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# A Non Randomized Controlled Study to Evaluate the Effect of Isotonic Handgrip Exercise on Blood Pressure in Normal Weight and Preobese Healthy Adults

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## Abstract

**Background:** The adverse health consequences of overweight and obesity in India leads to higher prevalence of diabetes mellitus and cardiovascular diseases. Also the compliance of people for routine form of exercise for BP control has not been very encouraging due to time, place etc constrains.

**Aim:** Therefore we conducted a nonrandomised clinical study to determine the short-term effects of isotonic handgrip exercise by using smiley balls on blood pressure in healthy normal weight and overweight adolescents with the objective to find a user friendly exercise which help in reducing blood pressure.

**Method:** A non randomized clinical study was conducted on 100 young normal-weight and pre-obese adults (50 Boys and 50 Girls) in the age group of 18–25 years. Isotonic handgrip exercise was performed at the rate 20 contractions/minute (2 sec contraction/1 sec relaxation) at maximal intensity for 10 minutes using **smiley ball**. Pulse rate and blood pressure parameters were tested at baseline and immediately after exercise in post-exercise recovery period.

**Result:** Statistically significant reduction was observed in systolic blood pressure(SBP) and mean arterial pressure (MAP) in both pre-obese boys and girls groups while pulse pressure & mean arterial pressure in normal weight girls after exercise regime.

**Conclusion:** We conclude that the exercise regime under consideration can produce some short-term beneficial effects with respect to blood pressure in especially pre-obese group of adults.

**Keywords:** *Isotonic Handgrip Exercise, Normal weight and pre-obese adults.*

## Introduction

It is known that regular dynamic exercise reduces blood pressure (BP) and helps in the prevention of hypertension by various mechanisms such as decrease in sympathetic nerve traffic, potentiation of baroreceptor reflex, decrease in arterial stiffness, increase in total systemic arterial compliance, increase in the release of endothelium-derived nitric oxide, and increase in insulin sensitivity. It has also been reported that an acute bout of dynamic physical exercise involving large muscle mass also results in lowering of BP lasting for 12–16 hours in the postexercise period, known as postexercise hypotension (PEH).<sup>1</sup>

The prevalence of overweight and obesity among children and adolescents has increased significantly in the developed countries during the past two decades and similar trends are being observed even in the developing world. Targeting adolescent age group for primary prevention can be justified for many reasons. The adverse health consequences of overweight and obesity in India leads to higher prevalence of type 2 diabetes and cardiovascular diseases.<sup>2</sup>

However, despite the strong evidences that recommend regular dynamic exercise involving large muscle mass (briskwalking and cycling) for lowering BP and prevention of hypertension, the compliance

of people toward such forms of exercise has not been encouraging owing to various possible reasons such as comorbid conditions (osteoarthritis and coronary artery disease) and other constraints (such as time, space, and economic constraints).<sup>3</sup>

It is, therefore, necessary to design an alternative feasible form of physical exercise involving a relatively lesser muscle mass, which can reduce BP and increase exercise compliance in the population.

Isotonic handgrip exercise is a simple, cheap, and feasible form of physical exercise involving relatively lesser muscle mass, which can be performed as per individual convenience with respect to time and place using simple equipment such as handgrip dynamometer, **smiley ball**. But, before we prescribe isotonic handgrip exercise to the population, it is essential to determine whether isotonic handgrip exercise decreases BP in post-exercise period as an acute short-term effect (PEH) and/or over a long term as a training effect. It is also essential to understand the mechanism underlying such BP-lowering effects of isotonic handgrip exercise. As scant literature is available, which illustrates the role of isotonic handgrip exercise in lowering blood pressure, we investigated a single bout of isotonic handgrip exercise for PEH.

### **Aim & Objectives of Study**

- To evaluate the effect of isotonic handgrip exercise on blood pressure
- To compare the effect of exercise on normal and pre-obese healthy adults in both genders.

### **Material and Method**

- **Study Design:** A Non randomized controlled study
- **Study Population:** Medical students of age group 18 to 25 years and both the genders
- **Sample size:** 100 students (50 girls and 50 boys including normal weight and pre-obese)
- **Inclusion criteria:**
  1. Age group 18 to 25 years boys and girls
  2. BMI -18.5 to 29.9 (normal weight-18.5-24.9; pre-obese -25 to 29.9 kg/m<sup>2</sup>)<sup>2,4</sup>

### **Exclusion Criteria:**

1. BMI less than 18.5 kg/m<sup>2</sup> and more than 30 kg/m<sup>2</sup>
2. Participants who were smokers, athletes and suffering from chronic illness

### **Method**

Present study was conducted on 100 voluntary participants (50 Boys and 50 Girls) at SBKS Medical Institute and Research Center (SBKS MIRC) after taking approval from Human Research Review Panel (HRRP) of SBKS MI & RC and Institutional Ethics Committee (SVIEC) of our institute and consent from participants.

Those medical students who were in the age group of 18 to 25 years and had BMI in the range of 18.5 to 29.9 kg/m<sup>2</sup> were included in study. The body weight (Wt) was measured bare footed to the nearest 0.5 kg and the height was measured using meter scale without footwear to the nearest 0.5 cm. Body Mass Index (BMI) will be calculated as the weight (kg) divided by the square of height (m<sup>2</sup>).<sup>1</sup>

The study participants were first tested for pre-exercise (baseline) pulse rate & blood pressure. This was followed by a bout of isotonic handgrip exercise for 10 minutes. After exercise, again pulse rate & blood pressure was taken.<sup>3</sup>

**Method of Measurement of BP:** The blood pressure was measured in the non-dormant arm in the sitting position with arm and back support. The blood pressure was recorded at the interval of 1 min till the difference between two consecutive blood pressure readings was <5 mmHg. The average of the two consecutive readings was used for statistical analysis. The systolic and diastolic blood pressure was measured by using digital sphygmomanometer<sup>1</sup>. Pulse pressure (PP) was calculated by using formula: systolic blood pressure (SBP) – diastolic blood pressure (DBP). Mean arterial pressure (MAP) was calculated by formula (Diastolic Pressure + 1/3 Pulse pressure (PP)).<sup>1</sup>

**Method of Performing Single Bout of Isotonic Handgrip Exercise:** The single bout of isotonic handgrip exercise was performed by the dominant hand of the participants using smiley ball for duration of 10 minutes continuously at an intensity of MVC. During the exercise the participant was asked to squeeze the ball for 2 seconds (Contraction phase) followed by release of the ball for 1 second (Relaxation phase) such that a compression cycle rate of 20/min (each cycle had 2 seconds contraction followed by 1 second of relaxation) is achieved. (1) Exercise was stopped if the heart rate rises above 85% MHR or Blood Pressure rises above 180/110 mmHg.<sup>1,3</sup>

**Statistical Analysis:** Mean and Standard Deviation of the study variables were calculated for Pre-exercise baseline and Immediate Post-Exercise Period. Student’s Paired t-test was used to study if any significant differences in study variables were observed between the Pre-exercise and the post-exercise periods. P value < 0.05 was considered as significant.

**Ethical Issues**

No ethical issue in this research project because of

- Intervention was simple handgrip exercise in the study

- Voluntary participation is in inclusion criteria.

**Feasibility Issues:**

- Sample size according to the time
- No ethical issues
- Data will collected rapidly, no complicated procedure involved

So this project is feasible in current set up and condition

**Observations:**

**Table 1: Subject Characteristics**

	Boys(n=50)		Girls (n=50)	
	Normal (n=27)	Preobese(n=23)	Normal (n=28)	Preobese(n=22)
Age(Yrs)	21.6 ± 1.63	20.7 ± 1.3	20.4 ± 0.64	20.6 ± 0.94
Weight (Kg)	63 ± 7.07	77.83 ± 8.37	50 ± 5.94	57.4 ± 4.16
Height (Cm)	172.3 ± 9.08	170.8 ± 11.3	158 ± 6.34	152 ± 9.45
BMI (Kg/M <sup>2</sup> )	21.1 ± 1.5	27.04 ± 1.41	20.02 ± 1.49	26.55 ± 1.4

Values indicate Mean ± SD \* Indicates significant difference between groups. P value < 0.05

**Table 2: Short-term Effects of Single Bout of Isotonic Handgrip Exercise on Blood pressure in boys**

	Normal weight			Preobese		
	Baseline	Post-exe	p-Value	Baseline	Post-exe	p-Value
Pulse Rate	82.27±6.56	84±6.9	0.35	83.63±5.34	86±6.5	0.184
SBP	122.4±4.89	119±6.37	0.087	129.1 ± 5.67	126±6.32	0.017*
DBP	76.6±5.87	75.2±5	0.35	81.4±7.33	78.2±6.89	0.135
PP	45.75±5.58	44±7.6	0.34	47.75±5.23	47±4.7	0.612
MAP	92±4.9	89.8±4.16	0.081	97±6.4	94±6.33	0.019*

Values indicate Mean ± SD \* Indicates significant difference between groups. P value < 0.05

**Table 3: Short-term Effects of Single Bout of Isotonic Handgrip Exercise on Blood pressure in Girls**

	Normal weight			Preobese		
	Baseline	Post-exe	p-Value	Baseline	Post-exe	p-Value
Pulse Rate	79.79±9.35	81.3±6.93	0.48	79.83±8.9	82.5±4.45	0.226
SBP	116±9.43	105.9±8.66	0.64	115±7.44	106.6±8.75	0.002*
DBP	71.6±6.4	71±5.76	0.709	74.7±6.99	75.3±7.11	0.784
PP	44±11	35±7.3	0.001*	40±9.7	31±10	0.005*
MAP	86.25±5.68	82.7±5.94	0.024*	106.6±8.75	85.7±5.98	0.045*

Values indicate Mean ± SD \* Indicates significant difference between groups. P value < 0.05

Table 1 shows subject characteristics age in yrs, weight (Kg), height (Cms) and BMI (Kg/M<sup>2</sup>) in both genders. As depicted in Table 2 and Table 3, in comparison to the pre-exercise baseline condition, systolic blood pressure (SBP), mean arterial pressure (MAP) in Preobese groups of both genders were found to be significantly different in the Post-Exercise period. Table 3 shows also significant reduction in pulse pressure (PP) and mean arterial pressure (MAP) in normal weight girls.

## Discussions

The current study indicates that Isotonic Handgrip Exercise performed for 10 minutes at an intensity of MVC at a Compression cycle of 20 contractions per minute can produce post-exercise hypotension (PEH) in preobese adults as compared to normal weight adults specially in girls.

PEH has been well documented in humans with both borderline hypertension and hypertension. However, its occurrence in normotensive humans is inconsistent because of lesser magnitude than in hypertensive individuals and compensatory mechanisms such as the Baroreflex, which are activated in normotensive.<sup>5</sup> JR Macdonald et al had found no difference in magnitude of hypotension following 30 min of cycle ergometry at 50% and 75% VO<sub>2</sub> peak in normotensive volunteers.<sup>5</sup> In contrast Pescatello et al were unable to document PEH in a normotensive.<sup>6</sup> These findings show that the effect of dynamic exercise on blood pressure in normotensive subjects is less clear. Our study has been conducted on healthy young normalweight adolescents aged 18-25 yrs and this could be possible reason for no significant results after a single exercise session.

Victor Ronald et al. found no significant difference in mean arterial pressure after rhythmic handgrip exercise at 10, 30 and 50 % of MVC and mild two-arm cycling at 0 to 20 watt, but at 40 and 60 watt intensity two arm cycling shows significant difference in arterial pressure response as compared to baseline values in recovery period.<sup>7</sup> Present study shows significant difference in systolic blood pressure and mean arterial pressure in Preobese adults after a single bout of 10 minutes isotonic handgrip exercise at maximal intensity.

Nilesh Patel et al. study showed no significant difference in blood pressure due to normotensive adolescents and lower intensity isotonic handgrip exercise with more relaxation as compared to contractions.<sup>1</sup> Current study also has no significance result in normal

weight group but has significant difference in Preobese group due to higher intensity exercise with more contraction duration as compared to relaxation during exercise session.

A recent meta-analysis of 18 prospective cohort studies has shown elevated the risk of cardiovascular disease (CVD) by 1.55 (relative risk, RR), coronary heart disease (CHD) by 1.50, and stroke by 1.71 in pre-hypertension group compared to normotensive group. It is one of the most common modifiable risk factors in CVD. Mean reduction of 3.0 mmHg in SBP could reduce mortality from CHD by 6% and from stroke by 9%. Another subanalysis for healthy and hypertensive studies showed mean reduction of 4.03 mmHg in SBP and of 2.96 mmHg in DBP.<sup>8</sup> The current study also reported significant reduction in SBP and MAP in Preobese groups in both genders.

Isotonic forearm exercise produces less of demand on heart than continuous isometric exercise. Marjorie et al. found that cardiovascular response produced by isotonic exercises is intensity dependant and exercise sessions which includes relaxation between contractions shows lower cardiovascular response.<sup>9</sup> Many studies done as rhythmic handgrip exercises show significant rise in Muscle Sympathetic Nerve Activity (MSNA) and blood pressure during exercise sessions of different intensity but post-exercise response is less significant.<sup>7</sup> In the present study, we have used protocol for isotonic handgrip exercise with less relaxation between contractions and which is probably responsible for the exercise to produce cardiovascular changes in post-exercise period specially in preobese adults.

Three mechanisms are thought to be responsible for the neural cardiovascular modulation during voluntary muscle contractions: an activation of higher brain centers ("central command") as well as reflex activity primarily involving inputs from chemo- and mechanoreceptor ("muscle metaboreflex") and baroreceptor afferents ("baroreflex"). The influence of each mechanism on the heart rate and blood pressure response to exercise depends on factors like recruited muscle mass, muscle fiber type, exercise intensity and the exercise mode.<sup>10</sup>

When oxygen delivery to active skeletal muscle is insufficient to meet the metabolic demands during dynamic exercise, metabolites (e.g., lactic acid, adenosine, potassium, diprotonated phosphate, and arachidonic acid products, among others) accumulate within the active muscle and stimulate group III and



IV afferent neurons. These sensory neurons project to the medulla oblongata and their activity elicits reflex increases in sympathetic nerve activity and systemic blood pressure in an effort to enhance blood flow to the ischemic muscle. Termed the muscle metaboreflex, this response is thought to provide important functional links between metabolism in active muscles and central hemodynamics during exercise. Our results show that increasing workload shifts the muscle metaboreflex threshold to higher blood flow levels and therefore reduces the change in blood flow necessary to elicit a reflex pressor response.<sup>11</sup>

### Conclusion

Present study reveals that a single bout of an isotonic handgrip exercise performed by dominant hand at an intensity of MVC using a **smiley ball** with contraction frequency of 20/minute for 10 minutes can produce post-exercise hypotension into the post-exercise period. However, further studies are required to determine if such exercise form can produce PEH in the hypertensive population and to study the long-term effects of training on blood pressure with such form of exercise in larger population.

**Study limitations and Future Perspective:** A major limitation of the study was that the participants were also normotensive individuals. Thus, it is essential to study the effect in Prehypertensive and hypertensive population. It is also required to determine if PEH can be produced by lowering the intensity, contraction cycle rate and duration of exercise. And finally, it is essential to study the effects of isotonic handgrip exercise training on blood pressure and cardiovascular autonomic functions in larger groups.

**Ethical Clearance:** Taken From Sumandeep Vidyapeeth Institutional Ethics Committee (SVIEC).

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**Conflict of Interest:** Nil

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# Expired Air Carbon Monoxide Levels as a Marker of Passive Smoking In Pregnant Women

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## Abstract

**Background and Aim:** Smoking directly or indirectly has serious impact on health of an individual. During smoking various chemicals and toxic substances are released into the air such as nicotine, carbon monoxide which can be inhaled by the people nearby indirectly. Pregnant mothers may passively inhale carbonmonoxide when exposed to such environment. Maternal Carbon monoxide passes through placenta and enters foetalblood combine with foetalhaemoglobin forming foetal carboxy haemoglobin. Foetal carboxy haemoglobin in turn makes oxygen unavailable to foetal tissues which could be the reason for various poor outcomes in unborn foetus such as low birth weight, intra uterine growth retardation with higher rates of perinatal mortality. This study is aimed at measuring the expired air carbon monoxide levels in passively smoking pregnant women that can be correlated with exposure to passive smoking.

**Method and Materials:** 100 antenatal women of gestational age between 26 to 34 weeks with and without exposure to passive smoking were measured for carbon monoxide levels in their expired air using simple, portable non invasive carbon monoxidemonitor.

**Statistical Analysis:** Data analysis was done with unpaired student t test. P value of <0.05 was taken to be significant.

**Results:** No significant statistical difference is observed between cases and control groups in expired aircarbon monoxide levels.

**Discussion and Conclusion:** The lesser half life of carbon monoxide in exhaled air. The longtime interval between the exposure and the time of testing, the metabolic changes associated with pregnancy could have made this test inconclusive. Though gold standard blood analytic method cannot be replaced by this breath monitors,it can be used as a simple portable noninvasive tool to educate pregnant women to avoid exposure to passive smoking.

**Keywords:** Expired air carbon monoxide, pregnant women, passive smokers, foetal carboxy haemoglobin.

## Introduction

According to the international consultation on environmental tobacco smoke (ETS) and child health,

maternal smoking is associated with many harmful effects on the outcome of unborn foetus. Low birth weight and growth retardation are common complications<sup>1</sup>. There is also 33% increase in perinatal mortality, 50% increase in idiopathic mental retardation. Other complications include sudden infant death syndrome<sup>2</sup>, attention deficit hyperactivity disorders and premature delivery. The components emitted during smoking cross the placenta and act as mutagens resulting in childhood cancers like acute lymphocytic leukemia and lymphoma<sup>3</sup>. In countries like India though maternal smoking is not as common

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as western countries indirect exposure to smoking is not uncommon. There is evidence that passively smoking pregnant women show harmful effects on outcome of pregnancy as those of smoking mothers. These include miscarriage, stillbirth, congenital malformations, low birth weight, intrauterine growth retardation, sudden infant death syndrome, and preterm delivery<sup>4</sup>.

The so called passive smoker inhale the side stream smoke arising from burning end of the cigarette which contributes to 85% of smoke present in the room. This side-stream smoke appears to have the lethal toxic gases in considerably higher concentrations than in the mainstream smoke<sup>5</sup>. Carbon monoxide and nicotine are the common gases emitted during smoking. On average 4% carbon monoxide is present in cigarette smoke<sup>6</sup>. In a smoke filled room a non smoker is exposed to 25-100 ppm carbon monoxide.

Carbon monoxide combines with maternal haemoglobin and form carboxy haemoglobin which in turn crosses the placenta and leads to foetal hypoxia. Nicotine in the smoke causes vasoconstriction aggravating foetal hypoxia. Nicotinic effects may be due to inappropriate stimulation of nicotine cholinergic receptors and its neuroteratogenicity<sup>7</sup>.

Carbon monoxide is a colourless, odourless gas produced from incomplete combustion of fossil fuels. Exhaust fumes from the vehicles, malfunctioning heaters, poorly ventilated fires and exposure to tobacco smoke are some of the commonest sources of carbon monoxide production. It enters the body mainly by inhalation. Haemmetabolism contributes to some of its endogenous production<sup>8</sup>. Carbon monoxide dissolves in the plasma and binds with haemoglobin, the oxygen transporting pigment. It has high affinity for haemoglobin about 240 times that of oxygen, shifting oxygen dissociation curve to left<sup>9</sup>. This results in unavailability and utilization of oxygen by the tissues. In foetus the affinity is still more about 172 times. This accounts for at least some of its toxic effects<sup>10,11</sup>. There is a close relationship between concentration of carbon monoxide in the expired air and maternal blood carboxyhaemoglobin (% COHb) concentration<sup>12</sup>.

The carbon monoxide is eliminated in exhaled breath due to dissociation of carboxyhaemoglobin allowing free carbon monoxide to be present in the expired air. This can be employed in estimating carbon monoxide levels in the blood by indirectly measuring

exhaled CO<sup>13</sup>. Also there is a direct link between the level of CO in an expectant mother's breath and the level of CO in her unborn foetus blood. This is known as "Fetal carboxyhaemoglobin" (%FCOHb)<sup>14</sup>. Proportion of **FCOHb** level is higher when compared to maternal COHb. This level has been found to be on average 1.8 times higher in the baby than in the mother<sup>15</sup>

Various methods are used to assess smoking exposure such as assays of cotinine (the major metabolite of nicotine) in urine and blood, hair nicotine, breath carbon monoxide analysis and self reporting. Measure of expired air carbon monoxide levels is a rapid, non-invasive and cheap method to assess exposure to passive smoking and can be used safely for antenatal clinic settings<sup>16</sup>. Breath CO monitors measure carbon monoxide in parts per million (ppm) in expired air and display breath concentration of CO reading along with its corresponding blood concentration levels.

Therefore, the present study is aimed at measurement of expired air carbon monoxide level (ppm) in passive pregnant smokers and corresponding % carboxy haemoglobin level (blood CO) using the non invasive Carbon monoxide check+ instrument.

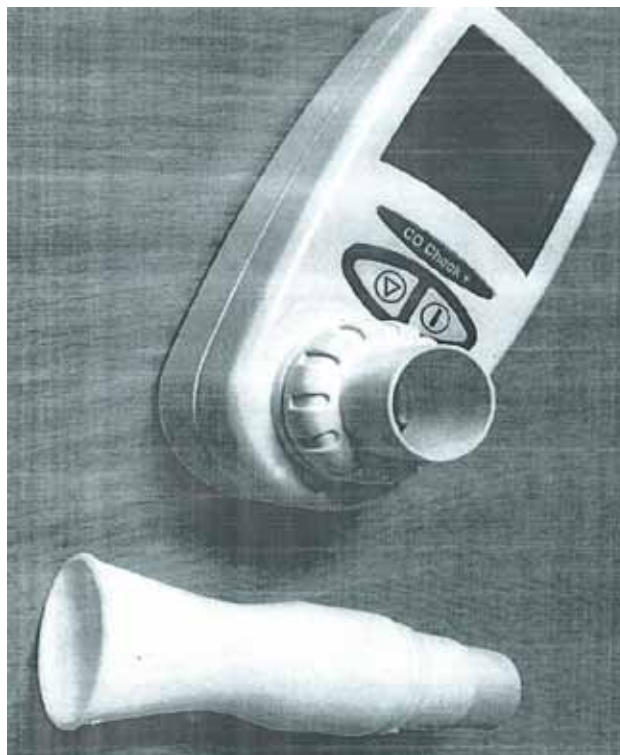
## Materials and Method

### Study Design: Prospective case control study

This study conducted in August 2014 in Govt Egmore maternity hospital after institutional ethics clearance. 100 pregnant women in the gestational age group between 26-34 weeks were selected randomly from morning antenatal clinic and classified into 2 groups those exposed to passive smoking (Exposure to other people's tobacco smoke) and those not exposed to passive smoking. **Inclusion criteria** for study selection: Pregnant women with gestational age 26-34 weeks (exposed as well as non exposed to passive smoking) **Exclusion Criteria:** Pregnant women who are active smokers, those exposed to cooking wood fire, known chronic obstructive pulmonary disease, pulmonary tuberculosis, acute or Chronic lung diseases, and other maternal related or general illness. All the participants included were informed about the study and a written and informed consent were obtained from them. They were subjected to complete general and systemic examination. The study and control group were subjected to non invasive assessment of expired air CO levels using CO check + instrument. After comfortably seated in the chair for a while the subjects were asked to inhale

deeply, hold the breath for 15 sec and exhale slowly into the disposable mouth piece attached to the CO check + instrument. Breath carbon monoxide reading were noted and reported as parts per million (ppm) .The CO Check + instrument detect carbon monoxide gas by means of an electrochemical gas sensor. They incorporate a graphic LCD displayer that display the carbon monoxide concentration level in a numerical format and/or colored indicators that correspond to various concentration

ranges like green 0-6 ppm indicates no exposure, yellow 7-10ppm indicates mild -moderate exposure and red 10-20ppm indicates severe exposure. The % COHb along with the results of CO she passed on to her unborn baby were also displayed in % FCOHb or foetalcarboxyhaemoglobin. All measurements were backed up with an appropriate colour code to show the risk of exposure. The sensitivity of the instrument is as low as 1 ppm.



**Figure 1: Carbon monoxide check plus instrument**

**Statistical Analysis:** The results of the above tests were evaluated statistically using Statistical Package for the Social Sciences (SPSS) software version 21.

**Results**

The mean and standard deviation of the variable were determined for the two groups. Independent student t test was employed as the Test of significance at 95% confidence interval and P value < 0.05 was considered as significant.

**Table 1: Comparison of CO in ppm in exhaled air between exposed and non exposed**

Variable	Group	N	Mean	SD	P value
CO (ppm)	Exposed	50	2.78	1.01	0.145**
	Non exposed	50	2.48	0.97	

\*\* p -value >0.05 not statistically significant

**Table 2: Comparison of % COHb in ppm between exposed and non exposed**

Variable	Group	N	Mean	SD	P value
%CO Hb(ppm)	Exposed	50	0.45	0.17	0.1186**
	Non exposed	50	0.39	0.15	
** p -value >0.05 not statistically significant					

Table 1 shows comparison of carbonmonoxide in ppm in exhaled air between exposed and non exposed to passive smoking which shows that the mean CO ppm in exposed group was  $2.78\pm 1.01$  and that of non exposed group was  $2.48\pm 0.97$ . There was no statistically significant difference between the two groups ( $p$ -value>0.05)

Table 2 shows comparison of % COHb between exposed and non exposed to passive smoking which shows mean % COHb was  $0.45\pm 0.17$  and that off non exposed group  $0.39\pm 0.15$ . There is no statistically significant difference between the two groups( $p$ -value>0.05).

### Discussion

Pregnant mothers fall under high risk groups. They transmit many substances to the unborn foetus compromising the outcome of the baby. These people when inhale carbon monoxide emitted from cigarette smoke passively tend to transmit the same to unborn foetus. Foetalhaemoglobin binds with carbon monoxide so that oxygen is made unavailable to foetal tissue. Exhaled air CO correlates better with blood CO percentage. Though many gold standard method are available to measure carbon monoxide levels, a non invasive simple method is used to find out the exposure to passive smoking in this study. The result of this study showed that expired air breath carbon monoxide analysis by breath monitors is less reliable in detecting exposure to passive smoking in pregnant women. In both case and control groups expired air CO levels were less than 6ppm which indicates no exposure ( $2.78\pm 1.01$  and  $2.48\pm 0.97$  respectively) and corresponding maternal blood % COHb< 0.96 ( $0.45\pm 0.17$  and  $0.39\pm 0.15$  respectively) shows highly insignificant results. This unreliability of the test may be related to some of the physiological changes that occur during pregnancy.

The clearance of some drugs, chemicals is altered during pregnancy which results in short half- life of carbon monoxide. This narrow window for detection

could be the reason for the negative results in this study (Koren G et al)<sup>17</sup>. The plasma half life of carbon monoxide is found to be 5 hours in air. Cotinine test is considered as most accurate method of measuring environmental tobacco exposure with longer half life about 9 hrs in pregnant women (Benowitz NL et al)<sup>18</sup>

As per the study by Frederiksen LW, Martin JE et al<sup>19</sup> shows the longer interval between an overnight sleep and the test results in the morning could explain the negative results.

