Chinese investigations<sup>9</sup>.

Iron uptake from the duodenum is limited in obese compared to normal weight children<sup>10</sup>.studies have shown that the Iron absorption was increased with loss of weight in children<sup>11</sup>.

## CONCLUSION

Serum iron is lower in obese children and they are at risk of developing iron deficiency anemia

**Source of Funding:** Self

**Ethical Clearance:** Taken from Scientific Ethical committee,Mahavir Institute of Medical Sciences,Vikarabad,Telangana.

**Conflict of Interest** – Nil

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# A Study of Blood pH and its Relation with Convulsions in Pregnant Women of MNR Hospital

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## ABSTRACT

**Introduction:** The physiological changes, together with the hormonal changes make pregnancy a psychological event for the women. Hyperventilation is defined as a condition in which the alveoli are ventilated at a greater extent than is necessary to maintain normal blood oxygen and carbon dioxide tensions it can be the result of an increase in the tidal volume or respiratory rate, or a combination of the two. Today, epilepsy is the most common neurological disorder during pregnancy, after migraine. **Material and method-** The subjects who were selected venous blood sample was collected using digital pH meter **Results and conclusion-** This study has been used to generate data on blood pH and its relation with convulsion in pregnant women of MNR Hospital, Sangareddy which shows that hyperventilation in pregnancy results in CO<sub>2</sub> wash out, this change will cause shift of body pH towards alkaline side, decreasing CO<sub>2</sub> (hypocapnea) and increasing neuronal excitability in the hippocampus to induce seizures. By controlling hypocapnea it will helpful to various gynaecologist as well as physcians to control antenatal, perinatal and post natal morbidity.

**Keywords-** PCO<sub>2</sub>, PAO<sub>2</sub>, HV-HYPERVENTILATION, PREECLAMPSIA, ECLAMPSIA.

## INTRODUCTION

The physiological changes, together with the hormonal changes make pregnancy a psychological event for the women. Hypocapnia (a low CO<sub>2</sub> level in the blood) is typical in modern people and is even more pronounced in the sick. It is the main cause of seizures. Today, epilepsy is the most common neurological disorder during pregnancy, after migraine. Comprehensive epidemiological studies reveal that the prevalence of epilepsy is 6.8%, and 0.3% to 0.5% of pregnancies are accompanied by epilepsy. Pregnant women with epilepsy are worried that antiepileptic drugs may have negative effects on the fetus, that convulsion frequency may increase, and that some

health problems, including epilepsy, may occur in their children. Furthermore, it is known that antiepileptic drugs increase the risk of congenital malformation.<sup>1</sup> (Among other factors, the main effects of hypocapnea caused by hyperventilation in relation to the seizures cause are: - increased excitability of nerve cells that lowers seizure threshold - reduced brain-oxygen level and increased cellular acidity (low pH in cells) - reduced glucose availability for the brain - worsened blood-glucose control - increased muscular tension - worsened ability to resist stress due to a weakened immune system.<sup>2</sup> Respiratory alkalosis (decreased in dissolved CO<sub>2</sub> caused by excessive artificial ventilation<sup>3</sup>.

## MATERIAL AND METHOD

The study was conducted in the Physiology Department, MNR Medical College, Sangareddy. 60 pregnant women in the Department of Obstetrics and Gynaecology, MNR Medical College and Hospital. The subjects who were selected and blood pressure was recorded with a standardized mercury

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sphygmomanometer and venous blood sample was collected using digital pH meter and questions asked i.e. gravida, trimester, previous stillbirth and, gestational age. All these parameters and history taking is done for first visit and second visit for same study subjects. Following detailed assessment of the subjects, they were screened for the presence of inclusion and exclusion criteria and dropped if any exclusion criteria were present. Study subjects were divided into two Group 1- 30 normal pregnant women of 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> trimester. Group 2- 30 pregnant women of 2<sup>nd</sup> and 3<sup>rd</sup> trimester who are diagnosed with preeclampsia. 1st visit: all trimesters, 2<sup>nd</sup> visit: 2-3 trimesters.