Also the relatively low levels of carbon monoxide in breath during passive smoking when compared to those of active smokers may be the reason of this unreliability. Studies by Ashford KB et al<sup>20</sup> showed that passive exposure to smoking results in far lower levels of carbon monoxide in the breath compared with active tobacco smoking.

Thus the findings of this study confirmed that there is no difference in breath carbon monoxide levels between the study and the compared group. And the levels of carbon monoxide in exhaled air as measured by breath monitors can be considered as an invalid tool. But still this method can be used for screening smoking exposure among pregnant population and educating them to avoid further exposure to smoking environment. However further evaluation regarding the method of measurement to reduce passive smoking exposure among pregnant women; more vigorous biomarker detection tests to quantify the smoking exposure are needed<sup>21</sup>

This study population not included occupational environmental tobacco smoke exposure and domestic wood fire exposure which are other common causes of smoke; it should extend to this group too. Sometimes just eliciting history of chronic exposure to passive smoking in pregnant women will alert the primary health care personnel to reinforce the required care.

Antenatal clinic settings represent an opportunity to motivate pregnant women for avoidance of further

smoking exposure whether passive or active. There are studies that confirm health education on the harmful effects of passive smoking was associated with a reduction of further exposure.

The limitations of this study include the use of electronic device rather than gold standard biomarkers.

### Conclusion

The result of this study concludes that analysis of expired air carbon monoxide levels by breath monitors not be useful as marker of exposure to passive smoking in pregnant women. Lesser half life of carbon monoxide, various physiological and metabolic changes associated with pregnancy, prolonged time interval between the test and exposure might have made this test inconclusive. But in many Indian scenarios the seriousness of passive exposure of smoking usually ignored among pregnant women, thus exposing unborn foetus to various serious consequences. So non invasive portable breath monitors can be used as a tool to educate pregnant women to aware of exposure to passive smoking at earliest to avoid complications in the foetus.

**Conflict of Interest:** No

**Source of Funding:** Self

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

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# Impact of Elective Percutaneous Transluminal Coronary Angioplasty (PTCA) on Heart Rate Variability in Patients with Coronary Artery Disease

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## Abstract

Coronary artery disease (CAD) is a major public health problem in India and is one of the leading causes of mortality and morbidity. Impairment of heart rate variability (HRV) has been observed in patients with CAD. Percutaneous transluminal coronary angioplasty (PTCA) is one of the common modalities of treatment of CAD. In this study we have studied the effect of successful elective PTCA on HRV parameters in patients with CAD.

**Material and Method:** HRV parameters in both time and frequency domain were studied in 30 male patients before PTCA, one day and three day after PTCA.

**Results:** - The time and frequency domain parameters between pre PTCA and 1<sup>st</sup> day post PTCA were not found to be statistically significant. Changes in the time domain parameter rMSSD and SDNN was statistically significant between pre and 3<sup>rd</sup> day post PTCA but no significant difference was observed in pNN50. A significant decrease in LF n.u and increase in HF n.u was observed between pre and 3<sup>rd</sup> day post PTCA.

**Conclusion:** Revascularization with PTCA results in improvement of the autonomic tone. Improved autonomic modulation is seen as early as 3<sup>rd</sup> day post PTCA. It indicates that restoring the blood flow reverses to some extent the autonomic modulatory effect of coronary artery disease.

**Keywords:** Coronary artery disease, heart rate variability, PTCA

## Introduction

Heart rate variability (HRV) is defined as the physiological variation in the duration of intervals between sinus beats<sup>1</sup>. The property of automaticity is intrinsic to the pacemaker tissue of the heart but the rate

and rhythm are influenced by the autonomic nervous system. HRV reflects the activity of the divisions of the autonomic nervous system on the heart rate (HR)<sup>2</sup>.

HRV is a non invasive, affordable and reproducible technique to measure the autonomic function. HRV is altered in conditions such as obesity, diabetes mellitus, asthma and also coronary artery disease(CAD).

Coronary artery disease is a major public health problem in India and is one of the leading causes of mortality and morbidity<sup>3</sup>. Impairment of HRV in CAD has been extensively studied and has been attributed to arrhythmias and risk of sudden cardiac deaths<sup>4,5,6</sup>. PTCA is one of the common modalities of treatment of CAD. Successful PTCA results in improved left ventricular

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(LV) systolic function, reduced anginal symptoms, increased exercise capacity and an increase in survival<sup>7,8</sup>.

However, there is limited information on the effect of successful PTCA on the recovery pattern of HRV. The importance of studying the recovery pattern of HRV following PTCA is to understand the mechanisms involved in arrhythmias and reperfusion injuries. Variable alterations in HRV have been documented in patients after undergoing PTCA. Some studies have shown a decrease in the parasympathetic tone associated with or without an increase in the sympathetic tone following revascularisation<sup>9,10</sup>.

The reason for these variations may be due to incomplete understanding of the manner in which obstruction to coronary artery and its reperfusion affects HRV.

Thus, in this study, influence of successful elective PTCA on the parameters of HRV in patients with uncomplicated CAD was evaluated. In addition, the alteration in HRV based on the artery involved is also studied.

## Material and Method

Male patients admitted to the hospital with TMT positive for inducible angina and angiography with a single vessel block of more than 70% were screened. Patients in the age group of 40-65 years and satisfying the exclusion criteria were recruited for the study. The control group consisted of age, sex and anthropometrically matched TMT negative subjects. The patients with diseases or on drugsthat altered the autonomic functions, athletes and obese individuals were excluded from the study.

The sample size of 30 was determined based on the projected availability of patients during the period of study.

Detailed protocol of the study, extent of their involvement, the right to terminate during the study and the possible complications was explained in patient's own language. An informed written consent was obtained from the participants.

Detailed history was taken followed by clinical examination of the subject and the findings were confirmed by the attending physician/cardiologist. Their

height was measured in meters using a stadiometer and weight was measured in kilograms using sensitive balance. BMI was calculated by using the formula weight in Kg/height in meter<sup>2</sup>. Waist circumference was measured at a level midway between the lowest rib and the iliac crest, the hip circumference at the level of the great trochanters and Waist hip ratio was calculated.

The HRV recordings were done between 9.00am to 12.00 noon of the day in a quiet semi-dark room to avoid the bias of changes due to circadian rhythm. HRV recordings were done a day before, 24 hours after and on the third day after PTCA.

The patient scheduled for HRV recording was instructed to abstain from rigorous exercise, consumption of alcohol, stimulants beverages like coffee or tea and smoking. The patient was rested for period of 15 minutes in supine position. 5 min ECG was recorded by placing the electrodes in right infraclavicular, left infraclavicular and left iliac regions. The ECG was analyzed using RMS Vagus HRV software (RMS, India).

The analysis from the HRV software provides information about time domain and frequency domain parameters.

**Statistical Analysis:** The data was expressed as mean + standard deviation. One way analyses of variance (ANOVA) were used to test the difference between groups.

## Results

The mean age of our study population was 53.97±6.42yrs. The mean BMI was 25.69±2.57kg/m<sup>2</sup> and 0.88±0.87 was the mean waist hip ratio. The resting heart rate was 88.77±4.20 beats/min.

The time and frequency domain parameters between before PTCA and 1<sup>st</sup> day post PTCA were not found to be statistically significant.

Time domain parameters rMSSDand SDNN was statistically significant between pre and 3<sup>rd</sup> day post PTCA but no significant change was observed in pNN50. A significant decrease in LF n.u and increase in HF n.u was observed between pre and 3<sup>rd</sup> day post PTCA.

No statistical significance was found between the involved artery and alteration of HRV.

**Table No. 1: Comparison of HRV pre, 1<sup>st</sup> and 3<sup>rd</sup> day post PTCA**

HRV parameters (n=30)	Pre PTCA	Post day 1	Post day 3
HR (beats/min)	88.77±4.20	89.60±2.71	84.73±3.89
SDNN(ms)	20.08±7.43	19.12±9.04	26.30±10.40
rMSSD	12.62±7.77	12.29±7.53	19.69±10.08
pNN50	1.92±4.19	1.78±5.50	2.34±6.17
LF ms <sup>2</sup>	51.50±44.95	49.50±39.55	42.70±33.93
HF ms <sup>2</sup>	21.47±19.00	23.00±22.74	36.26±34.95
LF (n.u.)	67.95±14.12	65.67±14.89	55.06±15.60
HF (n.u.)	31.98±14.12	34.28±14.91	44.83±15.77
LF/HF ratio	2.70±1.26	2.38±1.24	1.56±1.16

**Table No. 2: Comparison of HRV parameters pre, 1<sup>st</sup> and 3<sup>rd</sup> day post PTCA**

Dependent Variable	Comparison between	Mean Difference	Std. Error	Sig.	
SDNN(ms)	Pre	Post Day 1	0.959	2.33	1.000
		Post Day 3	-6.22	2.33	0.028
rMSSD	Pre	Post Day 1	0.33	2.20	1.000
		Post Day 3	-7.07	2.20	0.006
pNN50	Pre	Post Day 1	0.14	1.38	1.000
		Post Day 3	-0.42	1.38	1.000
LF (n.u.)	Pre	Post Day 1	2.27	3.84	1.000
		Post Day 3	12.88	3.84	0.004
HF (n.u.)	Pre	Post Day 1	-2.30	3.86	1.000
		Post Day 3	-12.84	3.86	0.004
LF/HF Ratio	Pre	Post Day 1	0.32	0.31	0.925
		Post Day 3	1.14	0.31	0.002

## Discussion

Alteration of HRV in CAD has been implicated in various adverse outcomes like arrhythmias and also death. Ischemia and infarction resulting from CAD are known to alter the functioning of the autonomic balance to the heart. However, little information exists on the modulation of autonomic system following PTCA, which is a common modality of treatment of CAD.

We have studied the effect of successful PTCA on the parameters of heart rate variability a day before, one day and three day after the procedure. Both the time domain and frequency domain parameters were assessed. In the time domain, SDNN, rMSSD, pNN50 were assessed and in the frequency domain L.F, H.F, and LF/HF ratios were analysed.

Time domain parameter SDNN assesses overall variation in the heart rate. rMSSD estimates the vagally mediated changes reflected in HRV, pNN50 correlated with parasympathetic nervous system activity. Frequency domain parameter L.F reflects baroreflex activity, H.F reflects parasympathetic activity and LF/HF ratio reflecting the sympathovagal balance<sup>11,12</sup>.

In our study, there was a significant improvement in rMSSD and SDNN accompanied by decrease in LF nu and increase in HF nu three day post PTCA. This indicates a decrease in sympathetic tone and a shift in the autonomic balance towards parasympathetic system.

In a similar study by Ali Aydinlar et al, found SDNN, rMSSD and HF to be increased and LF to be decreased immediately after PTCA indicating an increase in

parasympathetic tone associated with a decrease in sympathetic tone<sup>13</sup>.

A study by Agnieszka Janowska-Kulińska et al analysed the effect of PTCA before and 24 hours the procedure found a decrease in HF and increase in LF/HF ratio indicating an increase in the sympathetic tone and decrease in the balance between sympathetic and parasympathetic tone. They attributed this to changes induced by myocardial reperfusion which produced a pressure overload and strain on the myocardial fibers. Denervation of the myocardial nerve endings due to accumulation of potassium, adenosine, free radical and mitochondrial loading with calcium has also been hypothesized<sup>14</sup>.

Similar findings were reported in a study by Kanadasi et al. They assessed the HRV parameters 24h, 10 days and 30 days after PTCA and found a reduction in HRV 24 h after PTCA which was more significant in patients with previous Myocardial infarction. Recovery was only observed 10<sup>th</sup> day post PTCA<sup>9</sup>.

Variable alterations in HRV have been observed in the above studies with some showing changes in the autonomic regulation as early as 24 h after the procedure. Our study has shown an increase in the HF nu one day post PTCA. However statistical significance has not been achieved. Ethnicity could be implicated for the delayed recovery as Indians are more prone to CAD and at an earlier age than the western population. Further research is needed in this direction.

Studies by Tseng et al, Sedziwy E et al, Wennerblom B et al have shown a more gradual recovery of the HRV. Tseng et al showed an improvement in the sympathovagal balance 1 month post PTCA. Sympathovagal imbalance did not correlate with the severity of the CAD score in this study<sup>15</sup>. Sedziwy E et al<sup>16</sup> showed increase in parasympathetic control of the heart rate two weeks after successful PTCA. Beneficial increase influenced both parts of the autonomic nervous system.

Wennerblom B et al<sup>10</sup> studied the effect of complete revascularisation on the HRV parameters 1 month and 6 months after PTCA in patients with no previous myocardial infarction. They found partial normalization of vagal tone at 6 months post PTCA. Thus, concluding that ischemia may not be the only mechanism causing a reduction in HRV.

Incomplete restoration of the autonomic balance

could be due to still unrecognised factors influencing HRV other than the blood supply.

Location of the involved artery did not significantly alter the HRV parameters in our study. Similar results have been reported by Kanadasi et al<sup>9</sup>. However Agnieszka Janowska-Kulińska et al have reported statistically significant decrease of HF accompanied by an increase in LF/HF following angioplasty of the right coronary artery (RCA). PTCA of left anterior descending (LAD) caused no significant changes in the HRV indices and in circumflex artery there was significant decrease of time domain indices and a marked increase of the LF/HF ratio<sup>13</sup>.

Improvement in the autonomic balance in the above mentioned studies have occurred over varying time periods. Although the exact mechanism underlying these is not known, one can hypothesize these to be attributed to the differences in the selection of patient groups. Some studies included patients with previous MI and patients with complicated CAD while these being excluded in others. Presence or absence of diabetes mellitus which is known to modify autonomic function, could also have influenced the results. Presence of collateral circulation could be another factor influencing the results, as presence of collaterals would not cause a marked change in HRV parameters following restoration of blood flow. The reasons for these discrepancies could also be due to not completely understood underlying mechanism of HRV changes associated with myocardial ischemia and improved perfusion.

From this study we can conclude that myocardial ischemia is not the only mechanism altering the HRV since recovery of HRV does not take place immediately post PTCA. Delayed and incomplete restoration of HRV could explain prolongation in the recovery of the patients and susceptibility to arrhythmias post PTCA.

## Conclusion

Revascularization with PTCA results in improvement of the autonomic tone. Improved autonomic modulation is seen as early as 3<sup>rd</sup> day post PTCA. It thus indicates that restoring the blood flow reverses to some extent the autonomic modulatory effect of coronary artery disease.

Limitations of the study – The present study is of a short duration. A longer follow up of the patients is required to study the recovery pattern of HRV following PTCA.

**Ethical Clearance:** Taken from institutional ethical committee in M S Ramaiah Medical College

**Source of Funding:** Nil

**Conflict of Interest:** Nil

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# Effect of Progressive Muscle Relaxation on DASS Score and Reaction Time

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## Abstract

**Background:** Reaction time is a reliable indicator of rate of processing of sensory stimuli by central nervous system and its execution in the form of motor response. It is found to be affected by factors like physiological stress, mood and diseases like neuropathy & nerve injury. Various relaxation interventions have shown to reduce stress, but there are fewer reports about the effect of progressive muscle relaxation on stress and reaction time.

**Objective:** The present investigation was undertaken to study the effect of progressive muscle relaxation on visual and auditory reaction times (RTs) & on Depression Anxiety Stress Scale (DASS) Score.

**Method:** 66 students who had moderate to severe stress & who met the inclusion & exclusion criteria were enrolled in the study and were randomly divided into 2 groups- subjects & controls. 33 subjects were given progressive muscle relaxation training daily for 6 weeks. DASS score, visual & auditory reaction time were recorded before & after the training period. The readings were compared within the group & also with the age matched 33 controls who did not receive Progressive muscle relaxation training.

**Results & Interpretation:** In the study group, there was decrease in the DASS score after the training. There was a significant decrease ( $P < 0.001$ ) in visual RT (from  $255.4 \pm 58.0$ ms to  $208.6 \pm 37.8$ ms) as well as auditory RT (from  $225.2 \pm 061.4$ ms to  $187.9 \pm 038.5$ ms). whereas the change in reaction time & DASS score was not significant in the control group.

**Conclusion:** Decrease in DASS Score & reduction in reaction times following progressive muscle relaxation technique, shows that it is a very effective tool in decreasing the stress levels in individuals. Progressive muscle relaxation is an easy and inexpensive method as it may help students to concentrate better & excel in their studies.

**Keywords:** Stress, Reaction time, Progressive muscle relaxation, DASS Score.

## Introduction

Stress has become an inevitable component of one's life. The term "stress", coined by Hans Syle is defined as "the non-specific response of the body to any demand for

change." Gratuitous levels of stress results in detrimental consequences on the quality of life of an individual. These negative effects are manifested in physical health, learning ability and retention, interpersonal relationships, and behavior.<sup>1</sup> Some individuals even indulge in self-destructive coping mechanisms such as alcohol consumption or smoking in order to alleviate these effects of stress and anxiety. In order to prevent these unhealthy behaviors, various relaxation techniques have been used by the researchers and it has been shown that these techniques help the individuals in positive coping and also been shown to reduce stress.

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Method for relaxation can range from various breathing patterns to pleasant visual imagery exercises. These methods are also widely used to treat a variety of health problems including headaches, anxiety, and insomnia. Not only do relaxation techniques reduce bad habits and treat some common health problems, but they have also been proven to be effective for relieving stress and anxiety.<sup>1</sup>

Reaction time (RT) is a reliable indicator of rate of processing of sensory stimuli by central nervous system and its execution in the form of motor response. Auditory & visual reaction times are the frequently used RTs; shorter RT means better performance which is indeed a prerequisite for surgeons & other professionals. It is altered by number of factors; stress being one among them. Psychological distress such as stress, anxiety and depression are frequent among medical students the reason being peer pressure and academic burden which can affect their academic performance, physical health and psychosocial wellbeing.<sup>2</sup> 1<sup>st</sup> year medical students are exposed to huge syllabus in a shorter span of time, that induce stress in them. Though stress in minimal level is facilitatory to the brain, increased stress can be detrimental; it can manifest as Anxiety/Depression disorders. Increased stress increases reaction time & decreases the attention span & concentration.<sup>2</sup>

There is a need for intervention that can not only decrease stress, but also improve reaction time. In this measure, lot of interventions has been tried in the form of meditation, music, yoga, etc. Progressive muscle relaxation developed by Edmund Jacobson, involves tensing & relaxing the muscles, one group of muscle/one body part at a time, to bring about a feeling of physical relaxation.<sup>3</sup> Different relaxation techniques have shown to reduce stress, but less has been proven about the effect of this less known technique of progressive muscle relaxation on stress and reaction time.

Hence this study was designed to explore the effect of progressive muscle relaxation technique on

psychological stress and reaction time in 1<sup>st</sup> year Medical students.

Subjective assessment of stress was done by self rated DASS Score. It is a reliable indicator of the level of stress and is also easy to administer. DASS Questionnaire is a set of three self report scales designed to measure the negative emotional states of depression, anxiety and stress with 7 items per scale.<sup>4</sup> Reaction time measurement was used as objective assessment method to see the effect of Relaxation technique.

## Materials and Method

The present study was undertaken in a medical college in south India. The study was approved by the institutional ethics Committee. The informed written consent was taken from all the subjects who volunteered to participate in the study. 150 students were given the DASS questionnaire & proforma to fill. 130 students returned the filled forms.

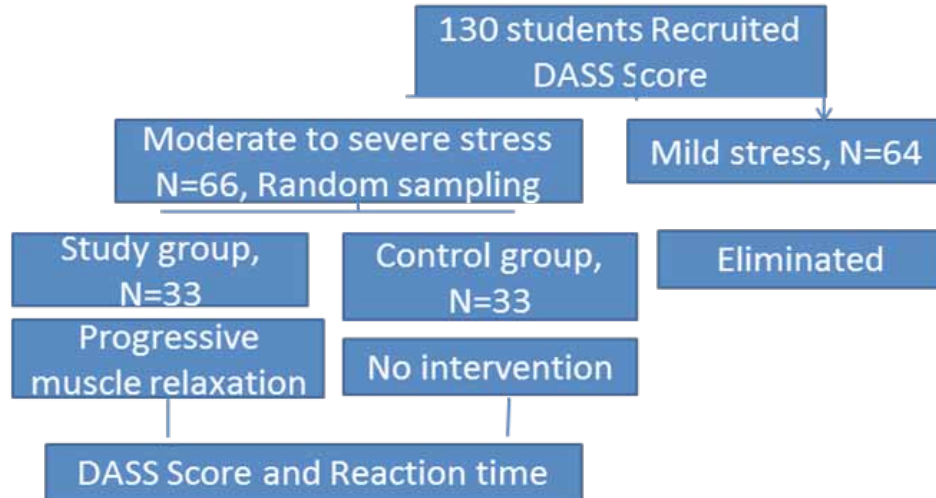
Detailed medical history of subjects was taken, which included history of present illness, past history, personal history (diet, sleep pattern, exercise & sports habits, vehicle driving, addiction). They were further recruited based on the following criteria:

**Inclusion Criteria:** Students who had moderate to severe stress scores that is between 19-33 scoring according to DASS score for stress.

Subjects aged between 17 – 21 yrs who gave informed consent.

**Exclusion Criteria:** Any medical or surgical disease (hypertension, diabetes, tuberculosis, psychiatric disorders, head injury, epilepsy, drug therapy etc.) E.N.T. diseases and ophthalmic diseases which would affect reaction time of the individual.

The following protocol was used.

**Study Protocol:**

**Reaction time recording:** The reaction time was recorded in a calm cool place between 11.00 am -12.30 pm hours of the day. **Reaction Timer:** The device used in the present study to measure Auditory and visual reaction time is a PC1000 reaction timer. PC1000 is a 1000 hertz square wave oscillator which has a soft key for 'start' and 'stop' function. This instrument has two components (A & B). Component (A) has a start button which is handled by the examiner only, second component (B) has a stop button which is handled by the subject. Small red LED light was used as visual stimulus. A 1000 Hz tone through the head phone was the auditory stimulus. Audacity software was used which records the reaction time in 0.001sec accuracy in wave format Visual Reaction Time (VRT) Measurement: The Examiner pressed the 'start' button in the component (A) & the subject was instructed to press the 'Stop' button in component (B) with the right index finger first as soon as he/she sees the red light in the instrument. Visual Reaction time was recorded in audacity software.

**Auditory Reaction Time (ART) Measurement:** The Examiner pressed the 'start' button and the subject was instructed to press the stop button with the right index finger first as soon as he hears the sound (1000 hertz's tone) through the head phone connected to it. Auditory Reaction time was recorded in Audacity software.

Five trials were given for measurement of VRT &

ART. The mean of the five reaction times was taken as the final reaction time.

**Progressive muscle relaxation technique:** Progressive muscle relaxation uses tension and release of muscles throughout the body to relax. It teaches how to relax the muscles through a two step process.<sup>3</sup> First, we systematically tense particular muscle groups in the body, next, we release the tension and notice how muscles feel when they are relaxed. These practice sessions were done using the video demonstration. The students were made to practice the muscle relaxation technique at the same time daily for 6 weeks under the guidance of researchers.

Students were given the handouts of the guidelines to perform the relaxation technique and also the video clipping, so as to facilitate them to continue their relaxation techniques at home/hostel in case of holidays or their absence.

**Results**

66 students were randomly divided into two groups as cases & controls with 33 members in each. Cases were those who practiced the progressive muscle relaxation technique for 6 weeks. Controls were those who did not practice relaxation.

Earlier to intervention the reaction time was not significantly different among both the groups. (Table 1).

**Table 1: Pre intervention reaction time in cases & controls**

Reaction time (in Milliseconds)	Cases	Controls	p value
Visual	255.4 ±58.0	255.0± 60.5	0.98
Auditory	225.2 ± 61.4	231.2± 48.2	0.66

p Value < 0.05 is statistically significant

**Table 2: Reaction time in Controls**

Reaction time (in Milliseconds)	N	Mean	Std. Deviation	p value
Visual Reaction time pretest	33	255.0	60.515	0.13
Visual Reaction time posttest	33	260.0	57.371	
Auditory Reaction time Pretest	33	231.2	48.293	0.86
Auditory Reaction time Posttest	33	232.1	47.125	

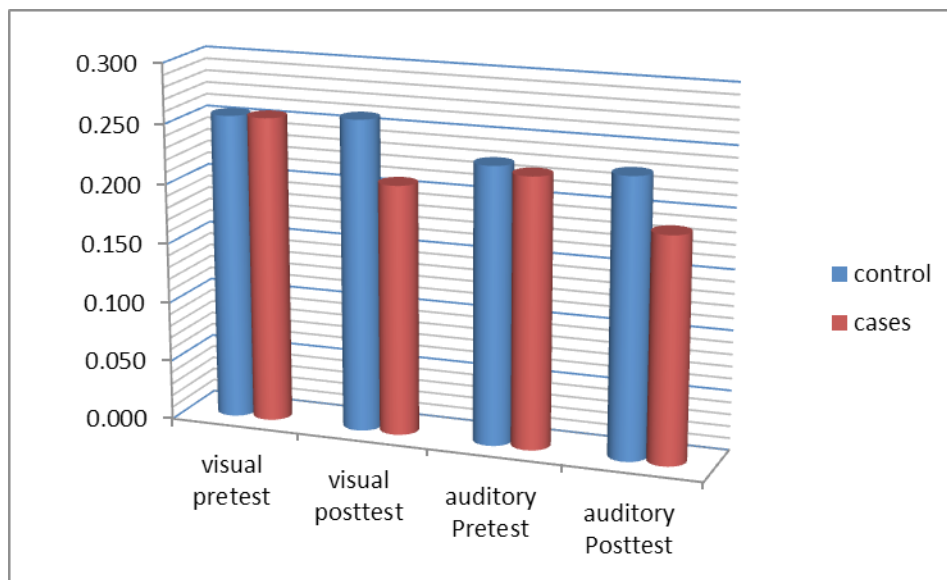
**Table 3: Reaction time in Cases before(Pre) & after(Post) the intervention**

Reaction time (in Milliseconds)	N	Mean	Std. Deviation	P value
Visual Reaction time pretest	33	255.4	58.478	0.01
Visual Reaction time posttest	33	208.7	37.828	
Auditory Reaction time Pretest	33	225.3	61.423	0.01
Auditory Reaction time Posttest	33	187.9	38.543	

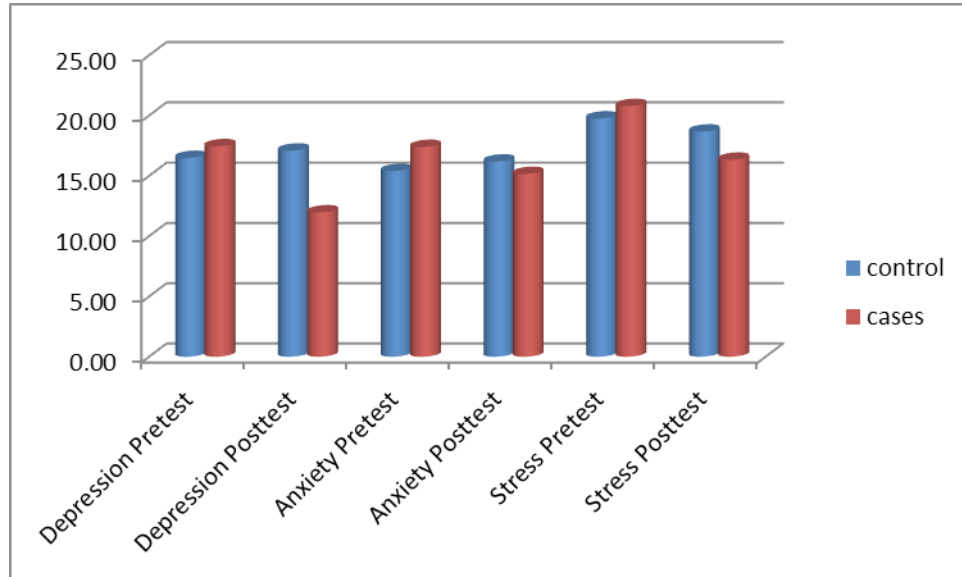
From the above tables 1, 2 & 3 it is clear that the baseline reaction times were not significantly different between the control and study group. After 6 weeks,

the reaction times decreased significantly in the study group who practiced progressive muscle relaxation as compared to controls.

**Effect of Progressive muscle relaxation on Reaction times:**





**Effect of progressive muscle relaxation on DASS Score:****Table 4: DASS Score after progressive muscle relaxation in cases in comparison with controls**

Mean Scores	Control	Cases
Depression Pretest	16.48	17.45
Depression Posttest	17.09	11.97
Anxiety Pretest	15.39	17.39
Anxiety Posttest	16.18	15.15
Stress Pretest	19.76	20.79
Stress Posttest	18.67	16.33

There was decrease in all the three scores; depression, anxiety & stress scores after PMR. The decrease in depression scores was much higher, followed by stress and anxiety scores.

**Discussion**

In the present study Pre intervention scores of reaction times and the DASS score between controls & cases were not statistically different. Post intervention, there was significant decrease in depression, anxiety & stress score in the study group. Similar findings were recorded in the study done by Praseeda P. et. al. wherein they found the post test mean score of academic stress was significantly lower than pre-test score which clearly proved that Progressive Muscle Relaxation was effective in reducing academic stress in classroom situation.<sup>6</sup>

Similar decrease in stress due to music was noted by Stephene Khalfa & others, where relaxing music decreased the Cortisol level.<sup>7</sup>

Stress is a word derived from the Latin word 'stringere', meaning to draw tight. Progressive muscle relaxation (PMR) technique is based on the premise that muscle tension is the body's psychological response to anxiety-provoking thoughts and that muscle relaxation blocks anxiety. This technique involves learning to monitor the tension in specific muscle groups by first tensing each muscle group. This tension is then released, as attention is directed towards the differences felt during tension and relaxation. PMR brings about a feeling of physical relaxation. When the body is physically relaxed, anxiety is also reduced.<sup>8</sup>

Researchers have found that medical science students have higher levels of anxiety & stress than other university students.<sup>9</sup> This could have negative effects on the student, their family members and their classmates. Thus practicing PMR on regular basis could bring down the stress & anxiety in medical students.

Studies have attempted to make correlations with

physiological and behavioral responses to stress and relaxation in order to improve performance abilities. A reduction in reaction time was seen with studies that reduced environmental stress.<sup>10</sup>

In our study, Post intervention, along with decrease in stress, there was significant decrease in both reaction times: visual & auditory. A decrease in reaction time indicates an improved sensory motor performance and could be due to an enhanced processing ability of the central nervous system. This effect on central nervous system could be due to greater and improved concentration power and inhibition of extraneous stimuli indicating better attention and less distractibility.

Similar findings were found by Borkar and Pednekar who studied the changes on Visual reaction time (VRT) and auditory reaction time (ART) before and after 4 weeks of pranayamic breathing exercises.<sup>11</sup>

Similar reduction in reaction times were also observed by Begum N., et. al. after short session of yoga, wherein the authors attributed it to the stimulation of the vagal nerves, restoring activities of Parasympathetic Nervous System (PNS) and the Gamma Amino-Butyric Acid (GABA) systems. Added to this, there is increase in the cerebral blood flow particularly in right hemisphere frontal lobe which may improve reaction time.<sup>12</sup>

Another study on yoga-based relaxation techniques in relation to attention speed and information processing showed a significant increase in scores on a six-letter cancellation task as competition. In this study by P. Subramanya and et. al., the supine rest group showed an increase in performance on tasks involving strict motor speed but not on a digit substitution task, which relies more heavily on cognitive function. This suggests differing effects of relaxation based on task type.<sup>13</sup>

Malathi and Parulkar demonstrated a significant decrease in reaction times both after single session of yogasana & also after 6 weeks of training.<sup>14</sup>

The medical students get trained to reduce their patient's stress and anxiety and thus improve patient's quality of life. In the meantime the health of medical students and their anxieties are being ignored. Practicing Progressive muscle relaxation technique can help the medical students not only in combating this anxiety and stress but also improve the reaction time, helping them in better judgment and quick action.

**Strength of the study:** The main advantage of using Progressive muscle relaxation technique which we observed was better compliance of students towards the practice of this procedure. The students could follow the steps well as it was a guided relaxation video. They neither felt sleepy nor distracted as they were actively involved in the contraction & relaxation of group of muscles as contrary to other techniques which use plain music.

## Conclusion

Our results show that practicing progressive muscle relaxation results in significant reduction in visual and auditory RTs and also the DASS Score. Thus we can conclude progressive muscle relaxation is a very effective tool in decreasing the stress levels in individuals. It will not only help students to concentrate better & excel in their studies but also is useful for sportsmen and surgeons.

**Limitations of this study:** The present study is a case-control study where the subjects were randomly selected from the population. The sample size is very small. The study can be extended to a larger population, so that results could be generalized.

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**Conflict of Interest:** None

**Sources of Funds:** Nil

**Ethical Clearance:** Institutional Ethical Committee, SIMS & RC, Bangalore.

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# Effect of Yoga on Cardio Respiratory Parameter in Medical Students

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## Abstract

The competition in medical field, unhealthy food and lack of exercise deteriorate the health of student. Yoga is the ancient technique to increase the concentration power, physical and mental well-being. We have done randomized control study on medical students between study and control group. The study group has practice yoga for 4 weeks whereas the control group hasn't. In the study group there is significantly improvement in cardio-respiratory parameters ( $p < 0.0001$ ). This study showed that regular yoga practice in medical students can decrease their stress and improve cardiac and respiratory function.

**Keywords:** Yoga, stress, medical students, cardio-respiratory parameters.

## Introduction

Medical students are confronted with a variety of life stressors from both college and home. The disproportion between sympathetic and parasympathetic discharge due to stress is associated with morbid condition like diabetes mellitus, hypertension and metabolic syndrome<sup>(1)</sup>.

Yoga is development of the union of mind and body through a combination of exercise respiration and meditation in order to achieve psychosomatic harmony<sup>(2)</sup>. Yoga consists of a holistic combination of postural exercise (Asana)<sup>(3)</sup>, relaxation and voluntary breathing exercise (pranayamas). The yoga has been studied in patient of diabetes, hypertension and post traumatic stress disorder for management<sup>(4)(5)(6)</sup>.

Hence it is pertinent to study of the effects of yoga on cardio respiratory physiology of medical students

in order to better understand its effects on healthy individuals and to provide the scientific basis for the possible use of yoga techniques as preventive and/or alternative therapy for health disorders<sup>(7)(8)</sup>.

## Material and Method

Study Design – Randomized control study

### Inclusion criteria:

1. Those who were ready to give written consent.
2. Those who were above 18 years.
3. Those who were apparently healthy on history and clinical examination.

### Exclusion criteria:

1. Those who had BMI  $< 18.5$  or  $> 24.99$
2. Those who were on any diet/exercise regime.
3. Those who were on any drugs that affect cardio-respiratory functions.
4. Those who had any disease/disorder that can affect physiological functions e.g. thyroid disorders, diabetes mellitus bronchial asthma any acute illness of respiratory or cardiovascular system.

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5. Those who were smokers and/or alcoholics.

Sample size-300

**Randomization:** Using simple random sampling, participants were allotted to intervention study group and control group.

**Intervention:**

- Four weeks yoga training program by a certified yoga teacher.
- Program included yoga training for 6 days a week with one-day rest.
- Graded yoga training schedule with weekly medical examination of subjects.

Week No.	Yoga training	Total daily duration
1	Joint relaxation exercise(JRE) for 15 minutes followed by shavasan for 5 minutes	15+5=20 minutes
<b>Weekly medical examination - 1</b>		
2	JREs for 5 minutes followed by pranayams for 10 minutes followed by shavasan for 5 minutes.	5+10+5=20 minutes
<b>Weekly medical examination - 2</b>		
3	JREs for 5 minutes followed by pranayams for 10minutes followed by suryanamaskar for 5 minutes followed by shavasan for 5 minutes.	5+10+5+5=25 minutes
<b>Weekly medical examination - 3</b>		
4	JREs for 5 minutes followed by pranayams for 10 minutes followed by suryanamaskar for 10 minutes followed by shvasan for 5 minutes	5+10+10+5=30 minutes
<b>Weekly medical examination - 4</b>		

**Pranayam included following:**

1. Anulomvilom pranayama
2. Bhramari pranayama
3. Shitali pranayama
4. Sitkari pranayama

General Parameters studied were Height, Weight and Body Mass Index. The cardiac parameter studied were Resting Pulse Rate (RPR), Resting Systolic and Diastolic Blood Pressure and Fitness Index (FI).

**Fitness index:** FI was calculated using the Harvard step test<sup>(9)</sup>.

$FI (\%) = (100 \times \text{test duration in seconds}) \div \text{by } (2 \times \text{sum of heart beats in the recovery periods})$

The Respiratory Parameters were measured using a computerized spirometer. The tests recorded were *tidal volume (TV)*, *slow vital capacity (SVC)*, *forced vital capacity (FVC)*, *forced expiratory volume in first second*

(FEV1), *FEV/FVC ratio*, *Peak expiratory flow rate (PEFR)*, *forced expiratory flow during middle 50% (FEF 25-75%)*, *Forced expiratory flow during 200-1200ml of expiration (FEF<sub>200-1200</sub>)* and *maximum voluntary ventilation (MVV)*. The respiratory parameters for efficiency measured were *Breathholding (BHT)*, *Maximum expiratory pressure (MEP)* and *40 mm Hg endurance test (40mmHg ET)*.

**Statistical analysis:**

To compare a mean of different variables each group i.e. intervention and control group (post-test value versus pre-test value), paired students test was used.

To compare mean of different variables between the groups i.e. intervention and control group unpaired students test was used.

The alpha level to determine significance was  $P < 0.05$  for all analysis.  $p < 0.05$  shall be considered highly significant.

**Findings:****Table 1: Anthropometric parameters in intervention group (paired student's test)**

Gender	Parameter	Pre yoga	Post yoga	P value
		Mean± SD	Mean ±SD	
Female (n=72)	Age(years)	18.50 ±0.71	18.50±0.71	-
	Height(cm)	156.46±5.29	156.46±5.29	-
	Weight(kg)	52.00±6.44	52.03±6.39	0.32
	BMI(Kg/m <sup>2</sup> )	21.22±2.26	21.24±2.25	0.39
Male (n=78)	Age(years)	18.60±0.62	18.60±0.62	-
	Height(cm)	170.72±7.64	170.72±7.64	-
	Weight(kg)	61.69±8.81	61.64±8.54	0.44
	BMI(Kg/m <sup>2</sup> )	21.14±2.46	21.12±2.37	0.47
All (n=150)	Age(years)	18.43±0.67	18.43±0.67	-
	Height(cm)	163.87±9.73	163.87±9.73	-
	Weight(kg)	57.04±9.14	57.03±8.96	0.72
	BMI(Kg/m <sup>2</sup> )	21.18±2.36	21.18±2.31	0.86

**Table 2: Anthropometric parameters in control group (paired student's test)**

Gender	Parameter	Pre yoga	Post yoga	P value
		Mean± SD	Mean ±SD	
Female (n=64)	Age(years)	18.09 ±0.29	18.09±0.29	-
	Height(cm)	158.94±5.51	158.94±5.51	-
	Weight(kg)	53.16±6.44	53.13±7.22	0.57
	BMI(Kg/m <sup>2</sup> )	20.97±2.04	20.96±2.01	0.62
Male (n=86)	Age(years)	18.23±0.48	18.23±0.48	-
	Height(cm)	171.00±6.43	171.00±6.43	-
	Weight(kg)	62.20±8.64	62.13±8.50	0.13
	BMI(Kg/m <sup>2</sup> )	21.22±2.23	21.20±2.19	0.15
All (n=150)	Age(years)	18.17±0.41	18.17±0.41	-
	Height(cm)	165.85±8.50	165.85±8.50	-
	Weight(kg)	58.34±9.23	58.29±9.13	0.13
	BMI(Kg/m <sup>2</sup> )	21.11±2.15	21.10±2.11	0.17

**Table 3: Cardiovascular efficiency in intervention group (paired student's test)**

Gender	Parameter	Pre yoga	Post yoga	P value
		Mean± SD	Mean ±SD	
Female (n=72)	RPR(beats/min)	75.31±5.39	73.04±5.26	<0.01
	SBP(mmHg)	116.75±8.68	114.44±5.43	<0.01
	DBP(mmHg)	75.95±4.28	74.06±4.49	<0.01
	fl (%)	69.93±12.10	71.31±10.34	<0.01

Gender	Parameter	Pre yoga	Post yoga	P value
		Mean± SD	Mean ±SD	
Male (n=78)	RPR(beats/min)	79.31±8.14	77.15±6.24	<0.01
	SBP(mmHg)	123.64±7.6	121.03±6.51	<0.01
	DBP(mmHg)	78.26±6.51	76.64±4.96	<0.01
	fl (%)	75.78±9.03	77.30± 7.14	<0.01
All (n=150)	RPR(beats/min)	77.39±7.22	75.18±6.13	<0.01
	SBP(mmHg)	120.33±8.81	117.87±6.84	<0.01
	DBP(mmHg)	77.13±5.65	75.40±4.90	<0.01
	fl (%)	72.97±10.98	74.42±9.29	<0.01

RPR – Resting Pulse Rate; SBP – Systolic Blood Pressure; DBP – Diastolic Blood Pressure; fl – Fitness Index

**Table 4: Cardiovascular efficiency in control group (paired student's test)**

Gender	Parameter	Pre yoga	Post yoga	P value
		Mean± SD	Mean ±SD	
Female (n=64)	RPR(beats/min)	76.16±5.35	76.67±4.50	0.11
	SBP(mmHg)	119.66±8.24	120.00±6.74	0.75
	DBP(mmHg)	77.25±4.21	77.03±3.80	0.77
	fl (%)	71.93±11.38	71.19±11.40	0.96
Male (n=86)	RPR(beats/min)	76.29±4.64	76.48±5.32	0.80
	SBP(mmHg)	120.95±6.54	119.16±5.93	0.07
	DBP(mmHg)	76.77±4.91	76.56±4.54	0.78
	fl (%)	77.05±7.32	75.06± 10.64	0.12
All (n=150)	RPR(beats/min)	76.23±4.94	76.56±4.97	0.54
	SBP(mmHg)	120.40±7.32	119.52±6.28	0.23
	DBP(mmHg)	76.97±4.62	76.76±4.23	0.69
	fl (%)	74.51±9.70	73.41±11.09	0.31

**Table 5: Respiratory function tests in intervention group (paired student's test)**

Gender	Parameter	Pre yoga	Post yoga	P value
		Mean ± SD	Mean ± SD	
Female(n=72)	TV(ml)	359.88±25.31	361.83±25.40	0.64
	SVC(liters)	3.10±0.45	3.47±0.57	<0.01
	BHT(seconds)	37.22±5.06	39.65±4.83	< 0.01
	MEP(mmHg)	64.11±11.24	65.31±11.58	< 0.01
	40mmHg ET(seconds)	28.04±9.57	30.40±10.21	< 0.01
	FEV1(liters)	2.50±0.52	2.62±0.71	< 0.01
	FVC(liters)	3.13±0.63	3.25±0.85	< 0.01
	FEV1/FVC(%)	79.86±2.98	81.56±3.86	< 0.01
	PEFR(L/sec)	382.72±26.63	383.88±26.40	< 0.01
	FEF25-75% (L/sec)	296.76±15.82	297.29±15.75	< 0.01
	FEF200-1200(L/sec)	346.58±14.79	347.11±14.83	< 0.01
	MVV(L/sec)	99.95±20.62	100.03±20.60	< 0.01

Gender	Parameter	Pre yoga	Post yoga	P value
		Mean ± SD	Mean ± SD	
Male (n=78)	TV(ml)	409.96±62.36	412.92±62.41	0.77
	SVC(liters)	4.00±0.53	4.30±0.50	< 0.01
	BHT(seconds)	41.77±9.08	45.01±9.54	< 0.01
	MEP(mmHg)	74.95±19.26	76.12±19.60	< 0.01
	40mmHg ET(seconds)	34.68±13.07	37.15±13.49	< 0.01
	FEV1(liters)	3.25±0.57	3.46±0.59	< 0.01
	FVC(liters)	4.07±0.66	4.30±0.71	< 0.01
	FEV1/FVC (%)	79.91±3.29	82.00±3.79	< 0.01
	PEFR(L/sec)	419.73±48.67	455.08±38.30	< 0.01
	FEF25-75% (L/sec)	298.32±16.52	298.88±16.48	< 0.01
	FEF200-1200(L/sec)	341.94±14.88	342.46±14.89	< 0.01
	MVV(L/sec)	129.99±23.95	130.42±23.94	< 0.01
All (n=150)	TV(ml)	385.92±54.27	388.40±54.55	0.69
	SVC(liters)	3.57±0.60	3.90±0.68	< 0.01
	BHT(seconds)	39.59±7.74	42.44±8.09	< 0.01
	MEP(mmHg)	69.75±16.78	70.93±17.08	< 0.01
	40mmHg ET(seconds)	31.49±11.96	33.91±12.46	< 0.01
	FEV1(liters)	2.89±0.66	3.06±0.81	< 0.01
	FVC(liters)	3.62±0.80	3.80±0.94	< 0.01
	FEV1/FVC (%)	79.89±3.13	81.79±3.82	< 0.01
	PEFR(L/sec)	419.73±48.67	420.90±48.62	< 0.01
	FEF25-75% (L/sec)	294.57±16.15	298.12±16.10	< 0.01
	FEF200-1200(L/sec)	344.17±14.97	344.71±14.95	< 0.01
	MVV(L/sec)	115.38±27.05	115.84±27.03	< 0.01

**Table 6: Respiratory function tests in control group (paired student's test)**

Gender	Parameter	Pre yoga	Post yoga	P value
		Mean± SD	Mean ±SD	
Female(n=64)	TV(ml)	357.84±26.10	358.67±25.74	0.87
	SVC(liters)	3.54±0.54	3.55±0.59	0.94
	BHT(seconds)	37.27±5.33	37.36±4.98	0.88
	MEP(mmHg)	62.91±11.06	62.30±11.19	0.10
	40mmHg ET(seconds)	27.05±9.72	27.39±10.28	0.35
	FEV1(liters)	2.80±0.52	2.75±0.60	0.45
	FVC(liters)	3.57±0.62	3.52±0.77	0.55
	FEV1/FVC (%)	78.34±3.33	78.13±3.34	0.71
	PEFR(L/sec)	390.27±26.56	390.31±26.61	0.86
	FEF25-75% (L/sec)	302.10±16.61	302.14±16.68	0.92
	FEF200-1200(L/sec)	345.81±15.00	345.29±15.90	0.21
	MVV(L/sec)	99.95±20.39	99.89±20.12	0.88



Gender	Parameter	Pre yoga	Post yoga	P value
		Mean± SD	Mean ±SD	
Male (n=86)	TV(ml)	395.08±48.07	396.56±63.02	0.86
	SVC(liters)	3.76±0.64	3.84±0.53	0.32
	BHT(seconds)	41.97±9.06	42.29±9.13	0.67
	MEP(mmHg)	72.58±14.43	73.45±18.86	0.46
	40mmHg ET(seconds)	35.20±12.86	34.95±13.32	0.68
	FEV1(liters)	2.97±0.65	3.02±0.63	0.65
	FVC(liters)	3.72±0.58	3.81±0.76	0.44
	FEV1/FVC (%)	79.93±3.31	79.06±3.27	0.10
	PEFR(L/sec)	393.34±36.12	392.85±35.96	0.12
	FEF25-75% (L/sec)	306.48±16.56	309.44±16.08	0.17
	FEF200-1200(L/sec)	349.66±15.70	350.00±15.18	0.49
	MVV(L/sec)	101.90±23.83	102.42±23.81	0.88
All (n=150)	TV(ml)	379.19±44.13	380.39±53.84	0.82
	SVC(liters)	3.67±0.61	3.72±0.57	0.43
	BHT(seconds)	39.96±8.02	40.19±8.00	0.78
	MEP(mmHg)	68.45±13.91	68.69±16.93	0.64
	40mmHg ET(seconds)	31.72±12.27	31.73±12.65	0.98
	FEV1(liters)	2.90±0.60	2.90±0.63	0.96
	FVC(liters)	3.65±0.72	3.69±0.78	0.67
	FEV1/FVC (%)	79.25±3.40	78.66±3.32	0.13
	PEFR(L/sec)	392.03±32.32	391.77±32.23	0.22
	FEF25-75% (L/sec)	304.61±16.67	306.32±16.68	0.17
	FEF200-1200(L/sec)	348.02±15.48	347.99±15.61	0.94
	MVV(L/sec)	101.07±22.37	101.34±22.28	0.89

## Discussion

**Cardiovascular efficiency parameters:** In our study RPR, SBP and DBP reduced significantly whereas Fitness Index (FI) increased significantly following yoga training in participant students compared to control group. The similar effect on cardiovascular parameters was found by Bhavanani et al wherein participants practiced pranayama such as sukhapranayams and Chandra nadi pranayama<sup>(10,11)</sup>. Many studies have reported that practice of pranayams increases parasympathetic activity and decreases sympathetic activity which may be responsible for these observed cardiac parameters<sup>(12)(13)(14)</sup>.

The calculated rate of breathing in different pranayams may have contributed to the beneficial cardiovascular effects seen in this study; previous studies have reported

that slow and deep breathing decreases the heart rate and heart rate variability possibly due to enhanced vagal tone on sinoatrial node<sup>(15)</sup>. Such enhanced vagal modulation may be responsible for decreased heart rate and blood pressure observed following yoga<sup>(16)</sup>. Bernardi et al reported that slow and deep breathing combines RR interval fluctuations with the rate of respiration and significantly increases their amplitude and thereby enhances baro-reflex competence<sup>(17)</sup>, such enhancement may have contributed to the lowering of blood pressure needs to be reduced. Thus yoga may serve as a simple cost effective adjunct in the management of hypertension in addition to the regular antihypertensive management. Jasmin et al found effect of yoga in diabetic patient to reduce pain with diabetic medication<sup>(4)</sup>.

The RPR and DBP were significantly lower post

yoga in our study. A similar decrease in RPR and DBP was reported by Bhavani et al. and Bhutkaretal<sup>(18)(19)</sup>. The main determining factor of DBP is peripheral vascular resistance which is regulated by sympathetic tone<sup>(20)</sup>. In the present study significant decrease in DBP and RPR in yoga group may be attributed to a decrease in peripheral vascular resistance due to reduced sympathetic tone. The SBP was found to be significantly lower in yoga group after training; Bhutkar et al also reported a similar decrease in SBP<sup>(19)</sup>. Interestingly Bhavanani AB et al reported an increase in SBP following fast Suryanamaskar practice which may be due to the speed of Suryanamaskar causing increasing venous return and cardiac output which resembles aerobic exercise<sup>(18)(21)</sup>.

The observed cardiovascular changes may also be due to the shavasan which is reported to reduce cardiac sympathetic modulation<sup>(22)</sup>. Yoga practices including relaxation (such as shavasan) improves autonomic modulation and enhances vagal dominance. Hence shavasan may be included in the non pharmacological management of hypertension.

**Respiratory efficiency parameter:** We found a significant increase in VC, BHT, MEP and 40 mmHg ET following yoga. While the change in TV was not statistically significant, similar results were reported by karmur et al and mahajan et al. yoga postures (asnas) such as suryanamaskar in our study increase skeletal muscle strength including those respiratory muscles<sup>(13,23)(24)</sup>. Hence lungs and thorax expand and collapse to the fullest possible. Such strengthening of the respiratory musculature contributes to the increased respiratory efficiency evidenced in our body.

**Pulmonary Function Tests:** Lung functions depend on many factors including neuropsychological factors and strength of respiratory muscles. We found that all the parameters i.e. FEV1, FVC, FEV1/FVC, PEF, FEF25-75%, FEF200-1200ml and MVV increased significantly after 4 weeks of yoga in healthy young adults' subjects, irrespective of the gender. Similar results were reported by various previous studies<sup>(18,19,23,25)</sup>. During yoga training especially during pranayama there are maximal inflation and deflation of the lungs which cause increased strength recruitment and endurance of respiratory musculature. Such maximal inflation and deflation act as a physiological stimulus causing secretion of prostaglandins and surfactants in the alveoli which thereby increase the lung compliance<sup>(26,27)</sup>. There is a reflex increases airway diameter and decreases

resistance to air flow which causes the dynamic PFTs to improve. All the participants were investigated by the same investigators under similar conditions.

## Conclusion

Yoga can be advocated as an adjunctive or alternative to conventional therapy for cardio respiratory disorders. We encourage yoga practice as physical activity during undergraduate curriculum in medical study to improve health condition of medical students.

**Conflict of Interest:** No

**Source of Funding:** Self

**Ethical Clearance:** Yes

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# Effect of Super-Brain Yoga on the Concentrating Ability of Students

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## Abstract

**Introduction:** Frontal and parietal association areas form the neural basis of concentration as evidenced by cellular studies of posterior parietal cortex in monkeys. Cerebral asymmetry, an evolutionary consequence, enhances brain output by synchronizing both hemispheres for maximal brain output facilitated by Super-brain yoga - a simple, effective technique to energize and recharge the brain, assessed with d2 test measuring attention, scanning and mental flexibility.

**Aim:** To assess the effect of Super-brain yoga on the concentrating ability of students by d2 test.

**Materials and Method:** 184 school children aged 11-13 yrs, physically fit & unaware of Super-brain yoga formed the study population.

**Methodology:** (1) d2 test consisting of: (i) **TN**: total number of characters (ii) **E1**: number of characters omitted (iii) **E2**: the number of errors (iv) **CP**: concentration performance  $CP = (TN - E2)$  was performed before and after Super-brain yoga to assess students concentrating ability. (2) Super brain yoga/yogic squats performed for 1 month.

**Results:** 'p' value < 0.05 statistical significance with paired 't' test.

**Conclusion:** Practicing Super-brain yoga for 1 month increased the concentrating ability of students.

**Keywords:** Cerebral asymmetry, d2 test, Super-brain yoga.

## Introduction

Concentration is focusing the mind on a specific issue at a given moment for learning. Frontal and parietal association areas form the neural basis of concentration as evidenced by cellular studies of posterior parietal cortex in monkeys<sup>4</sup>. Super-brain yoga by Master Chao Kok Sui with crossed hands, squatting up and down is thought to increase concentrating ability.

Cerebral asymmetry of human brain is the consequence of evolution. Complementary areas of

both hemispheres synchronize for maximal brain output and can be achieved with Super-brain yoga- simple, effective technique to energize and recharge the brain. Human brain has 100 billion brain cells which are technically called as "Neurons" & supporting cells as "Neuroglia". A complex system of neurons, **Corpus callosum** connects the brain's **Right hemisphere/Representational hemisphere/Non-dominant hemisphere** and **Left hemisphere/Categorical hemisphere/Dominant hemisphere**<sup>10</sup>.