### INCLUSION CRITERIA

Those subjects who were willing to participate in the study were included after obtaining informed consent. Normal healthy pregnant women in 1<sup>st</sup> (0-12 weeks), 2<sup>nd</sup> (13-26 weeks) and 3<sup>rd</sup> (27-40 weeks) trimester between 20-35 years of age. Pregnant women between 20-35 years of age who are Diagnosed with pre-eclampsia and eclampsia.

### EXCLUSION CRITERIA

1. Women aged less than 20 and more than 35 years.
2. Women with any organic cardiac disease.
3. Women with renal disease.
4. Women with severe anaemia.

**STUDY DESIGN** Prospective Study.

**STUDY INSTRUMENT** digital pH meter, 2CC syringe and standardized mercury sphygmomanometer

### STATISTICAL ANALYSIS<sup>4</sup>

All statistical analysis of the results were performed using Statistical package for Social Sciences (SPSS Inc, Chicago and III) 20<sup>th</sup> version. They are presented as number and percentages for categorical data and Pearson's chi-squared test ( $\chi^2$ ) was used to test the associations.

A "p" value of 0.05 or less was considered for statistical significance.<sup>5</sup>

## RESULTS

It is important to understand the normal physiological

changes occurring in pregnancy as this will help differentiate from adaptations that are abnormal.<sup>5</sup> There is a significant increase in oxygen demand during normal pregnancy. This is due to a 15% increase in the metabolic rate and a 20% increased consumption of oxygen. There is a 40–50% increase in minute ventilation, mostly due to an increase in tidal volume, rather than in the respiratory rate. This maternal hyperventilation causes arterial PO<sub>2</sub> to increase and arterial PCO<sub>2</sub> to fall.<sup>5</sup> Causes of hyperventilation in pregnancy:

1. Progesterone gradually increases during the course of pregnancy, from 25 ng/ml at 6 weeks to 150 ng/ml at 37 weeks gestation Progesterone acts as trigger of the primary respiratory centre by increasing the sensitivity of the respiratory centre to carbon dioxide.<sup>6</sup>

2. The progressive uterine distension is the major cause of lung volume and chest wall changes during pregnancy, which comprise elevation of the diaphragm and altered thoracic configuration. The enlarging uterus increases the end-expiratory abdominal (gastric) pressure (*Pga*), thereby displacing the diaphragm upwards.<sup>6</sup>

Since hyperventilation in pregnancy results in CO<sub>2</sub> wash out, this change will cause shift of body pH towards alkaline side, decreasing CO<sub>2</sub> Hypocapnia and increasing neuronal excitability in the hippocampus to induce seizures.

### In This Study, Association of Following Parameters Were Analyzed:- 1: Association of Eclampsia with pH by Using Chi-Square Test : 1<sup>st</sup> Visit pH

**a. Group 1:** Out of 30 normal pregnant women on their 1<sup>st</sup> visit fall under normal pH (7.31 to 7.41), 36.7% (11 out of 30) and remaining 66.7% (19 out of 30) showed pH shift towards alkaline pH (> 7.41).  $p > 0.05$ , this association is not significant.

**b. Group 2:** 2<sup>nd</sup> group of 30 pre-eclamptic women on their 1st visit 26.7% (8 out of 30) has normal pH (7.31 to 7.41) and 73.3%

(22 out of 30) were in alkaline pH (> 7.41).  $p < 0.05$ , this association is highly significant.

### 2<sup>nd</sup> Visit pH

**a. Group 1:** Out of 30 normal pregnant women,

3.3% (1 out of 30) has normal pH (7.31 to 7.41) rest all 96.7% (29 out of 30) were in alkaline pH (> 7.41).  $p > 0.05$ , this association is not significant.

**b. Group 2:** All subjects had pH value towards alkaline pH (>7.41) 100% (30 out of 30) for this group chi-square test is not applicable.

This results correlates with the reduction of long-latency responses is probably mediated by hypocapnia rather than by other nonspecific effects of HV. It is suggested that increased neuronal excitability caused by HV-induced hypocapnea leads to spontaneous and/or asynchronous firing of cortical neurons, which in turn reduces stimulus-locked synaptic events.<sup>8</sup>

This results correlates with showing a fall in the blood CO<sub>2</sub> and a rise in the blood pH during pregnancy, support the assumption that hyperventilation is one of the primary factors in the disturbed acid-base equilibrium<sup>9,10</sup>. have recently presented convincing.

**Table 1: Association of Eclampsia with pH 1<sup>st</sup> Visit (Group 1)**

Group 1	Normal pH	Alkaline pH	Total
Non-Eclamptic	9	11	20
Eclamptic	2	8	10
Total	11	19	30

Chi= 1.794, df=1, p value=0.180

**Table 2: Association of Eclampsia with pH 2<sup>nd</sup> Visit (Group 1)**

Group 1	Normal pH	Alkaline pH	Total
Non- Eclamptic	1	19	20
Eclamptic	0	10	10
Total	1	29	30

Chi=0.517, df=1, p value=0.472

**Table 3: Association of Eclampsia with pH 1<sup>st</sup> Visit ( Group 2)**

Group 2	Normal pH	Alkaline pH	Total
Pre-Eclampsia	4	9	13
Eclampsia	4	13	17
Total	8	22	30

Chi=4.455, df=1, p value=0.035

**Table 4: Association of Eclampsia with pH 2<sup>nd</sup> Visit (Group 2)**

Group 2	Normal pH	Alkaline pH	Total
Pre- Eclampsia	0	13	13
Eclampsia	0	17	17
Total	0	30	30

## CONCLUSION

This study has been used to generate data on blood pH & its relation with convulsion in pregnant women of MNR Hospital, Sangareddy, there are previous studies about pH, which shows that hyperventilation in pregnancy results in CO<sub>2</sub> wash out, this change will cause shift of body pH towards alkaline side, decreasing CO<sub>2</sub> (hypocapnia) and increasing neuronal excitability in the hippocampus to induce seizures. By controlling Hypocapnia it will helpful to various gynaecologist as well as physicians to control antenatal, perinatal and post natal morbidity. This study shows significant p-value between pH and the eclampsia.

**Conflict of Interest-** Nil

**Source of Funding-** Self

**Ethical Clearance** -It was given by the institution.

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# An Experimental Study to Screen the Effect of Dietary Garlic (*Allium Tuberosum*) on Hyperlipidemia

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## ABSTRACT

There is a strong association between hyperlipidemia especially hypercholesterolemia and development of atherosclerosis and coronary heart disease. Diet plays a significant role in the lipid metabolism and several studies have suggested conflicting views regarding the efficacy of Garlic in alleviating hyperlipidemia. Therefore, this experimental study was undertaken to assess the effect of Garlic on hyperlipidemia. Hyperlipidemia was induced in guinea pigs by administering diet rich in cholesterol for a limited duration. Hyperlipidemic animals were divided in two groups, one fed with normal diet and another fed with diet supplemented with crushed emulsified extract of *Allium tuberosum*. Animals showed the tendency to return to the eulipidemic state after cessation of hyperlipidemic diet. The normalisation of lipid levels was, however, more marked in animals fed with diet supplemented with garlic extract in comparison to animals fed with normal diet. This leads to a positive conclusion that garlic in raw crushed form is a hypolipidemic agent and thus it is suggested that garlic supplementation as a culinary agent should be encouraged.