One needs to exercise & nurture the brain just like physical exercise for the body to make the most of this miraculous tool<sup>9</sup>. The output of brain can be enhanced by creating & strengthening the synapse, junction between two neurons, through a physiological mechanism of "Synaptic plasticity". The brain can plasticize to maximal extent during childhood.

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d2 test of attention is EXECUTIVE FUNCTION TEST, categorized under neuropsychological test. The effect is assessed by d2 test measuring attention, scanning and mental flexibility. The current study focuses on the effectiveness of Super-brain yoga on the concentrating ability of students using d2 test as a key<sup>6</sup>.

### Materials and Method

A pre test and post test research design was carried out at Zilla Parishat High School, Hayathnagar, Hyderabad, Telangana. This study was granted approval by the Ethics Committee of Osmania Medical College. In this study, 184 male students of 7<sup>th</sup> & 8<sup>th</sup> classes were recruited and assigned Super-brain yoga. They were all explained about the study and motivated. Written consent was obtained from the Principal of the school. The students who were physically & unaware of Super-brain yoga and age of 11 to 13 years were included in the study, while students who were practicing Super-brain yoga and suffering with any musculo-skeletal conditions like polio, muscular dystrophies, etc., were excluded from the study.

d2 test consisting of: (i) **TN**: total number of characters (ii) **E1**: number of characters omitted (iii) **E2**: the number of errors (iv) **CP**: concentration performance  $CP = (TN - E2)$  was performed before and after Super-brain yoga to assess students concentrating ability. The test items consist of the letters *d* and *P* with one to four dashes, arranged either individually or in

pairs above and below the letter. The subject must scan across each line to identify and cross out each *d* with two dashes. The characters of letter *d* having 2 dashes above, 2 dashes below and 1 dash above and below (correct hits) are called "relevant items." All other combinations of letters and lines are considered "irrelevant", they should not be crossed out. The one-page d2 test form consists of 14 lines, each with 47 characters, for a total of 658 items. The subject is allowed 20 seconds per line.

The Superbrain yoga described by Master Chao kok Sui was practiced by students at the rate of 14 Yogic squats per day<sup>2</sup> for one month in specific hand position :i) Standing with feet slightly apart, greater than shoulder width and toes pointing forward, facing towards east. ii) Hands are crossed over each other, first left hand is crossed over upper body to take hold of right earlobe with thumb and fore finger, with thumb in front. Then right hand is crossed over left<sup>5</sup>. iii) While squatting down inhale through nose, hold the breath and exhale while coming back to standing position.

**Statistical Analysis:** Mean & standard deviation for all d2 test parameters were derived with paired 't' test 'p' value <0.05 was considered statistically significant and <0.001 as statistically highly significant.

### Results

'p' value from paired 't' test is highly significant for all the variables of d2 test for selective attention.

**Table: Comparison of d2 test parameters, before and after super-brain yoga.**

Parameters	Mean ± SD		Paired t test	p-Value
	Before	After		
<b>TN</b>	172.30 ± 80.969	203.91 ± 45.423	7.801	<b>0.001 HS</b>
<b>E1</b>	159.05 ± 64.768	119.77 ± 40.755	13.771	<b>0.001 HS</b>
<b>E2</b>	38.29 ± 35.433	25.68 ± 12.565	6.262	<b>0.001 HS</b>
<b>CP (TN-E2)</b>	132.23 ± 63.748	178.23 ± 40.755	13.914	<b>0.001 HS</b>

HS = Highly significant

TN: Total attempted E1: Number of letters omitted

E2: Errors CP (TN-E2) : Concentration performance

## Discussion

The present study reveals a significant improvement in concentration & selective attention of students after doing Super-brain yoga with 'p' value of 0.001 significance for all d2 test parameters. There is significance for all d2 test parameters ( $p = 0.001$ ). This is in agreement with "A comparative study of the effects of Super-brain Yoga & aerobic exercise on cognitive functions" the study done by Joseph Irvin Thomas, Venkatesh D<sup>2</sup> and study done by Srikanth N Jois, Lancy D' Souza, R Moulya on beneficial effects of Super-brain yoga on short term memory & selective attention of students<sup>7</sup>.

In the current study all measures of d2 test of selective attention with 'p' value of 0.001 statistical significance correlates with "Effect of repetitive yogic squats with specific hand position (Thoppukarnam) on selective attention<sup>7</sup> & psychological states" by Angelica Chandrasekaran et al.

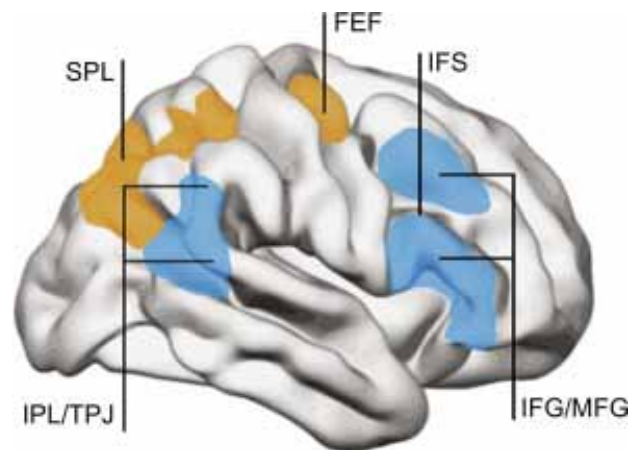
Brickenkamp and Zillmer (1998)<sup>8</sup> demonstrated that the d2 test correlates with Symbol Digit Modalities Test (Smith, 1973), Stroop Color Word Test (Stroop, 1935), and Trail Making Test Parts A and B (Reitan & Wolfson, 1985), all of which are measures of attention, scanning and mental flexibility. The construct validity and reliability of the test has been investigated with factor analysis and against criterion measures.

To demonstrate the validity of the d2 test, Bates and Lemay (2004) administered d2 test with several other neuropsychological measures to a relatively large participant sample. The tests with measures of attention, processing speed, abstract reasoning, verbal ability, visual spatial ability and working memory were employed in their study. Results were internally consistent as measured by Cronbach's alpha.

Attentional control is the ability to direct attention to only those stimuli that are relevant to our current goals, minimizing the influences that deviate to capture attention (Corbetta and Shulman, 2002). Weber et al. (2009) proposed theory of flow which suggests widespread synchronization between neural attention networks which are the basis for the complete absorption during flow state, which is based on tripartite theory of attention proposed by Posner et al.'s (1987); involving executive, alerting and orienting networks. The alerting network is responsible for initiating and maintaining attentiveness, while the orienting network directs

attention to a stimulus, both the networks are modulated by executive network. Optimal synchronization of alerting and orienting networks with executive network enhances attention to be highly goal-directed (Petersen and Posner, 2012). This organization is achieved through synchronized firing rates of neurons within attentional networks.

The importance of higher attentional processes during the flow experience is supported by neuroimaging research. Ulrich et al. (2016) have identified activation of multiple brain areas associated with the multiple demand (MD) network during flow in an arithmetic task. Prefrontal and parietal cortex areas, including inferior frontal sulcus, anterior insula, presupplementary motor area and in and around intraparietal sulcus form the MD network. Corbetta and Shulman (2002) identified areas of parietal and frontal cortex which form frontoparietal dorsal stream<sup>11</sup>. MD system serves to coordinate a series of multistep behaviors, guide selective focus to task-relevant information and provide cognitive control.



**Figure: Dorsal and Ventral networks of attention**

Projection from the temporoparietal junction (TPJ) toward inferior frontal gyrus (IFG) and middle frontal gyrus (MFG) form ventral network (*blue*), responsible for reorienting attention to salient stimuli. Projection from the superior parietal lobe (SPL) toward the frontal eye fields (FEF) form dorsal network (*orange*), responsible for voluntary allocation of attention. The MD system includes overlapping frontoparietal areas, from the SPL to the premotor cortex and inferior frontal sulcus (IFS).

Neurotransmitter activity of dopamine affects attentional processes. Dopamine pathways are primarily associated with reward networks and also modulate attentional focusing (Nieoullon, 2002), error

monitoring (Holroyd and Coles, 2002; Ridderinkhof et al., 2004) and response inhibition (Chambers et al., 2009; Congdon et al., 2008). Increased availability of dopamine D2 receptors in the striatum are functionally related to selective attention (Nieoullon, 2002). Dopamine action modulates response inhibition and impulse control for attentional control (Miyake et al., 2000).

The neural basis of concentration lies between the frontal and parietal association areas. When Super-brain yoga is practiced it is thought that, strengthening of synapses occur by physiological mechanism of synaptic plasticity due to increased synchronized firing rates of neurons within attentional networks and Corpus callosum as well which is responsible for synchronization of the two hemispheres giving out maximal output of the brain.

Master Choa kok Sui explains that the Super-brain yoga – YOGIC SQUATS when done correctly moves energy trapped in the basic chakras through the physical body's other major energy centers and finally up into the crown chakra that controls the pineal gland and overall brain health<sup>1,2</sup>. "Once the energy gets up to the forehead and crown chakras, it is transformed into subtle energy, which is utilized by the brain for its proper functioning," writes Master Choa kok Sui.

Jeremy Vanhelst et al., conducted a study on "Physical activity is associated with attention capacity in adolescents<sup>13</sup>" concluded that promoting Moderate-to-vigorous physical activity may have a beneficial effect on attention capacity in adolescents. In the present study students doing regular exercise were not excluded as exercise was part of their regular school schedule. Further a study comparing both Super-brain yoga and moderate exercise is to be done.

### Conclusion

Instituting Super-brain yoga for one month are effective in enhancing concentrating ability of students as evidenced by:

1. Significant **increase** in:

**TN:** Total number of characters attempted and

**CP = (TN-E2):** Concentration performance

2. Significant **decrease** in:

**E1:** Number of letters omitted and

**E2:** Errors committed

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**Ethical Clearance:** Taken from Osmania Medical College Institutional ethics committee

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**Conflict of Interest:** Nil

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# A Study of Blood Pressure Variation in Different Phases of Menstrual Cycle

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## Abstract

**Aim:** The aim of the study is to assess blood pressure variation in different phases of menstrual cycle

**Objectives:** To investigate any changes in blood pressure during different phases of menstrual cycle using mercury sphygmomanometer.

**Material and Method:** 30 apparently healthy female medical students aged between 18-23 years were selected for the study and blood pressure was recorded by auscultatory method using mercury sphygmomanometer during the different phase of menstrual cycle as follows: a) Menstrual phase (MP) (2nd day) b) Proliferative phase (PP) (11th day) c) Secretory phase (SP) (22nd day).

**Results:** Statistical analysis was done using SPSS 17.0 Software. To compare means of two independent groups, students t- test for independent samples was used. There was no significant difference in both systolic and diastolic blood pressure among different phases of the menstrual cycle

**Conclusion:** There was no significant difference in both systolic and diastolic blood pressure among different phases of the menstrual cycle. These findings should be re-examined with a larger study group.

**Keywords:** Blood pressure, menstrual cycle, Menstrual phase, Proliferative phase, secretory phase.

## Introduction

The menstrual cycle is a repetitive phenomenon occurring during the reproductive life of a female, that involves a patterned sequence of structural, functional and hormonal changes in the reproductive system. The menstrual cycle is characterized by cyclical fluctuations in the levels of Follicle stimulating hormone (FSH), Luteinizing hormone (LH), oestrogen and progesterone. These hormones are known to have an effect on oxygen carrying capacity, immune response, bleeding and also

changes in serum electrolytes which may be responsible for variable physical, psychological symptoms and autonomic changes.

The characteristic rhythmic changes in the rate of secretion of ovarian hormones produce corresponding changes in reproductive and other organ systems. Variation in Blood Pressure during different phases of menstrual cycle can also be attributed to the effect of ovarian hormones on cardiovascular function.

Data from studies indicate that progesterone, the natural progestin, has either neutral or depressor effects on blood pressure. For example, decreases in blood pressure with the progression of pregnancy are positively correlated with increases in progesterone<sup>1</sup>, oestrogen administration was known to promote vasodilator action by increasing prostacyclin and nitric oxide synthesis and the physiologic changes observed during the luteal phase of menstrual cycle are known to mimic early

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pregnancy<sup>1</sup>, These include a marked decrease in total peripheral resistance and a significant decrease in mean arterial pressure in the mid luteal phase<sup>2</sup>.

The incidence of coronary artery disease (CAD) and hypertension (HTN) is relatively low among women in reproductive age, with a sharp rise after menopause<sup>3</sup>. Oestrogen is also known to reduce the development of HTN in pre-menopausal women through peripheral actions such as up-regulation of endothelium derived vasodilator factors with simultaneous downregulation of vasoconstrictor factors. Oestrogen might protect against elevated arterial pressure by inhibiting sympathetic nervous activity<sup>4</sup>.

This study is carried out to find any blood pressure variation in different phases of menstrual cycle.

### Materials and Method

The study was carried out at Gandhi medical college, secunderabad 30 apparently healthy female medical students aged between 18-23 years were selected for the study.

#### Inclusion Criteria:

1. Normal regular menstrual cycles of 27-33 days.
2. Ovulatory cycles

#### Exclusion Criteria:

1. Subjects below 18yrs and above 23yrs of age.
2. Subjects with endocrinal & gynecological disorders, chronic diseases, allergic conditions.

3. Subjects with Diabetes.
4. Pregnant subjects.
5. Subjects with irregular menstrual cycle.
6. History of drugs intake affecting menstrual cycle.

All the subjects were explained about the BP recording and informed consent was taken

#### Recording of Blood Pressure:

Subjects were allowed to rest for half an hour in the laboratory before taking them to the examination couch. Blood pressure was recorded by mercury sphygmomanometer and littman stethoscope. Blood pressure was measured by auscultatory method. The equipment was checked and calibrated for its accuracy as per the recommendations by British hypertension society<sup>5</sup>.

Blood Pressure was recorded during the following Phases of Menstrual cycle:

- i. Menstrual phase (MP) (2<sup>nd</sup> day)
- ii. Proliferative phase (PP) (11<sup>th</sup> day)
- iii. Secretory phase (SP) (22<sup>nd</sup> day)

**Statistical analysis** was done using SPSS 17.0 Software. To compare means of two independent groups, students t- test for independent samples was used.

P value < 0.05 was considered as statistically significant

### Observation and Results

**Table 1: Comparison of Blood pressure in different phases of menstrual cycle S.B.P, Systolic Blood Pressure; D.B.P, Diastolic Blood Pressure**

Parameter	MP (Mean±S.D)	PP (Mean±S.D)	SP (Mean±S.D)	MPVsPP		MPVsSP		PPVsSP	
				T Value	P Value	T Value	P Value	T Value	P Value
S.B.P (mmHg)	108.33±8.34	109.67±10.66	107.47±11.36	0.542	P=0.62	0.334	P=0.83	0.78	P=0.44
D.B.P (mmHg)	63.67±8.9	67.33±9.07	65.33±8.6	1.578	P=0.14	0.735	P=0.74	0.876	P=0.48

There was no significant difference in both systolic and diastolic blood pressure among phases of the menstrual cycle

## Discussion

The human menstrual cycle involves physiological and biochemical changes. It is under the control of Hypothalamo-Pituitary-Ovarian (HPO) axis. Steroid hormones, oestrogen and progesterone which play a major role in menstrual cycle are controlled by an integrated HPO axis through release of FSH and LH.

In the present study, Systolic Blood Pressure (S.B.P) & Diastolic Blood Pressure (D.B.P) were increased in Proliferative Phase (PP) compared to other phases but were statistically insignificant. Findings of our study were in accordance with previous studies<sup>6,7</sup>.

However, Other studies showed that Resting SBP was significantly higher in the ovulatory phase than in other phases, but resting DBP did not differ significantly between phases<sup>12</sup>. In another study SBP was lower during the follicular phase while DBP and Heart rate (HR) were lower during the menstrual and follicular phases<sup>13</sup>. In a study carried out by McFetridge and Sherwood, Resting DBP was lower during luteal phase and higher during follicular phase whereas SBP remained uniform across the menstrual cycle<sup>14</sup>.

Data on the effects of estrogenic preparations on blood pressure are inconsistent, and include reports of blood pressure lowering<sup>9</sup>, blood pressure elevating<sup>10</sup> and blood pressure neutral effects<sup>11</sup>.

Unlike the natural oestrogen estradiol, the synthetic oestrogen, ethinyl estradiol increases blood pressure<sup>8</sup> by an increase in the hepatic synthesis of renin substrate, which leads to an increase in plasma angiotensin-II level and aldosterone mediated salt and fluid retention.

In a study, oral administration of natural progesterone significantly lowered blood pressure in six men and four postmenopausal women with mild to moderate hypertension who were not receiving antihypertensive drugs<sup>15</sup>. However with Natural progesterone administration, no change in blood pressure was recorded<sup>16</sup>.

The different findings may be attributed to various complex interactions among vasoactive mediators (e.g., nitric oxide, prostaglandins, prostacyclins, and the renin-angiotensin system), hemodynamic changes (i.e., cardiac output and systemic vascular resistance), and sex hormones (i.e., oestrogens and progesterone).

In our study, there was no significant difference in

both systolic and diastolic blood pressure among phases of the menstrual cycle. Simultaneous hormonal assays would offer an advantage to assess Blood pressure changes in different phases of menstrual cycle, if any. And also the present study was carried out on small number of subjects, further studies should be carried out with more number of subjects to substantiate our result.

## Conclusion

There was no significant difference in both systolic and diastolic blood pressure among phases of the menstrual cycle. The findings should be re-examined with a larger study group.

**Acknowledgement:** We would like to thank all the subjects who participated in the study

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**Ethical Clearance:** Taken from Scientific Ethical committee, Gandhi Medical College, Secunderabad, Telangana.

**Conflict of Interest:** Nil

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# Gender Differences in Response to Cold Pressor Test in the Age Group of 18-30 Years

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## Abstract

**Introduction:** The Cold Pressor Test (CPT) was first introduced by Hines and Brown in 1932. It was designed to measure the reactivity of the blood vessels to a standard stimulus<sup>2</sup>. Cold Pressor Test is an established challenge test of sympathetic vascular regulation. Sympathetic nervous system activity varies in males and females.

**Aim:** To study the response of Cold Pressor Test on blood pressure in normal healthy young adult males and females.

**Materials and Method:** After Institutional ethics committee approval, a total of 104 subjects in the age group of 18-30 years were recruited with informed consent for the study, after considering inclusion and exclusion criteria. Subjects were explained about the test procedure. Basal blood pressure (pre-test BP) was recorded after 20 min. of rest. Systolic and diastolic blood pressure was measured in mmHg (pre-test). Subject was asked to dip left hand till the wrist in cold water (4<sup>0</sup>-8<sup>0</sup> C) for 1 min. (minute). Blood pressure was recorded from right arm during the test, 1 minute, 2 minutes, 3 minutes and 4 minutes after the test.

**Result:** Basal BP was significantly higher in males than in females. Systolic BP(SBP) response, Diastolic BP response (DBP) to CPT were statistically higher in males compared to females.

**Conclusions:** The gender variations are seen due to differences in the sympathetic nervous system activity and due to the effect of sex hormones. Males are more prone to develop hypertension and other cardiovascular disorders when compared to females due to higher sympathetic activity.

**Keywords:** Cold Pressor test, sympathetic nervous system, males, females.

## Introduction

The subjects prone to develop hypertension in later life have a period of underlying sympathetic overactivity<sup>1</sup>. Increased sympathetic and decreased parasympathetic activities in young adults alters sympathovagal balance,

which could be the major mechanism in causation of pre hypertension<sup>3</sup>. Sustained sympathetic overactivity has been reported as among the primary mechanisms for genesis of essential hypertension<sup>2</sup>. The subjects at risk of developing hypertension can be identified by performing the CPT.

The Cold Pressor Test (CPT) was first introduced by Hines and Brown in 1932. It was designed to measure the reactivity of the blood vessels to a standard stimulus<sup>2</sup>. (CPT) is an established challenge test of autonomic vascular regulation. This study aims to study the gender variation in response to CPT, giving insight into level of sympathetic activity, and subsequent risk of developing hypertension in future and other cardiovascular disorders.

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**Materials and Method**

104 Subjects 18-30 years, studying in Osmania Medical College.

**Inclusion Criteria:**

Healthy Young adult offspring of

1. Normotensive parents.
2. Either one or both parents can be hypertensive.

**Exclusion Criteria:**

Subject with history of

1. Hypertension
2. Diabetes Mellitus
3. Cardiovascular disorders
4. Neuropathy
5. Addictions to tobacco/alcohol.

**Materials and Method**

Mercury Sphygmomanometer, Stethoscope, Thermometer, Timer, Ice water at 4°-8°C, Stadiometer, Weighing machine.

**Instructions:**

Subjects were asked

1. Not to have caffeine rich drinks at least 2-3 hour prior to test.
2. Not to perform Valsalva manoeuvre & isometric contraction during the test.

**Procedure:** After Institutional ethics committee approval, with informed consent subjects were recruited for the study. Details regarding the health status, past medical history, past cardiac surgeries, family history of hypertension (in parents), addictions were obtained. Subjects were explained about the test procedure. Basal blood pressure (pre-test BP) was recorded after 20 minutes of rest. SBP & DBP was measured in mmHg (pre-test). Subject was asked to dip left hand till the wrist in cold water (4°-8°C) for 1 minute. Blood pressure was recorded from right arm during test, 1 minute, 2 minute, 3 minute & 4 minute after the test.

**Statistical analysis:** Unpaired t test was used to find the significance between various parameters. p value <0.05 was considered as statistically significant (\*).

**Observation and Results:****Table 1: Characteristics of male and female subjects**

Parameter	Male	Female	p value
Number	45	59	-
Age (years)	19.78±2.89	20.50±3.97	0.294
Height (meters)	1.671±0.71	1.58±0.091	0.001*
Weight (kg)	59.89±10.71	56.93±11.54	0.185
BMI (kg/m <sup>2</sup> )	24.06±4.67	25.90±4.20	0.037

**Table 2: Comparison of Basal Blood Pressure (mmHg) between males and females**

Parameter	Male	Female	p value
Basal SBP	110±8.52	101.70±9.86	0.001*
Basal DBP	72.67±5.94	69.29±7.22	0.01*

**Table 3: Comparison of Systolic Blood Pressure response to CPT (mmHg) between males and females**

Parameter	Male	Female	p value
Basal SBP	110±8.52	101.70±9.86	0.001*
During test	127.42±9.48	119.12±10.76	0.001*
1min after test	115.91±11.26	107.56±11.79	0.001*

Parameter	Male	Female	p value
2min after test	109.20±9.12	102.10±10.35	0.001*
3 min after test	108.09±8.92	100.91±9.53	0.001*
4 min after test	107.46±9.18	99.93±9.07	0.001*
Maximum SBP	127.42±9.48	119.12±10.76	0.001*
Change in SBP	16.18±7.45	16.91±8.19	0.637

**Table 4: Comparison between Diastolic Blood Pressure (mmHg) response to CPT between males and females**

Parameter	Male	Female	p value
Basal DBP	72.67±5.94	69.29±7.22	0.01*
During test	86.98±10.50	69.29±7.22	0.01*
1min after test	77.87±8.81	72.10±9.67	0.002*
2min after test	73.91±6.22	69.53±8.41	0.005*
3 min after test	73.02±6.61	68.47±7.61	0.002*
4 min after test	72.89±6.61	68.03±7.40	0.001*
Maximum DBP	86.76±10.76	81.22±9.04	0.005*
Change in DBP	13.73±8.47	11.18±6.50	0.086

## Discussion

Males had higher basal SBP (110±8.52mmHg) & higher DBP (72.67±5.94mmHg) compared to basal SBP (101.70±9.86mmHg) & basal DBP (69.29±7.22mmHg) in females, which was statistically significant (p=0.001). This is in accordance with study done by Weinber & colleagues<sup>4</sup>, Khoury et al<sup>5</sup>, a meta- analysis study by Staessen et al<sup>6</sup>, The third National Health & Nutrition Evaluation Survey<sup>7</sup> (NHANESIII).

BP recordings during CPT & sequentially every minute thereafter up to 4 minutes, reveal statistically significant higher values in males compared to females. The higher SBP in males (127.42± 9.48mmHg) during CPT when compared to females(119.12±10.76mmHg) correlating with Srivastava et al<sup>8</sup> (P=0.001).

BP measurement continued after the removal of hand from the cold water in order to detect delayed pressor effect and duration of elevated blood pressure.

The SBP values 1 minute after the test were higher in males (115.91±11.26mmHg) than in females (107.56±11.79mmHg). The SBP values 2 minutes, 3 minutes, 4 minutes after test were significantly higher in males than in females.

The maximum SBP attained in males was higher (127.42±9.48 mmHg) than in females (119.12±10.76mmHg) & the p value was statistically significant (p=0.001). The change in SBP (Maximum SBP minus Basal SBP) was higher in females (16.91±8.19mmHg) than in males (16.18±7.45mmHg) and was not statistically significant (p=0.637). The change in SBP was higher in females correlating with Srivastava et al<sup>8</sup>.

The DBP response to CPT during test was higher in males (86.98±10.50mmHg) than in females (69.29±7.22 mmHg) & the values were statistically significant (p=0.001) correlating with Srivastava et al<sup>8</sup>. Males had higher DBP values 1 minute, 2 minutes, 3 minutes & 4 minutes after the test than females. Maximum DBP attained was higher in males than in females. The change in DBP was more in males (13.73±8.47mmHg) than in females (11.18±6.50 mmHg) in contradiction to Srivastava et al.<sup>8</sup> with a higher change in DBP in females than in males.

Mechanism of CPT induced increase in BP<sup>9</sup>: When the hand is immersed in cold water (4°-8°C), the A & C fibres are activated in response to pain, temperature sensation. These fibres ascend contralaterally as

anterolateral system which includes Spinothalamic, Spino-reticular & Spino-mesencephalic tracts. The nociceptive specific neurons ascend in the lateral mesencephalon, while the thermoreceptive specific neurons project to Dorsomedial part of ventro posteromedial nucleus of Thalamus. The ascending fibres of spinoreticular tract project to medullary reticular formation, via the Ionotropic Glutamate receptors activate the Rostral Ventrolateral Medullary (RVLM) neurons resulting in increased sympathetic nervous activity leading to increase in blood pressure, heart rate, total vascular resistance, muscle sympathetic activity<sup>10,11,12</sup>, decrease in forearm blood flow<sup>13</sup>. Thus, CPT increases the sympathetic nervous system activity to increase the BP which depends on the sympathetic tone.

#### **In the present study, males had higher:**

1. Basal SBP & DBP
2. SBP & DBP during the test
3. SBP & DBP 1 minute, 2 minutes, 3 minutes & 4 minutes after the test.

Gender variations to CPT is due to androgens, estrogen, progesterone.