**Keywords:** Hyperlipidemia, Hypercholesterolemia, *Allium Tuberosum* Garlic, Coronary Heart Disease

## INTRODUCTION

Much attention has been focused on hyperlipidemia because there is a strong association between hyperlipidemia especially hypercholesterolemia and development of atherosclerosis and coronary heart disease. Alterations in the relative concentrations of total serum cholesterol/ VLDL/LDL/HDL cholesterol are predictive of some diseases e.g., atherosclerosis, coronary heart disease etc.<sup>1</sup>

Approaches to management of hyperlipidemia can be both nonpharmacological and pharmacological. Each approach complements the other. However nonpharmacological approach should be viewed as basic approach. The management of hyperlipidemia aims to reduce the risk of coronary heart disease.<sup>2</sup>

Garlic, a unique plant, has a long and colorful history as both food and medicine and is highly valued as both. Local variety of garlic, botanically known as *Allium sativum*, is a widely distributed plant and used in all parts of the world as not only a spice and a food but also as a popular remedy. The variety in use by the population of Jammu & Kashmir is *Allium tuberosum*, locally known as 'THOME'.

Garlic and its preparations have been widely recognized as agents for prevention and treatment of cardio vascular and other metabolic diseases e.g., atherosclerosis, hyperlipidemia, thrombosis, hypertension and diabetes.

The use of garlic in diet to accord primary relief in arthritis and most importantly in heart diseases stands well documented in ancient literature. Garlic and its preparations have been widely recognized as agents for prevention and treatment of cardiovascular and other metabolic diseases, atherosclerosis, hyperlipidemia, thrombosis, hypertension and diabetes.<sup>3</sup>

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In modern era scientists have been trying to validate



many of these properties of garlic, especially in terms of the identity of the active components, their mechanisms of action and exploring the potential benefits as food supplements/as adjunct to specific therapy.

As knowledge of lipid biochemistry is important in understanding many current biomedical areas of interest; we undertook the present study to examine the effect of diet supplementation with emulsified crushed garlic (*Allium tuberosum*) on induced hyperlipidemia in guinea pigs. Any correlation, if existing, between the lipid status in the experimentally induced hyperlipidemia in guinea pigs and the supplementation of emulsified crushed garlic in diet was found out to delineate the role of *Allium tuberosum* in hyperlipidemia.

Studies prior to 1995 consistently concluded hypolipidemic action of garlic.<sup>4,5</sup> However studies after 1995, performed using enteric-coated preparation of raw garlic did not manifest any hypolipidemic effect.

These paradoxical observations warrant a systemic study to resolve the controversy, since it is a great challenge for scientists all over the world to make a proper use of garlic and enjoy its maximum beneficial effect, as it is the cheapest way to prevent cardiovascular disease. Since different varieties of garlic are consumed in different parts of the country hence scientific validation of biological effect of garlic on hyperlipidemic status, a risk factor for cardiovascular ailments, becomes all the more prudential.

## MATERIAL & METHOD

### EXPERIMENT PROTOCOL:

Sixteen guinea pigs, in-house bred siblings of either sex, were selected from the animal house of the institute after getting approval from the ethical committee. The study was performed in three phases:

**Pre Cholesterol Feeding:** Apparently healthy animals were given normal standard diet, normal in terms of quality and nutrition; consisting of raw wheat and pulse flour and little edible oil mixed with little green vegetable and pellets were formed of approximately 10 gms. These pellets and water or milk in separate flat container were made available ad-lib. The various parameters (body weight and serum lipid profile) were estimated to establish normal base line data.

**Cholesterol Feeding:** All sixteen guinea pigs were fed on cholesterol i.e., 500 mg/kg-body weight/day for four weeks to develop hyperlipidemia. Cholesterol was given orally in the form of emulsion prepared in edible oil through a feeding tube as per technique developed by Mathur and Mittal<sup>6</sup>, in addition to the usual diet. After four weeks, body weight of the guinea pigs was recorded and their serum lipid profile status was estimated in blood samples taken directly from heart without sacrificing the animal to confirm hyperlipidemia.

**Post Cholesterol Feeding:** Cholesterol feeding was stopped after the confirmation of hyperlipidemia, when it was observed that the serum cholesterol level rose 5 to 6 times from the base line pre cholesterol feeding status. To delineate the effect of *Allium tuberosum* on the induced hyperlipidemia, the guinea pigs were divided into three groups:

**Group 1:** (n =7) This group was maintained on normal standard diet described above till the end of the study to serve as control.

**Group II:** (n=9) This group was administered normal standard diet supplemented with emulsified crushed *Allium tuberosum* (2 mL/kg-body weight/day) for three weeks.

## GARLIC PREPARATION

**Crushed-Garlic Emulsion:** 200 gm of garlic (*Allium tuberosum*) was crushed in a grinder. This preparation was kept overnight in a muslin cloth at room temperature in a beaker containing distilled water to obtain an emulsion of garlic. This emulsion was then filtered through a muslin cloth and the filtrate evaporated in a desiccator to get the final emulsion of 100 mL. Thus 1 mL of this emulsion contained 2 gm of garlic content.

**Allicin potential:** As the average weight of the guinea pig was about 500 gm, so almost 1 mL of garlic extract (crushed or alcoholic) was administered to each animal daily. Since 1 mL of either garlic extract contained 2 gm of garlic content, therefore diet of each animal was supplemented with 2 gm of garlic. This quantity (2 gm) of garlic contains at least 5 mg of allicin or a total allicin potential of 2000 micrograms as per theoretical evaluation on the basis of data reported by Pizzomo and Murray<sup>7</sup> in case of *Allium sativum*. The content values with reference to *Allium tuberosum* were not available and therefore, on account of limitations involved in the

present study, the w/w values of alliin in *Allium sativum* were extrapolated for *Allium tuberosum*.

### PARAMETERS STUDIED

Body weight of the guinea pigs was measured in grams with the help of a standard beam scale. After an overnight fast of 10-12 hours, blood samples (2 mL) were drawn from the heart of each animal by using aseptic technique. Serum lipid profile i.e., Total Cholesterol, Triglycerides, HDL-Cholesterol, LDL-Cholesterol, VLDL-Cholesterol, LDL-C/HDL-C ratio and TC/

HDL-C ratio was estimated by using commercially available reagents and kits.

The data obtained for various parameters was subjected to statistical analysis. Arithmetic mean and standard deviation were calculated of all the parameters studied, to compute 't values' (student's t- test). On the basis of the t values, 'P values' (probability) were determined to make out the significance of variance between the mean values of individual parameters among the three groups of the subjects studied.

### RESULTS

Table 1 summarizes the values of body weight, total cholesterol and other parameters of lipid profile in animals before, and after they were administered diet supplemented with a known quantity of cholesterol.

**Table 1: Mean values of the various parameters studied in the pre cholesterol-feeding phase and after the cholesterol-feeding phase.**

Parameter Studied	Pre Cholesterol Feeding	Post Cholesterol Feeding	Statistical Significance Pre v/s Post Cholesterol Feeding
Body Weight (gm)	511.20 ± 49.27	542.80 ± 44.02	t value = 2.39 P value < 0.05 [S]
Total Cholesterol (mg/dL)	30.50 ± 6.08	114.47 ± 19.13	t value = 20.92 P value < 0.001 [HS]
Triglycerides (mg/dL)	38.48 ± 5.91	95.23 ± 10.29	t value = 23.91 P value < 0.001 [HS]
HDL-Cholesterol (mg/dL)	10.68 ± 1.52	11.48 ± 1.61	t value = 1.81 P value > 0.05 [NS]
LDL-Cholesterol (mg/dL)	12.13 ± 4.35	83.95 ± 16.73	t value = 20.77 P value < 0.001 [HS]
VLDL- Cholesterol (mg/dL)	7.70 ± 1.18	19.05 ± 2.06	t value = 23.90 P value < 0.001 [HS]
LDL-C/HDL-C	1.14 ± 0.40	7.34 ± 1.26	t value = 23.45 P value < 0.001 [HS]
T C./HDL-C	2.86 ± 0.46	10.01 ± 1.37	t value = 24.74 P value < 0.001 [HS]

This indicates a major contribution of cholesterol, supplemented in the routine diet, as one of the causative factors of increase in body weight; besides other possible factors like growth and development of the animal. Since

the diet and water were given ad-lib, therefore additional contribution of diet and water as factors responsible for increase in the body weight is ruled out.