Androgens play a crucial role in gender differences in blood pressure regulation. The probable mechanisms by which androgens lead to increased blood pressure are: Androgens lead to increased formation of Angiotensin II, which increases oxidative stress leading to production of superoxide, quenching of Nitric Oxide, and also reduce the renal vascular response to vasodilators, including residual Nitric Oxide (NO). Androgens also cause production of F<sub>2</sub>-isoprostanol which potentiates the effect of Ang II as a vasoconstrictor, and also stimulate endothelin-1 production to increase blood pressure further.<sup>14</sup>

Estrogens and their receptors play a crucial role in endothelium-dependent maintenance of vascular tone<sup>15-17</sup>. Estrogens cause relaxation via endothelium-derived hyperpolarizing factor (EDHF), by inducing vasodilator prostanoids (PGE<sub>2</sub>, PGI<sub>2</sub>), and by inhibiting endothelin-1 production<sup>18</sup>. Endothelial progenitor cells (EPCs) also play a crucial role in vascular response. Fertile females have higher levels of EPC than men. Intrinsic differences within the endothelial cells contribute to differences between men and women. Other factors that modulate autonomic

cardiac activity, may influence sex differences, such as inflammation, increased pain sensitivity to cold, and psychological disorders (e.g. depression). Healthy females have a lower central sympathetic neural output to periphery and a lower sympathetic vasoconstrictor drive when compared to healthy men.<sup>19</sup> Studies done at molecular level indicate that central actions of estrogens include facilitation of cholinergic transmission and activating the synthesis of a vital rate limiting enzyme choline acetyltransferase, involved in the formation of acetylcholine formation, and also have a role in sympathetic outflow inhibition<sup>20,21</sup>

Estrogen<sup>22</sup> decreases the production of cyclooxygenase derived products, reactive oxygen species, angiotensin-II & endothelin-1 which are vasoconstrictor agents. It inhibits smooth muscle cells by activating K<sup>+</sup> efflux & by inhibiting calcium influx through inhibition of L-type Ca<sup>2+</sup> channels and decreases myosin light chain phosphorylation and contraction of smooth muscle. Estrogen also inhibits proliferation of vascular smooth muscle cell.

Membrane-impermeant forms of estrogen act on cell surface estrogen receptors, leading to the activation of mitogen-activated protein kinase (MAPK) and increased cGMP production and nitric oxide (NO) release.

Estrogen acts on the renin-angiotensin system is at the formation of Ang II, at the Ang II receptors level and on Ang II-induced responses<sup>22</sup>. Estrogen increases gene expression and plasma levels of angiotensinogen and antagonizes the AT<sub>1</sub> receptor-mediated growth-promoting effects of Ang II in vascular smooth muscle cells. Estrogen acts on the endothelin-1 (ET-1) pathway at different levels: at its formation, at level of the receptors and on ET-1-induced responses.

The luteal phase is associated with a significantly reduced parasympathetic activity. The high progesterone levels during the luteal phase may have an inhibitory role on cardio-vagal activity<sup>22-25</sup>. Studies showed a higher sympathetic activity and a sympathovagal imbalance in luteal phase with an associated increase in parasympathetic activity in follicular phase, showing sympathetic predominance in luteal phase<sup>26-29</sup>. In physiological doses, progesterone acts as a sympathomimetic agent which induces nor epinephrine release<sup>30,31</sup>

The results corroborate with report by Weitz et al<sup>32</sup>, that the activity of sympathetic nervous system shows



gender specific differences with lower sympathetic nerve activity to muscle vascular bed in women as compared with men, the difference vanishes after menopause.

Thus, the female sex hormones together cause lower BP response to CPT in females.

### Conclusion

Males had significantly higher values for - Basal SBP & DBP, SBP & DBP values during the test, SBP & DBP values 1 minute, 2 minutes, 3 minutes & 4 minutes after test, Maximum SBP & Maximum DBP than females.

The gender variations are seen due to differences in the sympathetic nervous system activity and due to the effect of sex hormones. Males are more prone to develop hypertension and other cardiovascular disorders when compared to females due to higher sympathetic activity.

The Change in SBP & change in DBP values between males and females are not statistically significant.

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# Can GSR be Used as a Tool to Early Diagnose the Autonomic Dysfunction among GAD Patients

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## Abstract

**Background:** 14% of global disease burden goes to anxiety. Generalised anxiety disorder is one of the manifestations of anxiety. There has been many studies correlating the association of generalised anxiety disorder with cardiovascular mortality and morbidity. This contribution has been attributed to imbalance in parasympathetic and sympathetic stems of autonomic nervous system. This imbalance in autonomic activity can be measured by many invasive & non invasive method.

**Material and Method:** The present study was conducted on 60 subjects which were divided into 2 groups of 30 patients of generalised anxiety disorder and 30 age and sex matched normal subjects as control. Both groups were further subdivided based on gender with 15 subjects each. Basal galvanic skin response was recorded in all subjects and comparison was done among patients and controls.

**Result:** Basal galvanic skin response was decreased in both male and female generalised anxiety disorder patients when compared with controls. The difference was statistically non significant.

**Conclusion:** Basal galvanic skin response can not be used as an early diagnostic tool of autonomic dysfunction in generalised anxiety disorder.

**Keywords:** *Generalised Anxiety Disorder, Galvanic Skin Response, Autonomic Cardiac Function.*

## Introduction

Several mechanisms such as poor health habits like smoking, poor diet, sedentary life style etc associated with negative emotions – anxiety, anger and depression have been stamped as the reason for increased coronary heart disease but the main culprit seems to be potential direct effect of the negative emotions on progression

of atherosclerosis as well as lowering the threshold for ventricular arrhythmia and sudden cardiac death<sup>1</sup>. Credit of 14% of global disease burden goes to mental health disorders. Out of these anxiety contributes 4.7%<sup>2,3</sup>. Anxiety disorders have been associated with increased risk of cardiovascular morbidity and mortality. Several studies have predicted the increased risk of CHD in patients with phobic anxiety or panic disorders<sup>3</sup>. One of the health professionals follow-up study found increased risk of sudden CHD death rather than nonsudden. One of the hypothesis predicts this association as a dysregulation of autonomic nervous system control activity<sup>4-6</sup>.

Populations exposed to traumatic events have higher prevalence of major depressive disorder (MDD)

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and generalised anxiety disorder (GAD). MDD and GAD are highly morbid psychiatric disorders. MDD has been associated with increased mortality mainly due to CHD<sup>7</sup>. Other manifestations of anxiety have been linked with increased CVD mortality<sup>8,9</sup>. Anxiety has also been linked up with some chronic diseases like diabetes, hypertension and hypercholesterolemia<sup>3</sup>.

Possible mechanisms for the association of anxiety with CVD include changes in autonomic tone manifested as decreased vagal and increased sympathetic tone<sup>3</sup>. High level of anxiety has been associated with reduced heart rate variability (HRV). This reduced HRV predicts sudden cardiac death (SCD) in elderly and heart failure patients<sup>10</sup>. Increased incidence of SCD in population exposed to traumatic events has been implicated to increased sympathetic nervous system activity<sup>11</sup>.

There has been many studies showing reduced HRV predicting the severity of association of cardiovascular morbidity with GAD. But there are no or very minimal studies which has used galvanic skin response as a predictor of association of GAD with cardiovascular mortality & morbidity.

*Galvanic Skin Response:* Galvanic Skin Response (GSR) is a type of an electrodermal response that represents a change in the electrical conductivity of the skin caused by an increase in activity of sweat glands. GSR measures skin conductivity from the fingers or palms. It is affected in an anxious person because of increased sympathetic activity<sup>12</sup>. The activity of sweat glands in response to sympathetic nervous stimulation results in increase in conductance. Fowler et al have recommended that recordings can be taken from electrodes placed on the middle or distal phalanges of two fingers (either index and middle finger or index finger and thumb) or on the thenar and hypothenar eminences of palmar surface<sup>13</sup>.

Sympathetic activity and emotional arousal has a strong association. Tonic skin conductance changes with psychological arousal. It increases sharply when the subject wakes up and the it is increased further with activity, mental effort and especially in stress. GSR is an automatic response under so much control of cerebral cortex that it can be considered an indication of emotional status of the subject<sup>14</sup>.

GSR is the measure of the skin resistance to very small electric current passage. Meditation and relaxation procedures lead to a increase in skin resistance. High and

low skin resistance has direct association with relaxation and stress respectively. High resistance depicts a relaxed state of mind and low resistance means tension. But in psychotherapy session it is exactly opposite. Whenever repressed material comes to surface as in guilt or pain, the skin resistance will increase. In therapy session high skin resistance indicates tension. Suppressed emotions leads to building up of stuck energy in mind. When such emotions are restimulated by past events as in the case of generalized anxiety disorder, there will be increase in basal resistance. The wave like increase in skin conductance is known as Phasic skin conductance<sup>15</sup>. Cardiac automaticity is intrinsic to various pacemaker tissues but heart rate and rhythm is mainly under the control of autonomic nervous system. Parasympathetic influence on heart is by vagus nerve via acetylcholine release. Sympathetic effect on heart is by release of epinephrine and nor epinephrine. Under resting conditions, vagal tone is the main regulator of variations in heart rate<sup>16</sup>.

Most organs of our body has dual innervations from sympathetic and parasympathetic stems of autonomic nervous system, often mediating opposite effects. The relative balance between sympathetic and parasympathetic nervous system is regulated by afferent input directed primarily to the brain<sup>17</sup>. Fine spray type of nerve endings in carotid sinus and aortic arch, as the arterial baroreceptors, are the major contributors in regulation of blood pressure and heart rate. On stretching baroreceptors get stretched and send signals are from carotid sinus through glossopharyngeal nerve and from aortic arch through vagus nerve to the nucleus tractus solitarius in the medulla oblongata. From medulla, signals are sent to two centers- Inhibitory to vasomotor center and excitatory to the vagal center. Effects of stimulation of baroreceptors results in vasodilatation and decrease in heart rate. And this effect decreases the blood pressure by reducing peripheral resistance and cardiac output<sup>18</sup>.

Increased blood pressure results in reflex slowing of the heart via baroreceptor reflex. Increase in heart rate in response to lowering blood pressure results from decreased vagal inhibition and increased sympathetic activity. The vagal effects are immediate one but the sympathetic effects are delayed until several seconds. Pressure fall results in "Unloading" of arterial baroreceptors and reduced inhibitory influences on nucleus tractus solitarius and vasomotor center in medulla, resulting in increased sympathetic

and decreased parasympathetic tone, which produces vasoconstriction, increased cardiac contractility and increased heart rate.

Multiple ailments have their effect on autonomic nervous system. Impaired autonomic functions may be caused by diseases affecting either the CNS or peripheral autonomic nervous system. Various chronic disorders also have their effects on autonomic nervous system. Several studies have documented increased incidence of morbidity and mortality in patients suffering from various chronic disorders with autonomic irregularity<sup>19</sup>.

Assessment of autonomic activity-Tests of autonomic functions have been proposed, formulated and standardized by various workers. These tests are broadly divided into two categories:

**Invasive Tests:** Intraneural recording of postganglionic sympathetic activity, Response of autonomic nervous system to infusion of pressor agents, Plasma catecholamine levels estimation.

**Non-Invasive Tests:** Basal heart rate variability, Postural challenge tests (Head Up Tilt testing), Valsalva manoeuvre, Sustained handgrip test, Cold pressor test, Pseudomotor (sweating) function test.

A combination of these tests are generally employed in assessment of autonomic nervous system as some of these tests give information about the cardiac sympathetic functions whereas others give information about the cardiac parasympathetic functions<sup>20</sup>. Out of all these parameter, we included pseudomotor function test in the form of galvanic skin response in our study.

## Material and Method

The present study was conducted in Department of Physiology in collaboration with Department of Psychiatry, Pt. B. D. Sharma University of Health Sciences, Rohtak. The study was carried out on 60 patients of Generalised Anxiety Disorder (GAD) of 18-45 years of age of either sex. The normal age and sex matched subjects were selected from our staff members, medical students and healthy attendants accompanying the patients to the institute. The subjects and patients were divided into the following two groups:

Group I (60 in number) included normal healthy subjects. This group was further divided into two subgroups based on gender (30 each):

Group I<sub>A</sub>- 30 male healthy subjects.

Group I<sub>B</sub>- 30 female healthy subjects.

Group II(60 in number) included GAD patients diagnosed as per ICD-10 guidelines with Hamilton Anxiety Scale score of 28 (i.e. moderate anxiety), further divided into two subgroups based on gender (30 each):

Group II<sub>A</sub>- 30 male GAD patients.

Group II<sub>B</sub>- 30 female GAD patients.

**Exclusion Criteria:** The patients with the history of any other major illness (like diabetes, hypertension, myocardial infarction and hyperthyroidism) in the previous one year & history of drug intake for any other ailments in last one month were excluded.

**Tests Conducted:** In each individual basal GSR was recorded.

Recording of GSR was done by digitalised polygraph (POLYRITE D system, supplied by RMS India PVT. Ltd. Chandigarh). Individual customization of data was done after acquiring.

The following recommendations were followed to make the results reliable and interpretable.

**Sampling Rate:** The sampling rate in our machine was 256 Hz.

**Filters:** The machine was provided with different filters. Appropriate filters were chosen for GSR since the baseline shifting may affect the spectrum analysis.

High Filter- 2 Hz

Low Filter- 0.05 Hz

**Sensitivity:** Sensitivity knob was set on moderate high sensitivity (50 $\mu$ V).

**Sweep speed:** The screen speed was 30 mm/sec.

**Preliminary Preparation:** The subjects were informed about the whole procedure in detail in their own language to allay any fear or apprehension. Consent was taken from every individual to undergo the whole procedure. All the experiments were conducted in a particular time period (from 10 AM to 1 PM) to avoid the diurnal variations.

**Procedure:** All the subjects and patients were tested under similar laboratory conditions. They were allowed

to get familiar with the experimental and environmental conditions of the laboratory to allay any apprehension. After performing physical examination they were asked to lie down in supine position on wooden table. For recording GSR, two copper lined electrodes were attached on the palmar aspect of distal digit of the thumb and index finger of right hand after applying the conduction jelly. Then basal recording of GSR was taken for 5 minutes.

**Statistical analysis of data:** For interpretation of the results the data set of each group was analysed statistically. Basal GSR of Group I (normal healthy subjects) were compared with Group II (Patients of GAD) by unpaired student t test. The comparison was done among subgroups IA & IIA and IB & IIB. Significance of result was predicted based on the p value.

## Result

We observed the following result:

**Table I: Basal GSR among Normal Healthy Males (Group IA) and Male GAD Patients (Group IIA)**

Parameter	Group IA (Mean ± SD)	Group IIA (Mean ± SD)	P Value
GSR ( $\mu$ Mho)	2.135 ± 2.242	1.813 ± 1.959	0.555

Table I shows decrease in GSR among group IIA in comparison to group IA. The difference is statistically not significant.

**Table II: Basal GSR of Normal Healthy Female (Group IB) and Female GAD Patients (Group IIB)**

Parameter	Group IB (Mean ± SD)	Group IIB (Mean ± SD)	P Value
GSR ( $\mu$ Mho)	2.390 ± 2.230	2.071 ± 1.555	0.523

Table II shows decrease in GSR among group IIB in comparison to group IB. The difference is statistically not significant.

## Discussion

Manifestations of Anxiety have been associated with cardiovascular morbidity due to autonomic nervous system disturbance. It has been observed that longstanding anxiety markedly increases the risk of heart attack. The role of anxiety in hiking heart attack also goes beyond the effects of depression, anger, obesity, type A behaviour and other negative emotions<sup>21</sup>. Autonomic Nervous System plays major role in determining heart rate, stroke volume and peripheral vascular resistance to meet the appropriate requirement of body. Numerous studies have demonstrated the increased sympathetic and decreased parasympathetic nervous system activity hikes the risk of cardiovascular mortality and morbidity<sup>21</sup>.

Present study was carried out to assess and clinically evaluate autonomic functions by recording of basal GSR. This study was conducted on 60 patients of GAD and, age and sex matched 60 controls. They are divided

into two groups. Group I comprised of 60 controls who are further divided into two subgroups based on gender (IA comprised of 30 male controls and IB of 30 female controls). Group II comprised of 60 GAD patients who are further divided into two subgroups based on gender (IIA comprised of 30 male patients and IIB of 30 female patients).

As shown in the results in table I, there is decrease in GSR among group IIA (1.813 ± 1.959) in comparison to group IA (2.135 ± 2.242). This decrease in GSR among male GAD patients as compared to the male normal subjects indicates the decreased parasympathetic tone or increased basal sympathetic activity among GAD patients. Table II shows decreased GSR in group IIB (2.071 ± 1.555) as compared to group IB (2.390 ± 2.230). Similar to the results in group I, this also shows decrease of GSR in female GAD patients in comparison to normal female subjects.

Our study shows decreased GSR among both male and female GAD patients in comparison to their respective normal subjects. This shows that GAD either decreases the basal level of parasympathetic tone increases the basal sympathetic tone among the affected persons. But in both the scenario the difference is statistically insignificant.

### Conclusion

We concluded that though there is decrease in galvanic skin response among generalised anxiety disorder patients but statistically non-significant result does not warrant its use as an indicator for early diagnosis of autonomic dysfunction. Before coming to the final concrete conclusion there need to be done further research.

**Ethical Clearance:** Taken from PGIMS, Rohtak Institute Ethical Committee (IEC)

**Source of Funding:** Self

**Conflict of Interest:** Nil

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# Vitamin D, Oxidative Stress and Cognition in Diabetes Mellitus

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## Abstract

**Introduction:** Diabetes is a very common disease that affects almost all body systems. Of late recent studies have determined that vitamin D deficiency can cause several diseases such as diabetes, cardiovascular complications etc. Oxidative stress is an imbalance between free radicals and antioxidants in the body. Vitamin D is an antioxidant and its deficiency can cause oxidative stress. Thus vitamin D deficiency by itself and by causing oxidative stress can increase risk of developing diabetes

Hyperglycemia can affect cognition. Oxidative stress too can affect cognition. Studies done studying the role of vitamin D affecting cognition especially in diabetics are very few.

**Aim:** To determine the role of vitamin D in affecting cognition in Diabetic patients. To determine the correlation between vitamin D, oxidative stress and cognition in diabetes.

### Materials and Method:

Comparative cross sectional study

100 Diabetes patients were studied.

**Exclusion Criteria:** Hypertensives, alzheimers disease, dementia, bone diseases, epileptics, taking calcium or vitamin d supplements, hypo or hyperparathyroidism, thyroid disorders.

After obtaining ethical approval from the institution a structured questionnaire was given to all.

Fasting blood glucose levels was determined by GOD-POD Method.

HbA1c was determined by Immunoturbidometry.

Vitamin D was determined by ELISA method.

Oxidative stress (malonylaldehyde) was measured by manual TBARS(Thiobarbituricacid reactive substances method)

Cognition was assessed using Montreal cognitive assessment questionnaire.

All tests were carried out at the central lab of Sree Balaji Medical College and Hospital, Chennai.

Results were analysed using Microsoft excel.

**Results:** A negative correlation was seen between vitamin D and MOCA scores.

A positive correlation was seen between MDA and MOCA scores.

**Conclusion:** Vitamin D deficiency causes oxidative stress and affects cognition in diabetics. Vitamin D supplementation can be considered to reduce oxidative stress and thus improve cognition in diabetes mellitus patients.

**Keywords:** Diabetes, oxidative stress, vitamin D, Montreal cognitive assessment.

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## Introduction

Diabetes is a very common disease that affects almost all body systems. Of late recent studies have determined that vitamin D deficiency can cause several diseases such as diabetes, cardiovascular complications etc. Oxidative stress is an imbalance between free radicals and antioxidants in the body. Vitamin D is an antioxidant and its deficiency can cause oxidative stress.<sup>1</sup> Thus vitamin D deficiency by itself and by causing oxidative stress can increase risk of developing diabetes

Hyperglycemia can affect cognition. Oxidative stress too can affect cognition. Studies done studying the role of vitamin D affecting cognition especially in diabetics are very few.

Diabetes is characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term dysfunction and failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels. Recent studies have shown that diabetic patients are having low level of cognitive function in the form of mild cognitive impairment (MCI) which can lead to dementia and Alzheimer's disease.

### MCI is diagnosed when there is:

- (1) Evidence of memory impairment.
- (2) Preservation of general cognitive and functional abilities.
- (3) Absence of diagnosed dementia

### Study Design:

Comparative cross sectional study

No of Patients data Collected = 100 Nos

Diabetes Patients with no cognitive impairment data Collected = 43 Nos

Diabetes Patients with mild cognitive impairment data Collected = 57 Nos

## Materials and Method

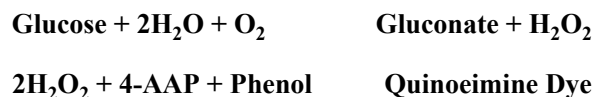
Ethical approval was obtained from the institution ethical committee.

5ml of blood was taken in fasting state from the diabetes patients and subjected to following tests.

### Plasma Glucose Level Estimation:

GOD – POD Method:

**Principle:** Glucose is oxidized by glucose oxidase (GOD) to produce gluconate and hydrogen peroxide. The hydrogen peroxide is coupled oxidatively with 4 amino- antipyrine (4-AAP) and phenol in the presence of peroxidase (POD) to yield a red quinoneimine dye that is measured at 505nm .The concentration of glucose is proportional to the absorbance at 505nm.



Absorbance of the colored solution at 505nm is directly proportional to the glucose concentration.

**(Reference Range: 70 – 110 mg/dl)**

**s Vitamin D Level Estimation:** Serum vitamin D levels estimated by immunoenzymetric assay (ELISA).

### Principle:

1. First 2 hours incubation step -vit.D present in calibrators,controls,samples is dissociated from binding serum proteins to fix on binding sites of a specific monoclonal antibody.
2. After 1 washing step a fixed amount of 250h vitamin D labelled with biotin in presence of horsedish peroxidase (HRP),compete with unlabelled 25OH vitamin d2 and 25OH vitamin D3 present on binding sites of the specific monoclonal antibody.
3. After 30 min incubation at room temperature the microtiter plate is washed to stop the competition reaction.

The DIA source 25OH vitamin D total ELISA is a solid phase enzyme linked immunosorbent assay performed on microtiter plates.

4. The chromogenic solution is added and incubated for 15 mins.

The reaction is then stopped with the addition of stop solution and the microtiter plate is then read at the appropriate wavelength.

5. The amount of substrate turnover is determined colourimetrically by measuring the absorbance, which is inversely proportionally to the total 25OH vitamin D(D2 and D3) concentration.

**(Reference range: 30 – 150 ng/ml)**

**HbA1c Level Estimation**

HbA1c Level estimated by Immunoturbidimetry

**Principle - Competitive binding****Reference Values:**

**Good control (5.6% - 7.0%)**

**Fair control (7.01% - 8.0%)**

**Unsatisfactory control (8.01% - 10.0%)**

**Poor control (>10.01%)**

**Estimation of Malonylaldehyde**

2 mL of blood was collected .Itwas centrifuged at 3000 rpm for 5 min. Then the serum MDA was measured using the method of Buege (1978).

The samples are kept in boiling water bath for 15 min.

To the diluted sample 1 mL of Trichloroacetic acid TCA-2-thiobarbituric acid (TBA)–HCl reagent is added. The reaction mixture is cooled and centrifuged. The supernatant is taken and the optical density of the pink colour formed is read at 535 nm.

The concentration of MDA in the sample is got by plotting the obtained absorbance against the standard graph. (normal range- 2.02-4.65 µM/L.)

**Assessment of Cognition**

Overview of the MoCA

Takes approximately 15 minutes to administer

Requires informed consent

It is a screening tool and not diagnostic

MoCA Scoring

- The total points was added.
- One point was added if the patient had less than 12 years of formal education. •
- Normal score is equal to or greater than 26/30. •

**Results**

All data was analysed using microsoft excel.

Unpaired student t test was performed. P value <0.05 was considered significant.

**Table-1: Demographic and Clinical Charecteristics of Total Type 2 Diabetic Patients with and Without MCI**

Number of Patients Male/Female	NO MCI N=43(43%) (28/15)	MCI N=57(57%) (19/38)	P Value
Age (Years)	48.55 ± 7.651	57.80 ± 5.664	<0.05
Duration of Diabetes (Months)	40.11 ± 36.198	156.42 ± 8.950	<0.05
RBS (mg%)	164.34 ± 38.999	210.12 ± 49.079	<0.05
HbA1c (%)	7.93 ± 0.462	9.18 ± 1.137	<0.05
Serum vitamin D level (ng/ml)	41.17 ± 11.544	21.97 ± 7.063	<0.05
MOCA Score	28.16 ± 1.252	20.70 ± 2.456	<0.05
MDA Levels	0.93 ± 0.39	2.65 ± 1.33	<0.05

**Table 2: Correlations of MOCA Score with Other Parameters in Total Patients with MCI (n=57)**

	MOCA Score	
	R	P
Log10[25(OH)d]	+0.512	0.001
Duration of DM in months	-0.103	0.444
RBS (mg%)	-0.062	0.647
HbA1c (%)	+0.003	0.984
MDA	+ 0.40	0.001

**Table 3: Comparison of Male and Female Patients with MCI**

Number of Patients	Male (n=19)	Female (n=38)	P Value
Age (Years)	58.68 ± 4.308	57.36 ± 6.240	0.412
Duration of DM (months)	167.36 ± 50.354	150.94 ± 62.719	0.326
RBS (mg%)	214.73 ± 49.252	207.81 ± 49.489	0.620
HbA1C (%)	9.24 ± 1.039	9.15 ± 1.195	0.781
Serum Vitamin D Level (NG/ML)	26.57 ± 5.695	19.68 ± 6.592	0.0001
MOCA Score	21.73 ± 2.921	20.18 ± 2.038	0.023
MDA Level	2.67 ± 0.45	2.98 ± 0.53	0.014

**Table 4: Average MOCA Score in All the Patients According to Different Serum Vitamin D Levels**

Range of serum vitamin D (ng/ml)	Average serum vitamin D level (ng/ml)	Average MOCA score	P value
<20(n=28)	15.9 ± 2.916	19.71 ± 4.243	0.0001
20-30(n=28)	25.79 ± 5.187	22.64 ± 3.234	0.009
>30(n=44)	42.18 ± 10.09	27.382 ± 2.470	0.0001

**Table 5: Association of Serum Vitamin D Level & Moca Score in Patients with MCI in Two Age Groups**

Age Group	Serum vit.D (ng/ml)	MOCA score	P Value
<50 yrs	23.51 ± 13.454	21.28 ± 3.400	0.43
>50 yrs	21.76 ± 11.766	20.62 ± 3.866	0.04

## Discussion

In our study, HbA1c positively correlated with MOCA scores. In a study by Roy et al., cognitive impairment was observed in 11.6% of the patients who had optimal glycemic control (HbA1c under 7%) and 30.2% with HbA1c 7% or above.<sup>5</sup> Khullar et al. showed that subjects having glucose levels >125 mg/dl had 1.73 times higher risk of developing neurocognitive impairment.<sup>[6,7]</sup> ACCORD-MIND trial done on 2977 type 2 diabetes subjects found a statistically significant age-adjusted association between HbA1c level and score on four cognitive tests.<sup>10</sup> Both clock in a box and clock-drawing test have been shown to inversely correlate with HbA1c.<sup>11</sup> Hence, our results are consistent with existing literature that poor glycemic control in type 2 diabetes is associated with cognitive decline.

The diabetes control and complications trial in type 1 diabetes demonstrated that improved HbA1c was related to improved cognition in nonamnestic domains.<sup>12</sup> Luchsinger et al. showed that improving HbA1c levels in an elderly population over a period of 5 years was associated with slowing down of global

cognitive decline.<sup>13</sup> Being a woman and longer duration of diabetes have been shown to be independent risk factors in previous studies.<sup>6</sup> Our study did not find any difference between sex or any relation to duration of diabetes perhaps because of inadequate sample size. The MoCA is now accepted as an excellent tool for brief cognitive screening measure and is freely available with multiple editions in various languages. The original MoCA reported a sensitivity of 100% and specificity of 87% in detecting mild AD using a cutoff score of 26.<sup>9</sup> Amnestic MCI (a MCI) is said to have a high likelihood of progressing to AD.<sup>10</sup> Hence, the differences noted in the MOCA scoring in our study could be suggestive of risk for development of AD in the future.<sup>15</sup> A study on the effectiveness of cognitive training program in people with MCI underlines the importance of early detection of MCI.<sup>16</sup> In summary, our study shows a high prevalence of undetected MCI in type 2 diabetes mellitus patients attending an outpatient clinic setting. A strong negative correlation was noticed between all parameters of glycemic control and MOCA scores representative of cognitive function. These observations make a strong case for routine screening of type 2

diabetes mellitus patients to detect MCI with a sensitive tool such as MoCA. Studies on the benefits of improved glycemic control on cognitive function would need to be performed in the future to help us understand the significance of our finding in the long-term management of these patients.

**Vitamin D and Oxidative Stress:** When vitamin D status is adequate, many of the intracellular oxidative stress-related activities are downregulated. Having suboptimal concentrations of serum 25(OH) D fails to subdue oxidative stress conditions, augment intracellular oxidative damage and the rate of apoptosis. The intracellular Nrf2 level is inversely correlated with the accumulation of mitochondrial ROS<sup>17</sup> and the consequent escalation of oxidative stress. Thus, Nrf2 plays a key role in protecting cells against oxidative stress; this is modulated by vitamin D. In addition, vitamin D supports cellular oxidation and reduction (redox) control by maintaining normal mitochondrial functions<sup>29-31</sup>. Loss in the redox control of the cell cycle may lead to aberrant cell proliferation, cell death, the development of neurodegenerative diseases, and accelerated aging<sup>31-35</sup>. Peroxisome proliferator-activated receptor-coactivator 1 $\alpha$  (PGC-1 $\alpha$ ) is bound to mitochondrial deacetylase (SIRT3). PGC-1 $\alpha$  directly couples to the oxidative stress cycle<sup>31</sup> and interacts with Nrf2. This complex regulates the expression of SIRT3; this process is influenced by vitamin D metabolites<sup>32</sup>. Calcitriol has overarching beneficial effects in upregulating the expression of certain antioxidants and anti-inflammatory cytokines<sup>33</sup>, thereby protecting the tissues from toxins, micronutrient deficiency-related abnormalities, and parasitic and intracellular microbe-induced harm<sup>34</sup>. It regulates ROS levels through its anti-inflammatory effects and mitochondrial-based expression of antioxidants through cell-signaling pathways<sup>34</sup>. In our study there was a positive correlation between vitamin D levels and MOCA scores. A positive correlation was also seen between HbA1c, MDA levels and MOCA scores.

Supplementation of vitamin D in Diabetic patients can prove to be beneficial in reducing oxidative stress, improving cognition as well as help control blood sugar levels.

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# Comparison of Different Teaching Method in First Year Medical Students: Both Subjectively and Objectively

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## Abstract

**Aim:** Our study was to compare three different teaching method in first year medical students.

**Objectives:** To assess the outcome and perception of students towards teaching method.

**Method:** 150 students were divided randomly in to 3 different groups as group A (attended Lecture), group B (attended demonstration) group C [attended small group discussion (SGD)], topic was Introduction to BP. As a pretest all the groups had answered a set of structured 25 MCQs, after attending their respective teaching sessions, immediately post test I and after 1 month post-test II was conducted. For subjective assessment likert scale was used.

**Statistical Analysis:** Paired t test was used for comparison. "P"<0.05 was considered as statistically significant.

**Results:** There was a significant difference between pre-test and post-test I, pre-test and post-test II in all the groups (P<0.001). When we compared post-test I and II, group A and B showed the significant difference (P<0.05), as their performance in post-test II has reduced, but there was no significant difference showed by group C between post-test I and II, as their performance in post-test II has not reduced. Subjectively also Students preferred SGD as better teaching method.

**Concussion:** Looking at results, clearly states that the SGD is better compared to other two method, both subjectively and objectively.

**Keywords:** Lecture class, demonstration, small group discussion, MCQs.

## Introduction

From the beginning didactic lectures are considered as the method of teaching for large group in medical students. It is challenging task to teachers to deliver the vast subject in a short time of lecture class about 40 to 50 minutes, and also to students where there is no interaction

and it promotes passive learning and fails to motivate the students. Since few decades many innovative modalities of learning are being implemented to make the learning process interesting like tutorials, seminars, case-based discussions, small group discussions<sup>1</sup>. Traditional didactic lecture is more passive and less effective as a teaching tool compared with active learning method like problem-based learning. But, a well-organized lecture can be one of the most effective ways to integrate and present information from multiple sources on difficult topics. So, assistance is needed to enhance the quality of lecture in the form of audio-visual aid like power point teaching which has now become the most popular package of teaching method<sup>2</sup>. Student motivation and performance improve when the instruction is adapted to student learning preferences and styles. According to the

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experience of the various authors, it was very difficult to retain the attention and interest of medical students in subjects, so there was introduction of problem based learning and small group discussion in addition to didactic lectures, and the students met this approach with enthusiasm. From this success, they began to consider ways to incorporate this technique into the traditional didactic lecture activities<sup>3</sup>. The emerging trend all over the world is to have a problem-based, integrated student-centred medical curriculum, demanding active participation from the students and facilitating self-directed learning<sup>4</sup>. Based on the previous research studies students have preference for chalk and talk<sup>5</sup>, chalk and talk with PPT<sup>6</sup>, small group discussion<sup>1,7</sup> and case based discussion<sup>3</sup>. So the aim of our study is to compare three different teaching modalities in first year medical students in Physiology (Lecture class, Demonstration and small group discussion (SGD)).

### Material and Method

Study was conducted in first year medical students of Subbaiah Institute of Medical Sciences, Karnataka, India, after taking informed consent from all the volunteers.

A batch of 150 students were considered for study, in which we have divided them in to 3 groups randomly and equally including both girls and boys. Group A had 23 boys and 27 girls(total 50), group B had 24 boys and 26 girls(total 50), and Group C had 24 boys and 26 girls(total 50).The topic chosen was introduction to BP, with no prior intimation.

Group A attended lecture class, group B attended demonstration and Group C attended SGD. All the session were conducted by only one faculty and conducted in sequence one after the other(to avoid teacher bias and also time bias).

**Objective Assessment:** Around 25 MCQ's were prepared related to the topic and all students

had answered to those MCQ's before and after their respective sessions. The test before the session was named as pre-test and the test after the session was post-test I and one more post test was conducted after one month that was named as post- test II. Pre-test, post-test I and Post-test II marks were compared in all the groups after respective sessions of teaching.

All the 150 student were asked to come to lecture class at sharp 8 o' clock, then we divided student in 3 groups (as previously mentioned, and students were not aware of which topic is going to be taken and also what kind of teaching method they were involved).Group A stayed in lecture class and other two groups went to their respect practical hall and seminar hall(to avoid topic bias we told one group to read thyroid and other to read cortisol as we will come and discuss).

In Lecture class group A attended pre-test, 20 min lecture class on Introduction to BP and post-test, so this took around 30 min. Then same faculty took pre-test, practical demonstration of BP for 20 min and post-test for group B. the last group was C, where pre-test was conducted, then SGD on BP which took more time (as it was discussion, we had to divided 50 students in to 6 small groups, provided them textbooks and manuals to read about introduction to BP for about 30 mins and then we discussed) and post-test was conducted for group C. (session were conducted by same faculty, for conducting pre and post other faculty were involved).

**Subject Assessment:** To find out the students perception towards the different teaching method, five questionnaires were given to each group as a feedback form, which they filled along with the post tests. To evaluate five point likert scale was used.

**Statistical Analysis:** Statistical analysis was done using paired "t" test to compare the pre test,post-test I and II of group A, group B and group C . A "p" value of less than 0.05 was considered as statistically significant.

**Results****Table 1: Paired t test (pre-test and post-test I)**

	Mean	N	Std. deviation	t value	P value
Group A (Pre-test)	8.15	47	2.797	-23.692	0.000*
Group A (Post-test I)	17.38	47	2.533		
Group B (Pre-test)	7.02	44	2.565	-17.991	0.000*
Group B (Post-test I)	15.84	44	2.658		
Group C (Pre-test)	6.72	46	2.697	-15.586	0.000*
Group C (Post-test I)	15.35	46	3.695		

\*= $P$  value  $<0.05$ **Table 2: Paired t test (pre-test and post-test II)**

	Mean	N	Std. deviation	t value	P value
Group A (Pre-test)	8.02	44	2.816	-13.169	0.000*
Group A (Post-test II)	14.18	44	2.912		
Group B (Pre-test)	6.88	41	2.472	-14.647	0.000*
Group B (Post-test II)	14.41	41	3.331		
Group C (Pre-test)	6.91	44	2.595	-16.885	0.000*
Group C (Post-test II)	15.27	44	2.944		

\*= $P$  value  $<0.05$

Table 3: Paired t test (post-test I and post-test II)

	Mean	N	Std. deviation	t. value	P value
Group A (Post-test I)	17.36	44	2.593	7.879	0.000*
Group A (Post-test II)	14.18	44	2.912		
Group B (Post-test I)	15.76	41	2.625	2.166	0.036*
Group B (Post-test II)	14.41	41	3.331		
Group C (Post-test I)	15.43	44	3.744	.277	0.783
Group C (Post-test II)	15.27	44	2.944		

\*=P value <0.05

Table 4: Likert scale (Subjective assessment)

Lecture class (47 students)	Strongly agree	Demonstration (45 students)	Strongly agree	Group discussion (45 students)	Strongly agree
Visualizing concepts	18(38%)	Visualizing concepts	36(80%)	Visualizing concepts	19(42%)
Sustaining interest	22(46%)	Sustaining interest	26(57%)	Sustaining interest	28(62%)
Remembering facts	20(42%)	Remembering facts	19(42%)	Remembering facts	27(60%)
Understanding better	22(46%)	Understanding better	25(55%)	Understanding better	33(73%)
Applying knowledge	17(36%)	Applying knowledge	22(48%)	Applying knowledge	25(55%)

## Discussion

Looking at our results all the groups have performed significantly better in post-test I compared to pre-test ( $P=0.00$ ) (Table 1) and also significant ( $P=0.00$ ) difference is there between pre-test and post-test II in all the groups (Table 2). But looking at the Table 3 where we have compared between post-test I and post-test II in which students performance has reduced significantly ( $P<0.05$ ) in post-test II in group A (who attended lecture) and group B (who attended demonstration) but post-test II performance is not reduced in group C (who attended discussion), this shows that small group discussion (SGD) is better way of teaching in which students can still remember the subject even after 1 month and performed well compared to other two teaching method. Our results were similar to the other studies<sup>1,7</sup>. In subjective assessment of our study, student strongly agreed that group discussion is much better than lecture class and demonstration type of teaching (table 4). So both objective and subjective assessments of our study showed group discussion is better way of teaching. There are studies who showed that small group discussion is better way of teaching in other subjects like in biochemistry<sup>8</sup> and in microbiology<sup>9</sup>. The study carried out by<sup>10</sup>, where instead of using MCQs they used short answer questions (SAQs) and assessed their retention after 15 days of small group discussion, they found it as very effective way of method of teaching. Small group discussion was advantageous because it motivates students to involve in discussion actively and helps for retention and also students were able to express their ideas and then receive input from the group members<sup>1,9</sup>. Small group discussion favours participation of everyone, as students are more comfortable in small groups. They are made to prepare specific tasks for the group to answer to the solution. All students when they work in groups, it enhances their reasoning abilities as well<sup>11</sup>. Some studies have even proved that integrated teaching is be more effective in improving the knowledge and attitude of students than traditional lecturing method, so integrated method of teaching needs to be implemented in the medical education curriculum for better understanding and best outcome<sup>12</sup>. In our study we used demonstration as one more method of teaching, where students post-test II performance was reduced, may be OSPE as assessment is better method of assessing with regards to practical demonstration of teaching BP, but to avoid the bias of assessment we used same MCQs method in our study.

Teaching in Physiology is followed always through lectures, practical's and tutorials. According to the university curriculum physiology is taught with the help of lecture classes, as delivering of more information to large group of students, but it is passive method and also teachers centred as a teacher it is not only duty to deliver large information to students but make sure that students should understand the information and can use that skilfully once they enters the clinical departments. Now a days there are so many types of teaching method including integrated teaching which actually help students to correlate the subjects and help them once they enter the final year.

**Limitations:** To avoid the other biases like, students should not know in which type of teaching they are going to attend, we didn't give any prior intimation to the group which came for discussion, as they need more time to read, we gave them little less time once the session started. For demonstration method of teaching we would have used OSPE, but we didn't used because assessment bias would have arrived.

## Conclusion

Our study indicates that small group discussion is better than the lecture class and demonstration type of teaching both subjectively and objectively. To teach Physiology small group discussion is better method, as most of the topics can be discussed but not all can be demonstrated and also retention is much better after small group discussion compared to other two method in our study.

**Conflict of Interest:** Nil

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**Ethical Clearance:** Got from institutional ethical committee.

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# Effect of Indian Classical Instrumental Music on Stress and Anxiety in Male Medical Students

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## Abstract

**Introduction:** Medical professionals, especially medical students face a lot of stress and anxiety due to various challenges at personal and professional levels. Stress and anxiety affect their health and well-being and causes mental disturbance. Blood pressure, heart rate, and other biochemical parameters show an abnormal rise due to stress and anxiety. Music therapy using Indian classical instrumental music can help relieve this stress and anxiety and bring the biochemical parameters to normal levels.

**Aim:** The purpose of the present study was to evaluate the immediate and short term effects of Indian classical instrumental music on stress and anxiety using both qualitative and quantitative measurements on male medical students.

**Materials and Method:** Fifty-six (56) healthy male medical students aged between 17-25 years were selected from Rajkiya Medical College, Jalaun (Uttar Pradesh), for a month-long music therapy which included listening to Indian classical instrumental music every day for thirty minutes. ADSS questionnaire, salivary cortisol, blood pressure, heart rate, and lipid profile other required parameters were recorded twice, once at baseline and another after one month of music therapy.

**Results:** Post music therapy values revealed a significant decrease in ADSS score anxiety ( $4.36 \pm 1.39$  v/s  $3.36 \pm 1.20$   $p < 0.0001$ ), depression ( $3.14 \pm 1.70$  v/s  $2.23 \pm 1.50$ ,  $p < 0.0001$ ) Stress ( $4.54 \pm 1.91$  v/s  $3.39 \pm 1.77$ ,  $p < 0.0001$ ) Salivary cortisol ( $4.04 \pm 1.39$  v/s  $3.33 \pm 1.35$ ,  $p < 0.0001$ ). Further, a significant decrease was recorded in systolic blood pressure ( $122.53 \pm 5.24$  v/s  $120.86 \pm 2.50$ ,  $p = 0.0005$ ) diastolic Blood Pressure ( $82.75 \pm 5.36$  v/s  $80.82 \pm 1.74$ ,  $p = 0.0045$ ) and heart rate ( $73.43 \pm 3.09$  v/s  $71.99 \pm 1.15$ ,  $p < 0.0001$ ).

**Conclusion:** The findings of the present study showed that listening to Indian classical instrumental music notably reduces salivary cortisol, blood pressure, heart rate, respiration rate, blood glucose, and lipid profile, and helps to relieve stress and anxiety in male medical students. However, further studies on a larger population are required to make a general policy to facilitate the better mental health of medical students.

**Keywords:** Stress, anxiety, young male medical students, music therapy.

## Introduction

Stress is an excessive and acute fear and worry about

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anything. Anxiety occurs in response to stress.<sup>(1)</sup> When a body suffers stress, it responds to it through physical, physiological or mental adaptation.<sup>(2)</sup> Many chronic and acute diseases are triggered by stress. It is also seen that stress acts as a cause for many cardiovascular diseases, and sometimes gives rise to fatal diseases like cancer.<sup>(3-5)</sup> In response to stress and anxiety in human body, the level of blood pressure<sup>(6)</sup>, heart rate<sup>(7)</sup>, respiration rate<sup>(8)</sup>, Salivary cortisol<sup>(9)</sup>, blood sugar<sup>(10)</sup> and lipid profile<sup>(11)</sup> increase.

In medical students, stress level is higher as compared to students of other profession because of the medical field, and this stress level keep on increasing throughout the course in medical college.<sup>(12,13)</sup> A number of stressors have been found to affect the well-being of medical students and they often result in psychological morbidities like depression and anxiety.<sup>(14)</sup> In a self-executed, questionnaire-based study, it was found that 39.1% medical students were distressed, 15.6% were suffering from anxiety, and around 12% were found to be depressed.<sup>(15)</sup>

Music helps in lowering anxiety and elevating mood. It also affects various physiological and biochemical factors such as blood pressure, pulse rate, respiration rate, lipids profile and blood sugar levels.<sup>(16)</sup> Indian classical instrumental music has been found to relieve stress in gastroscopy patients.<sup>(17)</sup> Indian *raga* improves attention and concentration in college students.<sup>(18)</sup>

However, the effects of Indian classical instrumental music on stress and anxiety of male medical students are still unclear, hence the present study was conducted to assess the effect of Indian classical music on stress and anxiety in male medical students.

## Material and Method

**Participants:** The current interventional type of study included fifty-six (56) male medical students from Rajkiya Medical College, Jalaun (UP). This study took place in the Department of Physiology. The inclusion criteria included a healthy first-year M.B.B.S. male students aged between 17-25 years. Students who were diagnosed with any medical disorders; undergoing medical treatment; having a dislike for music and preferred not to listen to it; suffering from severe anxiety and major depressive disorder; undergoing any Complimentary and Alternative Medicine (CAM's) were excluded from the study.

Pre and post music therapy the following parameters-ADSS questionnaire, salivary cortisol, blood pressure, heart rate, respiration rate, fasting blood sugar and lipid profile, were recorded. All the participants were made aware in detail about the objectives of the study. Informed written consent was taken from all the subjects that ensured that the participants could make an informed, voluntary and rational decision to participate. A demographic questionnaire was filled by them to gather information about their age, weight, and height. Psychological health, i.e., the overall level of

stress and anxiety in the subjects was measured by using responses to self-reported measures through the ADSS test questionnaire.<sup>(19)</sup>

**Measurements:** All of the physiological and biological parameters were recorded and calculated. These parameters were taken before noon. For salivary cortisol, the circadian fluctuations of hormone levels are pronounced in the morning hours and flatten throughout the day.<sup>(20,21)</sup>

**Music Intervention:** Music therapy was started for all the students after recording pre-music therapy parameters. All the subjects were asked to come at 7:30 am<sup>(22)</sup> sharp in a hall with a capacity of 60 to 70 persons. The hall was properly ventilated and isolated from noisy gathering places and distracting sounds, to provide easy access to all the subjects. Once settled on respective chairs from 7:30 am to 7:40 am, participants were asked to relax and make themselves comfortable. After 10 minutes, i.e., at 7:50 am, the Indian classical instrumental music (*Raga Desi-Todi* played on a flute by a renowned Indian musician, Pt. Hari Prasad Chaurasia)<sup>(23)</sup> was played by the attendants for the next 30 minutes<sup>(23,24)</sup> at 50 to 60 DB.<sup>(25)</sup> All the subjects were advised to keep their eyes closed during music therapy.<sup>(25)</sup>

Half an hour later, at 8:20 am, the music was stopped and the candidates were allowed to leave the room. This therapy took place in the same manner for a month.<sup>(23)</sup> On the fifteenth and the thirtieth day of music therapy, all the candidates were asked feedback about how they felt during the therapy. Students were free to contact for guidance if they had some queries or felt uneasy.

**Parameters:** Cortisol: For the analysis of cortisol, saliva was collected using small cotton swabs. Participants were given cotton roll to gently chew for 1 minute. After one minute, the stimulated saliva sample was collected by taking the chewed cotton roll and placing it into a small plastic tube. Saliva was analyzed by cortisol ELISA kit by BioVision, and this was conducted between 12:00 to 17:00 hours, to minimize the confounding effect of the hormonal diurnal rhythm.<sup>(26)</sup>

**Blood pressure:** Blood pressure was measured by the auscultatory method using a Sphygmomanometer.<sup>(27)</sup>

**Heart Rate:** Heart rate was recorded from the radial pulse.<sup>(28)</sup>

**Respiration Rate:** In the resting condition, the

respiration rate was calculated by counting the number of chest-rise per minute.<sup>(29,30)</sup>

**Glucose level:** It was estimated by the fasting method by the GOD-POD method.<sup>(30)</sup>

**Lipid Profile:** Lipid profile in the serum sample was measured by serum concentrations of the following parameters:

The serum concentration of total cholesterol was estimated by the enzymatic CHOD-POD method.<sup>(30)</sup>

The serum concentration of triglycerides was calculated by the GPO-PAP method.<sup>(30)</sup>

The serum concentration of high-density lipoprotein was measured by CHOD-POD/Phosphotungstate method.<sup>(30)</sup>

The serum concentration of low-density lipoprotein was evaluated by using Friedewald’s formula:  $LDL\ cholesterol = total\ cholesterol - HDL\ cholesterol - [triglycerides/5]$ .<sup>(31)</sup>

**Statistical Analysis:** Outcomes were expressed as Mean ± SD (standard deviation of the mean). The statistical data were analyzed and compiled using SPSS 21st version software manufactured by IBM USA. To compare values obtained before and after the musical intervention, the student’s paired t-test was applied. A p-value<0.05 was considered significant.

### Results

Results of the present study included data of various parameters in all the participants before and after music intervention. For all given parameters, degree of freedom: 55, 95% confidence interval was considered.

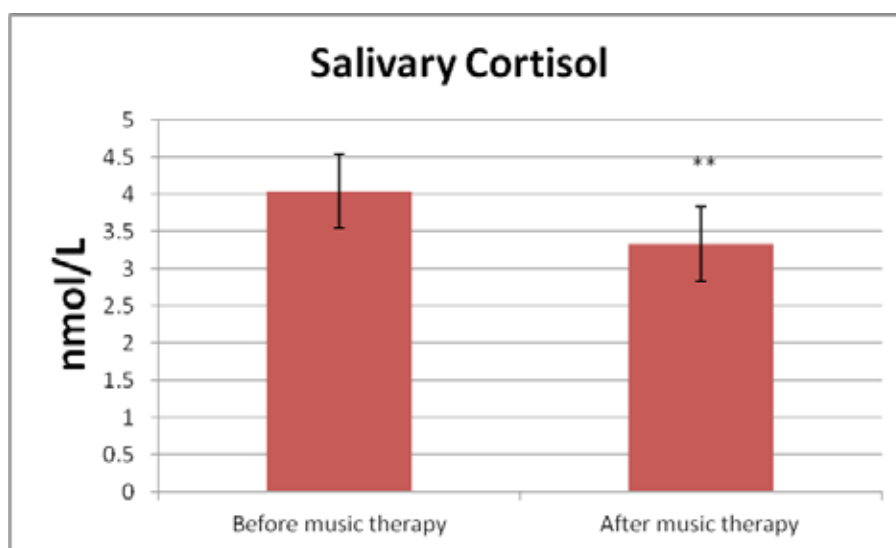
**Table 1: ADSS scores pre music therapy and post music therapy.**

	Pre Music Therapy	Post Music Therapy	p-value
Anxiety (0-9)	4.36±1.39	3.36±1.20	<0.0001**
Depression (0-9)	3.14±1.70	2.23±1.50	<0.0001**
Stress (0-9)	4.54±1.91	3.39±1.77	<0.0001**

Values expressed as Mean+/-SD. \*\*highly significant.

The level of anxiety, depression, and stress ranges from 0-9. Above 9, the condition is severe.

Table 1 suggests that mean ADSS score for anxiety (4.36 ±1.39 v/s 3.36 ± 1.20), p<0.0001 depression (3.14 ± 1.70 v/s 2.23 ± 1.50), p<0.00001 and stress (4.54±1.91 v/s 3.39±1.77), p<0.0001 was significantly decreased in male medical students after participating in music therapy for one month.



\*\*p<0.0001 Highly significant

**Figure 1. Mean difference in salivary cortisol pre music therapy and post music therapy.**



It is evident from Figure 1 that the baseline values of salivary cortisol significantly depleted from  $4.04 \pm 1.39 \text{ nmol/L}$  to  $3.33 \pm 1.35 \text{ nmol/L}$  ( $p < 0.0001$ ) in male medical students post music therapy a month later.

**Table 2: Blood pressure pre music therapy and post music therapy**

Parameter	Pre Music Therapy	Post Music Therapy	p-value
SBP(mmHg)	122.53±5.24	120.86±2.50	0.0005**
DBP(mmHg)	82.75±5.36	80.82±1.74	0.0045**

Values expressed as Mean+/-SD. SBP= systolic blood pressure, DBP= diastolic blood pressure, \*\* highly significant

Further, A significant decrease in systolic blood pressure from  $122.54 \pm 5.24 \text{ mmHg}$  to  $120.86 \pm 2.50 \text{ mmHg}$  ( $p=0.0005$ ) and diastolic blood pressure from

$82.75 \pm 5.36 \text{ mmHg}$  to  $80.82 \pm 1.74 \text{ mmHg}$  ( $p=0.0045$ ) was recorded after one month musical therapy. (Table 2).

**Table 3: Heart rate, respiration rate and fasting blood sugar pre music and post music therapy**

Parameter	Pre Music Therapy	Post Music Therapy	p-value
HR(beats/min)	73.43±3.09	71.98±1.15	<0.0001**
RR(/min)	14.46±1.94	12.64±0.94	<0.0001**
FBS(mg/dL)	91.61±10.35	87.32±8.69	<0.0001**

Values expressed as Mean+/-SD. \*\*highly significant. HR=heart rate, RR=respiration rate, FBS= fasting blood sugar

Table 3 shows a significant reduction in heart rate, respiration rate and fasting blood sugar post music therapy. There was a significant decrease of  $1.45 \pm 1.94$  beats/min, ( $p < 0.0001$ ),  $1.82 \pm 1.00$ /min, ( $p < 0.0001$ ) and

$4.29 \pm 2.66 \text{ mg/dL}$ , ( $p < 0.0001$ ) in heart rate, respiration rate and fasting blood sugar respectively in male medical students after month-long music therapy.

**Table 4: Lipid profile pre music therapy and post music therapy.**

Parameter	Pre Music Therapy	Post Music Therapy	p-value
TC(mg/dL)	199.33±33.54	198.98±33.42	<0.0001**
TG(mg/dL)	115.44±24.49	114.16±24.91	<0.05*
HDL(mg/dL)	45.96±6.43	46.66±6.50	<0.0001**
LDL(mg/dL)	128.88±30.09	128.53±30.04	<0.0001**

Values expressed as Mean+/-SD. \*significant \*\* highly significant. TC=total cholesterol, TG=triglycerides, HDL= high-density lipoprotein, LDL=low-density lipoprotein.