As is clear from the data, hyperlipidemia was induced in the guinea pigs, when they were fed with diet supplemented with cholesterol for four weeks. All lipid parameters increased significantly except the HDL-cholesterol.

Table 2 depicts the serum lipid profile levels in hyperlipidemic and guinea pigs maintained on normal diet after the cholesterol feeding phase. It indicates significant reduction lipid profile parameters except Triglycerides, HDL-Cholesterol and VLDL-Cholesterol in Group I animals after cessation of hyperlipidemic diet.

**Table 2: Statistical Analysis of Mean Serum Lipid Profile levels in Hyperlipidemic and Guinea Pigs maintained on normal diet**

Parameter Studied	Hyperlipidemic	Group I	Statistical Significance Hyperlipidemic v/s Group I
Total Cholesterol (mg/dL)	114.47 ± 19.13	94.49 ± 8.71	t value = 3.96 P value < 0.001 [HS]
Triglycerides (mg/dL)	95.23 ± 10.29	91.84 ± 5.86	t value = 1.12 P value > 0.05 [NS]
HDL-Cholesterol (mg/dL)	11.48 ± 1.61	11.43 ± 0.98	t value = 0.10 P value > 0.05 [NS]
LDL-Cholesterol (mg/dL)	83.95 ± 16.73	64.70 ± 7.70	t value = 4.34 P value < 0.001 [HS]
VLDL- Cholesterol (mg/dL)	19.05 ± 2.06	18.37 ± 1.17	t value = 1.12 P value > 0.05 [NS]
LDL-C/HDL-C	7.34 ± 1.26	5.70 ± 0.91	t value = 3.85 P value < 0.001 [HS]
T.C./HDL-C	10.01 ± 1.37	8.32 ± 1.09	t value = 3.42 P value < 0.01 [VS]

\*Group I : Maintained on normal diet.

Table 3 depicts the serum lipid profile levels in hyperlipidemic and guinea pigs administered diet supplemented with emulsified crushed garlic after

the cholesterol feeding phase. It indicates significant reduction in all lipid profile parameters Group II animals after cessation of hyperlipidemic diet.

**Table 3: Statistical Analysis of Mean Serum Lipid Profile levels in Hyperlipidemic and Guinea Pigs administered diet supplemented with emulsified crushed garlic**

Parameter Studied	Hyperlipidemic	Group II	Statistical Significance Hyperlipidemic v/s Group II
Total Cholesterol (mg/dL)	114.47 ± 19.13	37.20 ± 8.88	t value = 15.57 P value < 0.001 [HS]
Triglycerides (mg/dL)	95.23 ± 10.29	50.80 ± 13.18	t value = 9.16 P value < 0.001 [HS]
HDL-Cholesterol (mg/dL)	11.48 ± 1.61	12.89 ± 1.76	t value = 2.11 P value < 0.05 [S]

**Cont... Table 3: Statistical Analysis of Mean Serum Lipid Profile levels in Hyperlipidemic and Guinea Pigs administered diet supplemented with emulsified crushed garlic**

<b>LDL-Cholesterol (mg/dL)</b>	83.95 ± 16.73	14.15 ± 5.72	t value = 18.12 P value < 0.001 [HS]
<b>VLDL- Cholesterol (mg/dL)</b>	19.05 ± 2.06	10.16 ± 2.64	t value = 9.16 P value < 0.001 [HS]
<b>LDL-C/HDL-C</b>	7.34 ± 1.26	1.09 ± 0.38	t value = 22.16 P value < 0.001 [HS]
<b>T.C./HDL-C</b>	10.01 ± 1.37	2.87 ± 0.46	t value = 22.74 P value < 0.001 [HS]

\*Group II: Diet supplemented with crushed garlic.

### CONCLUSION

Various beneficial aspects have been attributed to this supplementation by garlic like acceptability of the nutritional raw ingredients, some medicinal benefits like prolongation of cardio-vascular integrity, protection from generation of tumours. Various species of garlic are used in the world out of which *Allium tuberosum* is used in northern hilly areas of the country as one of the culinary agents. Several studies have been performed for scientific validation of beneficial effects of garlic supplementation.<sup>8,9,10,11</sup> This study indicates that at the end of three weeks, after hyperlipidemia production, the serum total cholesterol fell significantly in the group which was maintained on normal diet. This fall indicates that biological machinery tends to bring hyperlipidemic state towards eulipidemic state after stoppage of cholesterol supplementation after lapse of three weeks. This fall in serum total cholesterol level is of higher magnitude in Group II animals which indicates that supplementation of diet with garlic quickens very significantly with a process of achieving eulipidemic state in a span of three weeks after stoppage of cholesterol feeding in comparison to the group which was not given garlic supplementation establishing the useful role of garlic supplementation in supporting the biological machinery to achieve a eulipidemic state. If the values of group I are compared with those of group II the pattern indicates that crushed garlic supplementation with diet is effective in achieving the eulipidemic state.

Out of the total cholesterol, HDL-Cholesterol has been not associated with atherogenic tendency while the LDL-Cholesterol and/or VLDL-Cholesterol complex has been held responsible for relatively higher atherogenic tendency but the conversion of LDL-C/VLDL-C to HDL-C reduces the atherogenic risk.<sup>12</sup>

Post garlic treatment for three weeks after stopping cholesterol supplementation changed the lipid profile status of the animals studied. This reduction is due to withdrawal of cholesterol supplementation in diet, indicating that resorting to cholesterol free diet helps in reducing possible atherogenic risk. When the values of non-garlic supplementation group, after three weeks, were compared with the values after stoppage of cholesterol supplementation, it was confirmed that the withdrawal of high cholesterol diet relatively increases the status of HDL-Cholesterol while there is a gradual decrease in LDL-Cholesterol and VLDL-Cholesterol percentage.

Analysis of LDL-Cholesterol/HDL-Cholesterol and serum T.C./HDL- Cholesterol ratio exhibit rising pattern. The T.C./HDL-C ratio increased by 3.5 times while LDL-C/HDL-C ratio increased by 6.4 times after cholesterol feeding. This indicates that the rise in LDL-C/HDL-C was no mark in comparison to T.C./HDL-C. After stoppage of cholesterol in non-garlic treatment, T.C./HDL-C ratio was decreased by 1.2 times after three weeks of stoppage of cholesterol supplementation and without starting garlic supplementation. While garlic supplementation reveal more fall than non-garlic supplementation, fall was more marked in LDL-C/HDL-C ratio.

Our study is in total agreement with notion advanced by Clouatre Dallas<sup>13</sup> that “the general public has been led to believe that all primary active constituents are in the lipophilic fractions of garlic such as alliin, allicin, ajoene, etc. this is contrary to the scientific findings - it has been known for more than a decade that the odourless water-soluble fractions of garlic are equal to the oil-soluble fractions in their effects.” The observations are tallying with those of

Ackermann<sup>8</sup> and Bordia<sup>4</sup>. The present study indicates that cholesterol feeding increases total cholesterol and the three fractions viz., HDL-C, LDL-C and VLDL-C. The supplementation of garlic as a culinary agent in raw crushed form is effective in supporting the biological mechanism to move towards eulipidemic state. This leads to a positive conclusion that garlic in raw crushed form is a hypolipidemic agent. In view of this it can be safely suggested that garlic supplementation as a culinary agent should be encouraged.

**Conflict of Interest-** Nil

**Source of Finding-** Self

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