Further, a significant decrease of TC ( $p < 0.0001$ ), TG ( $p < 0.0001$ ) and LDL ( $p < 0.0001$ ) was observed in all the participants after following one month music therapy. On the other hand, a significant increase of  $0.69 \pm 0.1 \text{ mg/dL}$  with a p-value  $< 0.0001$  was recorded in post HDL value compare to pre HDL value. A decrease in TG with a p-value of 0.05 was recorded.(Table 4)

## Discussion

Stress is a general response of the body to any mental or physical pressure on it.<sup>(32,33)</sup> Stress is a condition or a feeling that one experiences upon the realization that the expectations from him/her overshadow the social as well as personal potentiality and resources the individual

can put forth.”<sup>(34)</sup> Anxiety, on the other hand, acts as a response to stress both in moderation and in excess. In moderation, stimulates an anticipatory and adaptive response; and if it exceeds, it will destabilize and dysfunction the individual.<sup>(35,36)</sup> Although anxiety is as familiar and probably as devitalizing as depression,<sup>(37)</sup> it has managed to achieve less recognition and is often unseen and undertreated in the general population.<sup>(38)</sup>

Indian medical students suffer more stress and anxiety than other professionals.<sup>(39, 40)</sup> This is also because the Indian medical education system is slightly different from other regions, especially because of its selection process.<sup>(41)</sup> Moreover, the influence of one’s family in deciding the career in the medical field too plays a role in growing stress, anxiety and depression in Indian medical students.<sup>(39, 42)</sup> Furthermore, Indian medical students rarely seek professional help, mostly because of embarrassment and taboo concerning mental health.<sup>(43)</sup>

The present study recorded a significant decrease in the ADSS score of anxiety, depression, and stress in male medical students after following one month of music therapy. The results of the current study were very similar to the findings of the previous study of Prakash K et al.<sup>(44)</sup> as they revealed that the music therapy made the subjects feel less stressed and anxious. These significant changes of ADSS score in all three parameters seem to be due to one-month music therapy as a prior study suggests that listening to the music may have increased the HPA axis activation and assisted the faster recovery of the ANS, which further reduced stress and anxiety in the subjects.<sup>(16)</sup>

Further, a significant decrease in salivary cortisol was observed in the present study. These findings are very similar to the earlier studies of YC Hou et al<sup>(45)</sup> and Shaji John et al.<sup>(46)</sup> YC Hou et al<sup>(45)</sup> recorded that providing music during hemodialysis is an effective complementary therapy to relieve the frequency and severity of adverse reactions, as well as to lower salivary cortisol levels. Besides, Shaji John et al<sup>(46)</sup> found that there was a significant decrease in salivary cortisol levels in participants after music training.

This decrease in salivary cortisol secretion as recorded in our study may be due to reduced secretion of ACTH from the pituitary or decreased secretion of cortisol from the adrenal glands due to a reduction in stress and anxiety.<sup>(47)</sup> It has been suggested in studies

that salivary cortisol is one of the reliable physiological markers of stress which is decreased by music via inducing the activity of HPA-Axis.<sup>(16,47)</sup>

Stress is seen as the main threat of health mentally as well as physically.<sup>(48)</sup> The decrease in stress can reduce this threat and facilitate better well-being of the human body and mental health.<sup>(49)</sup> This decrease of stress observed in our study might be helpful to facilitate an improved mental state of mind in male medical students as a low level of stress has been found associated with better use of the mental ability.<sup>(50)</sup> As a response to stress, the endocrine system begins to release cortisol. Therefore a decrease in salivary cortisol marks the decrease in stress and anxiety.<sup>(51)</sup>

Our study recorded a significant decrease in blood pressure after following one month of music therapy. Similar results were shown in a study conducted by Wendy E. K. and Nikki S.<sup>(52)</sup> Wendy E. L and Nikki S observed that the exposure to music prevented the stress-induced increases in physiological parameters such as subjective anxiety, heart rate and systolic blood pressure in both male and female subjects. This decrease in blood pressure as observed in the present study might be due to music listening evoke some relaxation response which in turn helps in decreasing the blood pressure.<sup>(53)</sup> Another possible mechanism of action could be that music intervention resulted in heightened brain dopamine levels via a calmodulin-dependent system. This rise in dopamine levels restrained sympathetic activity via dopamine-2 receptors which in turn reduced blood pressure.<sup>(54)</sup> Besides, it was observed that the heart rate changed to normal post music therapy for a month. This may be due to the reason that classical music tends to relax the body and stimulate the parasympathetic nervous system, thereby changing the abnormal heart rate to normal.<sup>(55)</sup> It was also perceived that listening to music for a month regulated the respiration rate. Siritunga et al found a similar result in their study.<sup>(56)</sup> Music acts on the central and autonomic nervous system and the endocrine system sympathetic activity with concurrent activation of parasympathetic drive and a reduction in the stress-hormone release, hence regulating the respiration rate.<sup>(56)</sup> Also, fasting blood sugar levels were seen to improve post music therapy. Madhuri Sharma et al<sup>(57)</sup> found related derivation in their previous study. Classical music, a refined sound, stimulates the cell activity in the pancreas and normalizes the blood sugar release, hence reducing the increased blood glucose levels.<sup>(58)</sup>

A reduction in triglyceride, LDL with an increase in HDL concluded that music therapy lowers blood lipid levels. This finding was consistent with the results of the previous study conducted by Madhuri Sharma et al. (57) However, the reasons for such an effect of classical music on the lipid profile is unknown.

### Conclusion

Findings of the present study suggest that salivary cortisol, anxiety, depression and stress level were significantly decreased in male medical students after following music therapy with Indian classical instrumental music (*raga*) for a month. Moreover, a significant decrease in blood pressure, heart rate and fasting blood sugar levels were recorded after music therapy. Therefore, we strongly suggest music therapy for medical students suffering from stress and anxiety to decrease the stress level and enhancing mental health. However, further studies on a larger population are required to make a general policy to facilitate the better mental health of medical students.

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**Conflict of Interest:** We certify that there is no conflict of interest.

**Ethical Clearance:** The Ethical clearance was taken from the ethical committee of Rama Medical College, Hospital & Research Center, Kanpur, Uttar Pradesh

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# Correlation of Changes of Blood Pressure and Intra Ocular Pressure After Isometric Handgrip Exercise Test in Young Adults

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## Abstract

**Objective:** To correlate the blood pressure changes to intraocular pressure before and after Handgrip exercise

**Method:** Healthy young male adults in the age group of 18-22 years were selected among general population. Sample size was 40. Heart rate and intra ocular pressure (IOP) were recorded at rest and after isometric Handgrip test

**Results:** Handgrip predictably raised Systolic blood pressure has increased significantly from resting of  $113.40 \pm 5.75$  to  $133.50 \pm 4.29$  ( $p < 0.001$ ) immediately after Handgrip exercise SBP has returned back to resting level within 10 min after exercise.

Diastolic blood pressure has increased from significantly from resting of  $72.45 \pm 4.37$  to  $89.95 \pm 3.28$  ( $p < 0.001$ ) immediately after Handgrip IOP has returned back to baseline level within 10 min after exercise ( $p < 0.001$ -sig).

Right eye IOP has decreased significantly from resting  $16.27 \pm 1.54$  to  $13.34 \pm 1.32$  ( $p < 0.001$ ) after handgrip exercise.

Left eye IOP has decreased significantly from resting  $16.28 \pm 1.55$  to  $13.04 \pm 1.19$  ( $p < 0.001$ ) immediately after handgrip exercise; ( $p < 0.05$ ).

Blood Pressure is significantly and negatively correlated with IOP (Pearson's correlation coefficient,  $r = -0.352$ ).

**Conclusion:** Isometric Handgrip exercise induces raise in blood pressure and simultaneously lowers IOP which were significant. Hence may prove useful in normotensive glaucomatous patients

**Keywords:** Blood Pressure, Intraocular pressure, Handgrip dynamometer

## Introduction

Glaucoma is chronic progressive optic neuropathy caused by a group of ocular conditions which lead to damage to optic nerve with loss of visual function. Most

common risk factor is raised intraocular pressure<sup>(1,4,5)</sup>. Relationship between isokinetic exercise & IOP showed significant lowering of IOP after exercise.<sup>(2,3)</sup>

**Aims and objective:** To correlate the blood pressure changes to intraocular pressure before and after Handgrip exercise.

## Materials and Method

Fourty healthy young male adults in the age group of 18-22 years with BMI of  $18-22.9 \text{ kg/m}^2$  were

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selected from general population. Blood pressure and IOP were recorded at rest and after isometric Handgrip test. Subjects with pre-existing refractive error, acute and chronic conjunctivitis, glaucoma, migraine were excluded from study.<sup>(7)</sup>

#### Materials:

- Schiotz tonometer
- Handgrip dynamometer
- Sphygmomanometer.

#### Parameters:

- Study was carried out in physiology department
- Intraocular pressure in mm hg in supine position using standard steps.
- Weight in kilogram. & height in meters were measured. BMI=Weight in kg/height in meter<sup>2</sup> was calculated to group them as normal weight.
- Systolic and Diastolic blood pressure.
- Maximum voluntary contractions(MVC) was assessed and subjects were asked to carry out endurance isometric exercise at 40% of their MVC

#### Study Method:

It is a Cross sectional study.

Ethical clearance was obtained from institutional ethical committee. Prior to the procedure written and informed consent was obtained from all the subjects.

#### Inclusion Criteria:

- Young healthy adults males in the age group of 18-22yrs
- Non obese BMI 18 – 22.9 kg/m<sup>2</sup>.
- Normotensive < 130/80mm Hg.
- Non smoker
- Non alcoholic
- Euglycemic

#### Exclusion Criteria:

- Pre-existing refractive errors.
- Contact lens wearers.
- Glaucoma.
- H/o Migraine.

- H/o Conjunctivitis acute or chronic.
- Any systemic illness affecting IOP.

The exercise was performed in a well-ventilated room. Participants were instructed not to consume beverages nor a heavy meal in previous 4hours or participate in any vigorous activities 24 hour before test.

Isometric endurance contraction at 40% of the individuals MVC was executed with Handgrip dynamometer.

In order to minimize the bias of diurnal variations of IOP and other parameters, the studies were conducted between 3pm to 4pm.

At the reporting time subjects were asked to relax in supine position for 5min. Baseline IOP was recorded. Subjects executed MVC contractions of 1 second duration at 1 minute interval for 3 times. Maximum of these is considered as their MVC .Then endurance contraction at 40% of their MVC is made. Intraocular pressure and Blood pressure were measured in supine position immediately (within 30 sec), at five, at ten, at fifteen minutes after exercise.

**Statistical Analysis:** Mean and Standard deviation was calculated for isometric Handgrip exercise test in young adults. Paired t-test was applied at 5% level to test the significance of changes in above parameters(Using Epi-Info) Microsoft Excel and EPI-INFO package were used for data entry and statistical analyses respectively.

Correlation was calculated using Pearson's correlation test.

#### Result

Right eye IOP has decreased significantly from resting 16.27±1.54 to 13.34±1.32 (p<0.001) immediately after handgrip exercise IOP has returned back to baseline level within 15 min after exercise.

Left eye IOP has decreased significantly from resting 16.28±1.55 to 13.04±1.19 (p<0.001)immediately after handgrip exercise IOP has returned back to resting level within 15 min after exercise.

Systolic blood pressure has increased significantly from resting of 113.40±5.75 to133.50±4.29 (p<0.001) immediately after handgrip exercise SBP has returned back to resting level within 10 min after exercise.

Diastolic blood pressure has increased from resting of 72.45±4.37 to 89.95±3.28 (p<0.001) immediately after handgrip IOP has returned back to baseline level within 10 min after exercise (p=0.001-sig).

**Table 1: Mean and SD of IOP of right & left eye between the two groups after Isometric hand grip Exercise**

	Duration	Hand Grip	P Value
Right eye IOP	Resting	16.27±1.54	>0.05
	1 min exercise	13.34±1.32	<0.001*
	5 min postexercise	14.61±1.36	<0.001*
	10 min postexercise	16.03± 1.43	<0.001*
	15 min pt exercise	16.28±1.55	>0.05
Left eye IOP	Resting	16.28±1.55	>0.05
	1 min exercise	13.04± 1.19	<0.001*
	5 min postexercise	14.39±1.28	<0.001*
	10 min postexercise	15.79±1.44	<0.001*
	15 min pt exercise	16.11±1.65	>0.05

Data presented as mean & SD

\*Statistically significant p < 0.05

**Table 2: Mean and SD of SBP & DBP between the two groups after Isometric hand grip Exercise**

Parameter	Duration	Hand Grip	P Value
Systolic BP	Resting	113.40±5.75	>0.05
	1 min postexercise	133.50±4.29	<0.001*
	5 min postexercise	130.25±4.13	<0.001*
	10 min postexercise	113.50±5.65	>0.05
	15 min post exercise	113.50±5.65	>0.05
Diastolic BP	Resting	72.45±4.37	>0.05
	1 min postexercise	89.95±3.28	<0.001*
	5 min postexercise	87.20± 3.38	<0.001*
	10 min postexercise	72.60±4.53	>0.05
	15 min post exercise	72.60±4.53	>0.05

Data presented as mean & SD

\*Statistically significant p < 0.05

**Table 3: Correlations between SBP, DBP & IOP Right & left eye after immediate exercise**

	ISBP	IDBP
<b>Pearson Correlation</b>		
RIIOP	-0.299	-0.155
Sig. (2-tailed)	0.061	0.340
LIIOP Pearson Correlation	-0.031	-0.107
Sig. (2-tailed)	0.850	0.510

Blood pressure is significantly & negatively correlated with IOP (Pearson's correlation coefficient,  $r = -0.352$ ).



## Discussion

Rise in blood pressure has been suggested to be mediated primarily by the central command which is related to number of motor units activated and to reflex effects from active muscle mechanoreceptors. Inhibition of inhibitory cardiac vagal nerve activity also contributes.<sup>(8)</sup>

Isometric Hand grip exercise stimulate ocular sympathetic nervous system to increase the facility of outflow and thus decreases IOP. Also epinephrine stimulates synthesis of cAMP. Activation of cAMP decreases IOP by decreasing aqueous humour production.<sup>(6,9)</sup>

Also After Hand grip exercise there is rise in blood lactate levels. Increased Lactate levels causes outflux of water from eye which is responsible for fall in IOP.

## Conclusion

Isometric handgrip exercise induces raise in blood pressure and simultaneously lowers IOP and both were significant. Hence may prove useful in normotensive glaucomatous patients.

**Conflict of Interest:** Nil

**Ethical Clearance:** Ethical clearance was obtained from the institutional ethical clearance committee.

**Funding:** Self

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# Assessment of Hormonal Essay in Non-Obstructive and Obstructive Subject in Central India

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## Abstract

Infertility is slowly become major issue for married fertile couple in India. Now a days due to availability of many techniques and tests we came to know that male partner is equally contributed for infertility. The term “Azoospermia” is defined as the complete absence of sperm in the ejaculate, is identified in approximately 1% of all men and in 10 to 15% of infertile males. The various causes of infertility include disturbances in both partners (26%), disturbances in female partner (39%) and disturbances in the male (20%). No identifiable cause was found in 15% of cases. Around 1.5% azoospermic infertile male attributed to obstruction of the excurrent genital tract. In this study we focus on some etiological facts of azoospermia that plays important role in causing male infertility. We found that the level of hormones FSH and LH are significantly raised as compare to control group. Also, the testosterone level is significantly less in azoospermic subject as compare to control group. When we separately compared the hormonal profile of Obstructive and non-Obstructive subjects we found the level of Gonadotropic hormone FSH and LH is significantly high in Non-obstructive Azoospermic patients while the testosterone hormone level is significantly low in Non-obstructive Azoospermic subjects. It indicates the primary hypogonadism. This study depicts the hormonal profile in infertile male that will be helpful in further assessment and management of such individuals.

**Keyword:** Infertility, Obstructive and non- Obstructive azoospermia, Gonadotropic hormone FSH and LH, Spermatogenesis.

## Introduction

In every species the progeny carries the gene that is must for continuation of species. In India since so many decade one more new issue is arises which is related to big issue in society that is male infertility.

According to World Health Organization, “Infertility is the inability of a sexually active non contracepting couple to achieve pregnancy in one year”. Infertility

is a common condition with important psychological, economic, demographic and medical implications. Demand for infertility services has grown substantially even though the prevalence of infertility has been stable.

The term “Azoospermia” is defined as the complete absence of sperm in the ejaculate, is identified in approximately 1% of all men and in 10 to 15% of infertile males.<sup>1</sup> This diagnosis must be confirmed by centrifugation of a semen specimen for 15 min at room temperature with high-powered microscopic examination of the pellet and a centrifugation speed of at least 3,000 rotation per minute. The semen analysis should be performed according to the 2010 WHO guidelines, and at least two semen samples obtained more than two weeks apart should be examined.<sup>2,3</sup>

The various causes of infertility include disturbances in both partners (26%), disturbances in female partner

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(39%) and disturbances in the male (20%). No identifiable cause was found in 15% of cases. Around 1.5% azoospermic infertile male attributed to obstruction of the excurrent genital tract.<sup>4</sup>

Azoospermia may be due to variety of condition and complete history, physical examination, hormonal profile, genetic and imaging resources will be necessary not only to establish the cause but also to direct the couple towards the best treatment option suitable. The evaluation of a patient with azoospermia is performed to determine the etiology of the patient's condition.

Hormones evaluation is essential parameters in giving a definitive diagnosis in infertile males.<sup>5</sup> Abnormal hormone production has been noted as a male causative factor in male infertility and hormonal replacement could play a corrective role.<sup>6,7</sup> The most essential hormones to be evaluated include, follicle-stimulating hormone (FSH), luteinizing hormone (LH) and testosterone.<sup>8</sup> Merino, G et.al., suggested that decrease in sperm count is associated with low testosterone level.<sup>9</sup> Changes in FSH and LH levels could result in abnormalities of spermatogenesis in patients with low sperm counts.<sup>10</sup>

Spermatogenesis is regulated by luteinizing hormone (LH) produced by the pituitary gland. LH binds to receptors on Leydig's cells and stimulates testosterone production, which in turn binds to sertoli cells to promote spermatogenesis. Follicle stimulating hormone (FSH) is also essential because its binding to sertoli cells stimulates testicular fluid production and synthesis of intracellular androgen receptor proteins.<sup>10</sup> Testosterone regulates its own secretion by negative feedback mechanism. It acts on hypothalamus and inhibits the secretion of luteinizing hormone-releasing hormone (LHRH). When LHRH secretion is inhibited, LH is not released from anterior pituitary, resulting in the stoppage of testosterone secretion from testes. On the other hand, when testosterone production is low, lack of inhibition of hypothalamus leads to secretion of testosterone through LHRH and LH.

Some causes of Azoospermia are potentially correctable, other conditions are irreversible but still possibly treatable by assisted reproductive techniques using the husband's semen and finally some causes are irreversible and not amenable to any form of treatment demanding donor semen or adoption in order to constitute a family.

With the above background the present study

was conducted to find out the association between Azoospermia and Gonadotropin levels.

## Material and Method

The current cross sectional study was conducted in department of physiology, rural based Medical College during study period from September 2011 to August 2013. The total numbers of subjects studied were 100 in reproductive age group (21 to 45 year). The semen samples were obtained from male partner of infertile couples attending the reproductive biology unit of department of physiology. They were referred from gynecology department. Samples were analyzed as per WHO guidelines (**WHO laboratory Manual, 2010**).<sup>2</sup> We obtained an informed written consent from all study participants. The use of confidential patient data in this study was fully within the recent guidelines. During the above mentioned period 603 patients visited the reproductive biology unit, out of which 120 patients were diagnosed as Azoospermic. Out of 120 azoospermic patients 30 were follow up cases. They were excluded and 90 freshly diagnosed patients were selected for study. The patients were interviewed about their case histories, their reproductive problems, and their family background.

**Inclusion criteria:**-Subjects belonged to active reproductive age group and which demonstrated azoospermia in semen analysis.

**Exclusion Criteria:** Unmarried male attended reproductive unit for complaints other than infertility & vasectomized subject.

The semen samples were collected after a sexual abstinence of 3 to 5 days (**Preidt. R., 2003**).<sup>11</sup> The most preferred method for collection of sample was Masturbation (Self-Stimulation), another method was Coitus interruptus (withdrawal of penis just prior to ejaculation during sexual intercourse). The semen specimen was collected in a small, clean wide mouthed jar of 10 to 20 ml. In subject with absence of spermatozoa in semen three consecutive semen analyses were performed at an interval of one month each. Finding of absence of spermatozoa was confirmed by centrifuging the sample at 3000 rpm for 15 min and examining the sample under compound microscope.

Macro & microscopic examination of Semen sample were performed. Patient had been explained regarding the procedure. After proper exposure of genital region,

examination was performed with the patient in standing and supine position. A complete generalized & local examination had been done.

Subject was send to clinical biochemistry laboratory for hormonal profile (FSH, LH & free testosterone) and the data was collected. All the data was abstracted on a standardized data collection form. We used a spreadsheet to enter the data electronically and used statistical software SPSS.

## Observation and Results

**Table 1: Distribution of study subjects**

Study Subjects	No. of patient	Percentage (%)
<b>Type of Azoospermia</b>		
Obstructive Azoospermia	36	40
Non-Obstructive Azoospermia	54	60
<b>Total</b>	<b>90</b>	<b>100</b>

\*Control group = 50

In this study, 90 Azoospermic patients were classified on the basis of Pathophysiology diagnosed in Sonological findings as Obstructive and Non-Obstructive Azoospermic patient.

We found 36 cases had obstruction and were classified into Obstructive Azoospermia while in 54

cases obstruction was not seen and were classified into Non- Obstructive Azoospermia.

We observed that out of 90 azoospermic patients, 40 % of patient was of Obstructive Azoospermia and remaining 60 % was of Non- Obstructive Azoospermia. [Table 1].

**Table 2: Comparison of Hormonal profile among study groups**

Hormonal profile	Control (n=10)	Azoospermic (n=90)	z-value	p-value
FSH ((mIU/ml)	17.87±1.76	24.03±9.09	2.12	0.036, S, p<0.05
LH (mIU/ml)	8.07±1.29	12.68±8.54	1.69	0.093, NS, p>0.05
Testosterone (pg/ml)	31.41±3.84	23.23±33.42	0.76	0.444, NS, p>0.05

The Mean FSH levels in Azoospermic were significantly High (24.03±9.09) as compared to that of control (17.87±1.76) with p<0.05. The comparison of

the Mean LH levels and free Testosterone in between Azoospermic and Control were found to be insignificant (p>0.05). [Table 2].

**Table 3: Comparative hormonal profile among different subgroup of Azoospermia**

Sub Group	FSH (mIU/ml) (Mean ± SD)	LH(mIU/ml) (Mean ± SD)	Free Testosterone (pg/ml) (Mean ± SD)
Obstructive Azoospermia (n=36)	18.37±4	8.04±2.28	36.54±48.95
Nonobstructive Azoospermia (n=54)	28.36±9.57	16.26±9.80	13.35±8.91
z-value	6.66	5.76	2.8
p-value	0.000, S, p<0.05	0.000, S, p<0.05	0.000, S, p<0.05

By comparing Hormonal levels in Obstructive and Non-obstructive Azoospermic patients, We found that Mean FSH level in Non-obstructive azoospermia was significantly high ( $28.36 \pm 9.57$ ) as compare to in Obstructive Azoospermia ( $18.37 \pm 4$ ) with  $p < 0.05$ . Also, the Mean LH level in Non-obstructive azoospermic subjects was significantly high ( $16.26 \pm 9.80$ ) as compare to in Obstructive Azoospermic subjects ( $8.04 \pm 2.28$ ) with  $p < 0.05$ . The Mean Free Testosterone level in Obstructive Azoospermic subjects was significantly high ( $36.54 \pm 48.95$ ) as compared to in Non-Obstructive Azoospermic subjects ( $13.35 \pm 8.91$ ) with  $p < 0.05$ . [Table.3]

### Discussion

Most of the time in developing countries like India females is blamed for infertility issue though the fact is that male partner is equally contributed regarding infertility.

In this study we focus on some etiological facts of azoospermia that plays important role in causing male infertility. We found that the level of hormones FSH and LH are significantly raised as compare to control group. Also, the testosterone level is significantly less in azoospermic subject as compare to control group.

When we separately compared the hormonal profile of Obstructive and non- Obstructive subjects we found the level of Gonadotropic hormone FSH and LH is significantly high in Non-obstructive Azoospermic patients while the testosterone hormone level is significantly low in Non-obstructive Azoospermic subjects. It indicates the primary hypogonadism.

### Conclusion

This study depicts the hormonal profile in infertile male that will be helpful in further assessment and management of such individuals.

**Conflict of Interest:** None Declared

**Source of Funding:** No funding Sources

**Ethical Clearance:** The study was approved by the Institutional Committee.

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# Knowledge, Attitude & Practise of Voluntary Blood Donation in Students of BRLSABVM Medical College, Rajnandgaon, Chhattisgarh

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## Abstract

There is a crucial need for awaring people regarding blood donation all over the world. According to Census 2011 Rajnandgaon city, Chhattisgarh has 1,63114 population and only around 1000 Voluntary Blood Donation done per year<sup>3</sup>. This depicts the lack of knowledge and social awareness regarding the blood donation among the population of Rajnandgaon (C.G).

**Material and Method:** The present study is a cross sectional study and conducted among the students of the various graduate and post graduate colleges of Rajnandgaon, to assess their knowledge and attitudinal variables towards voluntary, non-remunerated blood donation.

**Sample Size:** It is calculated by using Kish and Lisle formula for cross-sectional studies<sup>8</sup>. Four hundred students were selected to participate in this study

**Result:** After analyzing the data, we observe that the overall knowledge regarding blood donation in study population was found to be 46.11%. Most amazing thing is that 370 student out of 400 never did blood donation (92.5%). Overall 30.60% of students have negative attitude towards blood donation which includes generalized tiredness after blood donation (10.12%), hypovolemic anemia(15.12%) and decrease in Immunity is (5.36%). Around 69.40% of students have positive attitude and are willing to donate blood if they are demanded for blood donation (39.40%), 3% of non-donors not know much importance of blood donation and 27% don't know the procedure and place where to do blood donation.

**Conclusion:** Through this study we suggest that appropriate motivational campaign should be launched immediately among this young section of the population to convert this favourable "attitude" towards blood donation into a regular "practice" in order to increase the voluntary blood donation in Rajnandgaon.

**Keyword:** *Voluntary blood donation, knowledge, attitude, practice, hypovolemic anemia.*

## Introduction

"More blood, more life<sup>1</sup>," lifesaving theme of World Blood Donor Day 2011 to express the importance of the crucial need for awaring people regarding blood donation all over the world. According to WHO, Around 93 million blood donors are donating blood annually all over the world<sup>2</sup>. Blood donation is one of the important

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components in saving the lives of many people in routine and emergency situations like in major surgery, pregnancy and childbirth, trauma etc. It has been observed that low income countries have nine times less donation as compared to high income countries, while day by day in the developing countries like India; the demand for blood supply has progressively increased. Various evidences indicate that there is a major shortage of blood and blood products in these countries.

As per the world health organization the minimum 1% blood donation must be done to meet the demand in given population, but according to Census 2011 Rajnandgaon city, Chhattisgarh has 1,63114 population and only around 1000 Voluntary Blood Donation done per year<sup>3</sup>. This depicts the lack of knowledge and social awareness regarding the blood donation among the population of Rajnandgaon (C.G). Acceptable age for blood donation is between 18 and 65 years<sup>4</sup>. It has been worldwide reported that college students can be a very good source of quick and accessible quality blood, if they are motivated and recruited well as potential voluntary blood donors<sup>5</sup>. However, information from various blood banks in Rajnandgaon has shown that only one-thirds of college students reported to have ever donated blood with varying reasons but many do not donate any longer.

The common reasons faced by college going students regarding Blood donation may be tight schedule at college, lack of knowledge and awareness of blood donation, fear of needle or infection, and lack of opportunity. In many countries, to understand factors that influence blood donation blood donation surveys have been used<sup>6</sup>.

There is no current published information on factors influencing voluntary blood donation among young people and among college students in Rajnandgaon. To fill that information gap, the study aimed at determining awareness, level of knowledge, and attitudes towards voluntary blood donation among university students by using the case of Rajnandgaon region. Such information would be vital in planning for raising awareness and helping young people to donate blood in the country.

#### **Aims and Objectives:**

**Objectives of the study were as follows:** To assess

the level of knowledge regarding blood donation, among the undergraduate and post graduate college students in Rajnandgaon, CG.

To assess the attitudes, regarding the blood donation.

To assess the awareness about practices regarding blood donation.

### **Material and Method**

The present study is a cross sectional study and conducted among the students of the various graduate and post graduate colleges of Rajnandgaon, to assess their knowledge and attitudinal variables towards voluntary, non-remunerated blood donation. The Rajnandgaon city has average literacy rate 86.83% with high rate (94%) of college enrolment<sup>7</sup>.

**Including and excluding criteria:** We included all healthy adult male and female participating students willing for study. We excluded all visiting students and those who do not have consent to participate or having health related issues.

**Sample size:** It is calculated by using Kish and Lisle formula for cross-sectional studies<sup>8</sup>. Four hundred students were selected to participate in this study and interviewed face to face on various aspects of blood donation using a structured questionnaire.

**Data Collection Tools and Procedures:** Questionnaires used in this study were self administrative paper-pencil questionnaire type. The filled questionnaires were collected by hand by the investigators on the same day after distributing them to the students. The questionnaire were developed from review from other pilot studies<sup>9,10</sup>. Questionnaire contains questions related to socio-demographic characteristics of participants.

Few questions were based on the awareness and knowledge of blood donation. While few questions was related to attitudes towards blood donation which were of "yes/no" answer type, and some questions were on attitude towards blood donation. A part of questionnaire was devoted to collect the source of information regarding blood donation and lastly there are questions related to reasons for donating and not donating blood with open ended type questions.

**Observation and Results****Table No. 1: Knowledge towards blood donation**

Knowdge	Percentage (%)
Age criteria for blood Donation	67.82
Weight criteria for blood donation	70.34
Blood pressure range for blood donation	54.22
Maximum blood donation per year	36.25
Quantity of blood volume withdraw each time	20.63
Up to how many days blood can be store	16.40
What are the different blood groups	70.31
Lives saved through each unit of blood	26.21
Benefits of blood donation	52.81
Over all percentage of knowledge	46.11

**Table No. 2: Attitude towards blood donation**

Attitude	92.5% (Never did blood donation)
Blood donation causes generalized tiredness	Negative 10.12%
It causes hypovolemic anemia	Negative 15.12%
It hampers the Immunity	Negative 5.36%
Nobody asked or motivated for blood donation	Positive 39.40%
Non-donors don't know importance of blood donation	Positive 3%
Don't know what to do and where to do blood donation	Positive 27%

**Table No. 3: Practice on blood donation**

Reason for not a regular donor	7.5%
Lake of privacy while procedure	28.37%
Students felt uneasiness after donation	9.23%
Students donated blood only once	7.5%
Never asked or motivated again for blood donation	62.40%
Willing to voluntary donate blood if they are demanded for blood donation	69.40%

After analyzing the data, we observe that the overall knowledge regarding blood donation in study population was found to be 46.11%(Table 1). Most amazing thing is that 370 student out of 400 never did blood donation (92.5%). Overall 30.60% of students have negative attitude towards blood donation which includes generalized tiredness after blood donation (10.12%), hypovolemic anemia(15.12%) and decrease in Immunity is (5.36%) (Table 2). Around 69.40% of students have positive attitude and are willing to donate blood if they are demanded for blood donation (39.40%), 3% of non-donors not know much importance of blood donation

and 27% don't know the procedure and place where to do blood donation. We observed that only 30 students (7.5%) donated blood once(Table 2). We found that amongst the blood donors,28.37% of donors said there is lake of privacy while procedure, around 9.23% felt uneasy after donation and 62.40% said they are never asked or motivated for blood donation(Table 3).

**Discussion**

Day by day due to population growth hike and other factors like medical emergency, Road traffic accidents the demand of blood and its substitute is tremendously



increased. In spite of available resources and facilities the number of voluntary blood donation is quit less. As per the world health organization the minimum 1 % blood donation must be done to meet the demand in given population, but according to Census 2011 Rajnandgaon city, Chhattisgarh has 1,63114 population and only around 1000 Voluntary Blood Donation done per year. This depicts the lack of knowledge and social awareness regarding the blood donation among the population of Rajnandgaon (C.G).It has been worldwide reported that college students can be a very good source of quick and accessible quality blood, if they are motivated and recruited well as potential voluntary blood donors. So in this study we tried to fill that gap between demand and attitude towards voluntary blood donation.

In the above study we found that students have average knowledge (46.11%) regarding blood donation. If we educate them regarding health benefit of blood donation and eradicating the myth towards the various false fact like donation causes anemia, weakness we can change the negative attitude (30.6%) in them.

The common reasons faced by college going students regarding Blood donation are lack of knowledge and awareness of blood donation, fear of needle or infection, and lack of opportunity (39.40%). By motivating them this figure can be easily added in voluntary blood donation.

### Conclusion and Implication

Recruiting a sufficient number of safe blood donors in India is an emerging challenge especially with the increase in demands as a result of an increase in population size and an increase in the number of medical facilities. The present study was conducted in Rajnandgaon city in order to understand the various factors contributing to beliefs, attitudes, and level of knowledge associated with blood donation and transfusion that will help the blood centre in building and maintaining an adequate and safe blood supply. The current study was aimed to reflect a general lack of information regarding donation policies and practices among the surveying individuals.

It is also possible that an increased availability of correct information on donation requirement to more eligible potential donors may help persuade some of them to donate. Through this study we suggest that appropriate motivational campaign should be launched immediately among this young section of the population

to convert this favourable “attitude” towards blood donation into a regular “practice” in order to increase the voluntary blood donation in Rajnandgaon.

**Conflict of Interest:** None Declared

**Source of Funding:** No funding Sources

**Ethical Clearance:** This study was approved by the Institutional Committee.

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# Alterations in Semen Quality by Antioxidants in Stressed Healthy Individuals

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## Abstract

The study was conducted to evaluate the role of antioxidants in current therapeutic use on stress induced by exercise on sperm profile. 68 unmarried healthy medical students between 18-24 years of G.S.V.M Medical College who with informed written consented as volunteers for study duration of 90 days. Their identities were strictly confidential and were selected randomly. Complete and fresh specimen semen was collected by accepted method of masturbation after abstinence for more than 3 days. Two samples were taken first on commencement and second on conclusion of 3 month period. The subjects have been divided into 4 groups of 17 subjects in a single blinded study. Group I- control and were administered placebo capsules containing arrow root powder. Group II-administered placebo capsules and performed moderate exercise daily for 30 minutes for 3 months on a bicycle ergo meter with persistent increase in heart rate 30 to 50 per minute above their basal heart rate. The subjects carried out exercised for 30 minutes or exercise was stopped due to pain. Group III- carried out the exercise with the same protocol as in group II and were given 1 capsule daily of antioxidant preparation, ECarotin (Franco Indian Pharmaceuticals, India) composition Beta carotene 10 mg, vitamin E acetate I.P. 25 mg, Vitamin C I.P.150 mg, Selenium dioxide monohydrates U.S.P. 75 mcg, Zinc sulphate monohydrate U.S.P. 61.8 mg (equivalent to elemental zinc 22.5 mg). Group IV - Subjects of this group did not perform any exercise and were administered 1 capsule daily of ECarotin. The following physical features of the ejaculate were recorded Color, Liquefaction Time, pH, Volume Microscopic Examination included Sperm Count, Motility, Sperm morphology while Biochemical estimation done for seminal fluid Fructose. A statistically significant ( $p < .005$ ) decrease in sperm count in group II (exercising and taking placebo) and group III (exercising and taking [ECarotin]) but group IV taking only (ECarotin) showed a significant increase in sperm count ( $p < 0.05$ ). Group's III and IV consuming antioxidant (ECarotin) showed a significant increase in the number of motile sperms ( $p < 0.05$ ) and a significant decrease in the number of non-motile/dead sperms ( $p < 0.05$ ). Most of the subjects had pus cell in mild to moderate quantity.

**Keywords:** Antioxidants, stress, Sperm count, Sperm Motility, Sperm morphology.

## Introduction

Stress in modern times is a multidimensional omnipresent disease creating issue not addressed by most of us unless disease is established. Infertility cases are increasing in the society resulting in a plethora of

anti-fertility treatment options available. The seminal profile has been shown to be influenced by numerous physical as well as psychological factors. One of the important conditions is stress, which has marked influence over quality and quantity of sperm production. However, the role of antioxidants in current therapeutic use has been sparingly explored on semen quality of humans. In the literature, the seminal profile has not been explored in response to exercise producing transient stress. Moreover, the effect of antioxidants has not been explored on stress induced changes in sperm profile. The present study has therefore been aimed to explore the effects of antioxidants on quality and quantity of sperm

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production and also to find out their effects on stress induced changes in sperm profile of human volunteers.

## Materials and Method

Present study has been conducted on 68 unmarried healthy male medical student between 18-24 years. Their identities remain strictly confidential and had been selected randomly for the study. Consent was taken in format as prescribed and ethical clearance was given by ethical committee.

The following anthropometric parameters like height, weight, BMI, pulse rate, blood pressure, and respiratory rate were recorded. Complete and fresh semen specimen was collected from volunteers through accepted method of masturbation with abstinence period of 3 days.

The specimen was obtained in a sterilized, wide mouth, screw capped containers, to avoid contamination. The time and date of collection was registered on the containers and preserved at standard temperature, physical and microscopic examination on the same day

The following physical features of the ejaculate were recorded Color, Liquefaction Time, pH, Volume Microscopic Examination included Sperm Count, Motility, Sperm morphology while Biochemical analysis of seminal fluid was done for Fructose estimation. **The Volunteers have been divided into 4 groups; each group containing 17 subjects in a single blinded study.**

**Group I** – Volunteers in this group served as control and were administered placebo capsules containing arrow root powder for duration of 3 months. **Group II** – Volunteers in this group performed moderate exercise daily for 30 minutes for 3 months on a bicycle ergometer until they refused to continue exercising, any further on account of fatigue. Heart rate during exercise monitored persistently 30 to 50 per minute above their normal pulse rate. Generally the subjects carried out fast exercise for 7 to 10 minutes slowing down for rest, repetitively until had to stop due to fatigue/pain. Subjects of this group were also given arrow root powder placebo capsules.

**Group III** – Volunteers in this group carried out the exercise with the same protocol as in group 2 and were given 1 capsule daily of antioxidant preparation, ECarotin (Franco Indian Pharmaceuticals, India) containing Beta carotene 10 mg, vitamin E acetate I.P. 25 mg, Vitamin C I.P.150 mg, Selenium dioxide monohydrates U.S.P.

75 mcg, Zinc sulphate monohydrate U.S.P. 61.8 mg (equivalent to elemental zinc 22.5 mg). **Group IV** - Volunteers of this group did not perform any exercise and were administered 1 capsule daily of ECarotin for 3 months.

The average value and their standard deviations of each parameter were calculated. The significance of difference between the standard deviations of means in different sets of observations would be assessed at 95% level of confidence, by applying Student's 't' test ( $p < 0.05$ ) The different groups in any study for 3 months are as follows

### The above groups have been compared as follows in the presentwork:

Group I & Group IV – This comparison has been carried out to reveal whether ECarotin alone can influence the seminal profile.

Group I & Group II – This comparison has been necessary to find out if the exercise stress alone has a significance on semen profile.

Group II & Group III – This comparison has been done to explore whether the intake of ECarotin for 3 months can influence the exercise response (as revealed by comparison of Group I and Group II).

## Observations and Results

The average age of the group is  $19.56 \pm 0.62$  years. The color of the ejaculate was found to be light cream or yellowish in 10% of the participating subjects while in 90% of the subjects it was white or off-white. The odour was typically of fishy type in all the subjects. The viscosity was watery or watery-mucoid in most of the subjects while in some it was found to be thick mucoid.

### After 3 months of study:

**Volume:** semen in all the four groups showed insignificant change and was within normal range in the initial samples taken and post analysis after 3 months of study. Statistically the 'p' value was insignificant.

**pH:** The pH in various groups was in alkaline range and the 'p' value was insignificant.

**Liquefaction Time:** The Liquefaction Time in the group I (Control group taking placebo) and group IV (taking antioxidants (ECarotin) only) volunteers showed no significant change in the liquefaction time. But the

group's doing exercise irrespective of antioxidant (ECarotin) or placebo showed a statistical significant increase in liquefaction Time.

**Sperm Count:** The sperm count in the group I of control subjects taking placebo showed no significant change, group II (exercising group taking placebo) and group III (exercising group taking antioxidants [ECarotin]) showed a statistically significant decreased ( $p < 0.05$ ) while group IV taking only antioxidants (ECarotin) showed a significant increase in sperm count ( $p < 0.05$ ).

**Motility:** The motility of the sperm was studied after 2 hours of semen liquefaction, which was classified as motile, sluggishly motile and non-motile. The group's III and IV consuming antioxidant (ECarotin) showed a significant increase in the number of motile sperms ( $p < 0.05$ ) and a significant decrease in the number of non-motile/dead sperms ( $p < 0.05$ ). The group 1 (control students taking placebo) and the group II (exercising students taking placebo) volunteers showed a statistically non-significant change in motility.

**Morphology:** The morphology of the sperms were categorized into normal and abnormal forms. The abnormal forms seen were round head, pin head, bifurcated head, long tapering heads, bifurcated tail, kinked tail, no tail and lack of acrosomal cap. After 3 months of study there was no significant change in morphology in any group.

**Miscellaneous examinations of semen:** The pus cells were 3-8 cells per HPF in 98.4% of the volunteers in the initial sample taken but after 3 months of study 82.6% of the volunteers showed 3-5 pus cells/HPF. However only 2 volunteers had 20 –33 pus cells/HPF in initial sample which decreased to 8-12 pus/HPF in the sample taken after 3 months. The semen fructose level was found to be between 263 to 489 mg%, which is within normal range. The clumping of sperms was present in the sample of 8 subjects (++++). Only 18% of the subjects revealed epithelial cells as 1-2/H.P.F. in initial sample which increased to 3-4/H.P.F. after 3 months of study. No Trichomonas was seen in any sample.

Some interesting findings were observed during the questionnaire session of the students after 3 months of study.

**Vitality (Feeling better):** Maximum number of

volunteers taking antioxidants reported that they felt better than before and many wanted to continue the preparation being given to them.

**Appetite:** Maximum number of students taking placebo reported an increase in appetite while most of the students taking antioxidants reported no change in appetite behavior.

**Memory:** Some of the students taking antioxidants reported a better retention power.

**Sleep:** A large number of students taking placebo gave history of increased desire to sleep while most of the group of students taking antioxidants reported no alteration in sleep pattern.

**Desire for sex/Libido:** There was a significant increase in the number of volunteers who gave history of increased desire for sex/increased libido who were taking antioxidants. Less so in the exercising group but more in the group taking antioxidants only.

## Discussion

In the present study, we found that the volume of the ejaculate in most volunteers averaging between 2.3 ml-3.0 ml, which is in accordance with the reported values of Falk and Kaufman<sup>[1]</sup> (1950), Bhushan et al (1978)<sup>[2]</sup> range of volume was ranging from 1.0-7.2 ml.

The pH of seminal fluid was found to be alkaline (8.4-8.6) in nature which is similar to findings of Bhushan et al (1978)<sup>[2]</sup> in Indian subjects.

The average Liquefaction time in our study was found at an average to be between 14.4-18.7 minutes, which corroborates with the findings of Lunenfeld and Glezerman (1981)<sup>[3]</sup>.

The sperm counts, in our study, in the exercising individuals were noted to be decreased. These changes may be attributed to the negative effects of stress produced due to exercise because of the generation of reactive oxygen species. Our findings also corroborates with publication of JAMA (1988)<sup>[4]</sup> in which Vietnam Veterans had decreased sperm count and also decreased number of morphologically normal sperms due to war-psychology. Fenster L et al (1997)<sup>[5]</sup> and Clarke RN et al (1999)<sup>[6]</sup>, both of the studies showed an inverse relationship between semen quality and the stress produced due to emotionally charged situations. Comhaire FH et al (2000)<sup>[7]</sup> who observed that vitamin

A and vitamin E supplementation decreases the reactive oxygen species in oligozoospermic men. We have found a decrease in sperm count in the exercising individuals while an increase in sperm count in the individuals taking antioxidants. This finding is supported by observations made by Clarkson PM et al (2000)<sup>[8]</sup> who observed that exercise increases reactive oxygen species but trained athletes receiving antioxidant supplements showed evidence of decreased oxidative stress.

The motility of the sperms in our study was found to be significantly increased while the number of non-motile sperms decreased significantly after taking antioxidants. Our findings of increase in motility is supported by reports of Omu AE et al (1998)<sup>[9]</sup> in which they suggested that Zinc increases the motility and the sperm count in men with asthenozoospermia while Scott R et al (1998)<sup>[10]</sup> who emphasized that Selenium increases motility in sub-fertile men.

In our study, we found that the semen contained a higher percentage of normal and a lower percentage of morphologically abnormal sperms and there was no significant difference between the initial and the final samples. This is supported by the study of Bartoov B et al (1981, 1982)<sup>[11]</sup> in which observed that the morphologically normal sperms have a frequency of 58% or more as found in our study. The semen fructose levels in our study were found to be within normal range (243-489 mg% as recommended by Eliasson and Treichl (1971)<sup>[12]</sup> who reported semen fructose levels to be normal within 150-600 mg% range. The pus cells in the semen sample of north Indian males are indicative of sub-clinical infection for which they do not take any treatment.

### Conclusion

The volume of the ejaculate in the volunteers showed no significant change in the initial sample or after 3 months of study in all the groups.

The pH of the ejaculate in the volunteers showed no significant change in both the samples and in all the groups suggesting that antioxidants have no effect of on pH.

The Liquefaction time of semen in the volunteers who were exercising showed a significant increase in it thus demonstrating that exercise has induced a negative effect on liquefaction time. The volunteers who were taking only antioxidants showed no change in

liquefaction time.

Antioxidants may increase sperm count but the count of morphologically normal and duration to stay motile increases significantly.

The exercising volunteers who were taking placebo (group II) showed a significant decrease in sperm count due to oxidative stress induced by exercise. The same observation was made in the exercising volunteers taking antioxidants (group III) but the decrease in sperm count was comparatively lesser than group II of volunteers who were doing exercise and taking placebo. So, antioxidants negate the effect of exercise induced stress.

Maximum percentage of North Indian males were having normal sperm count. Exercise induced stress can lead to the normal sperm count of the individuals at the lower normal range (20–36 million/ml) to an oligospermic state. So, exercise led to a significant decrease in sperm count.

The motility of sperms increased significantly after taking antioxidants while the number of non-motile sperms decreased significantly after taking antioxidants.

Maximum number of North Indian males showed pus cells in the semen sample indicative of sub-clinical infection.

The semen fructose level in North Indian subjects was found to be within normal range.

Desire for sex/libido, feeling better and the memory was reportedly better in the volunteers taking antioxidants.

**Conflict of Interest:** Nil

**Source of Funding:** Self

**Ethical Clearance:** Study was approved by Ethical Committee.

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# Effect of Occupational Exposure to Pesticides on Cardiovascular System among Farmers: A Comparative Study

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## Abstract

**Background:** Agriculture a very complex occupation, apart from increasing productivity also brings huge loss to community in terms of its ill effects on human health, plant and animal life. Adverse effects on cardiovascular system is likely to occur due to aggressive use of agrochemicals such as pesticides.

**Objectives:** To Study Changes in ECG due to pesticide exposure among farmers and to Compare these ECG Changes in those farmers with that of normal non-exposed subjects.

**Materials and Method:** A 12 lead ECG was recorded in apparently healthy male farmers exposed to pesticides and normal non exposed age matched male control subjects and analysed for parameters such as heart rate, PR interval, QTC interval, QRS axis, ST and T wave changes and compared.

**Results:** This study reports significantly increased heart rate, prolonged QTC interval and non-specific ST and T wave changes among pesticide exposed farmers compared to non-exposed control subjects.

**Conclusion:** The current study concludes that there is a need to detect these ECG changes as early as possible so that future risk of cardiovascular disease can be prevented by adapting precautionary measures during application of pesticides. Some form of continuous cardiac monitoring seems advisable. Farmers can be advised to quit toxic chemical fertilizers & pesticides and opt Organic ones to be safe from the hazardous implications of exposure.

**Keywords:** ECG; Pesticides; Farmers; Cardiovascular disease.

## Introduction

Agriculture is the backbone of the Indian economy. Agriculture plays a key role in the overall economic output of a country. Since in this modern era of agriculture, because of insufficient rains and due to parched irrigation, there is a persistent crop loss due to invasion by pests, diseases and weeds before the harvest. These losses affect the livelihood of a farmer and their

families. To get best crop yield there was introduction of chemical fertilizers & pesticides. In making India a self-sufficient nation in food grain production day by day resulted in rampant usage of chemical fertilizers & pesticides bringing delineation of soil with toxic chemicals entering the food chain building-up toxicity with huge loss to community in terms of its ill effects on humans, plant and animal life due to aggressive use of agrochemicals<sup>[1][2]</sup>.

Farmers, mostly being illiterate or ignorant, may not consider the precautions while using pesticides, might inhale, as well ingest or inculcate the toxic chemicals from them by one way or the other due to unhygienic conditions.

Chemicals like endosulfan, tetradifon, pyridaben,

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lead and many other organo-phosphates are present in pesticides<sup>[3]</sup>.

Cardiovascular diseases are one of the leading causes of death worldwide<sup>[4]</sup>. Nearly 50% of world's labour force is into agricultural sector<sup>[5]</sup>. The presence of these toxicants in farmers has the potential to affect cardiovascular health as well as electrocardiogram<sup>[6]</sup>. The present study is designed to detect the ill effects of exposure to fertilizers and pesticides on cardiovascular function.

ECG Recording is a simple non-invasive technique. Hence, this study is taken up to detect the Electrocardiographic changes in Asymptomatic Toxicant-Exposed Farmers, to study prevalence of such abnormal ECG findings and thereby predict future cardiovascular complications as early as possible. Farmers can be advised to quit toxic chemical fertilizers & pesticides and opt Organic ones to be safe from the hazardous implications of exposure.

### Methodology

This study was undertaken in 60 apparently healthy male farmers, aged between 20 to 40 years with history of working for at least 6-8 hours a day for twice a week for more than 10 years of exposure to pesticides in the farm. Sixty-two apparently healthy male, control subjects were selected randomly from general population. We approached Rural Farmers' associations and APMC Farmers' association for selection of study subjects. Subjects will be matched for age, height and weight. Subjects with History of smoking, Alcohol intake, Diabetes mellitus, Hypertension, Anemia, Abnormalities of Vertebral column and thoracic cage, Pulmonary tuberculosis, Bronchial asthma, Chronic bronchitis, other respiratory diseases, Cardiac diseases, Abdominal or chest surgery were excluded from the study.

After taking written consent and asking detailed history about pesticide exposure with series of questionnaires like type of pesticides, mixing procedure, use of protective measures like gloves and masks, frequency of application etc Institutional Ethics Committee (IEC) Clearance was obtained before beginning the study.

A detailed assessment of individual subjects was done using pre-structured pro-forma. Data acquisition

was done in the morning. A detailed physical examination of subjects was done followed by systemic examination and ECG recording. Physical examination of all the subjects included temperature, blood pressure, respiratory rate and pulse rate.

Detailed history was taken which included a comprehensive history on usage of agrochemicals

1. Type & composition (mix or solitary; pyridaben\ chlorpyros\urea\organochloride\ammonia and many other compounds)
2. Duration of usage-daily\weekly\quantity\mixture
3. Precautions considered-masks\clothing\footwear\ eye wear\post-work sanitation
4. Type of dispersal-by-hand\spray\tablets

Resting pulse rate was recorded from the radial artery. Blood pressure was recorded using a mercury sphygmomanometer with the appropriately sized cuff from the right brachial artery in supine position.

ECG was recorded in those individuals who are selected for the study. The instrument used for recording ECG is Computerized NIVIQURE Digital 12 Lead ECG machine. Special emphasis was given on Heart Rate, PR Interval, QTc Interval, QRS axis, ST Segment, T Wave changes and all parameters will be analysed. ECG signals were obtained from all the leads. The following ECG parameters have been considered in this study.

Standard ECG reference values will be taken from Wagner G.S: Interpretation of normal electrocardiogram.

**Statistical Analysis:** Continuous variables will be expressed as mean  $\pm$  standard deviation (SD). Categorical data will be presented as frequencies and percentages. Differences between the groups will be compared using the two-tailed unpaired student's t-test. Comparisons of categorized variables between groups will be performed using the  $\chi^2$  test. All tests of statistical significance will be two-sided and  $P < 0.05$  will be considered to indicate a statistically significant difference. The statistical analyses will be conducted with SPSS 20.0

### Results

The results obtained were expressed as mean  $\pm$  standard deviation. The study showed the following results which are tabulated and analysed.



**Table 1: Age-wise distribution of subjects in each group**

Age (Yrs.)	Farmers	Controls	Total
25-29	20	26	46
30-34	16	26	42
35-39	23	31	54
40-44	32	15	47
45	9	2	11
Total	100	100	200
Chi Square Test: P<0.000			

**Table 2: Incidence Of occurrence of Heart Rate changes in farmers exposed and controls:**

HR (bpm)	Farmers	Controls	Total
Normal	88	98	186
Abnormal	12	2	14
Total	100	100	200
Chi Square Test: P<0.01, Significant			

**Table 3: Incidence of occurrence of changes in the ST segment in farmers exposed and controls:**

**A. With respect to the number of subjects showing the changes:**

ST Segment	Farmers	Controls	Total
E	3	0	3
IE	97	100	197
Total	100	100	200
Fisher's exact test: P<0.003, Not Significant			

\*where E-Elevation & IE-Iso-Electric

**B. With respect to the leads and amplitude:**

Farmers			
Lead	No. Cases	Max (Amp)	No. Cases
I	1	0.5mm	2
II	2	1mm	1
III	1		
V3	2		
V4	2		
V5	1		

**Table 4: Incidence of occurrence of changes in T Wave in farmers exposed and controls**

T Wave	Farmers	Controls	Fisher's exact test	Significance
I	0	0	-	-
I	0	0	-	-
III - I	2	0	P<0.311	NS
III-F	5	7		

T Wave	Farmers	Controls	Fisher's exact test	Significance
avR	0	0	-	-
avL	0	0	-	-
avF	0	0	-	-
V1	3	0	P,0.123	NS
V2	2	0	P<0.249	NS
V3	1	0	P<0.500	NS
V4	0	0	-	-
V5	0	0	-	-
V6	0	0	-	-
<b>Total</b>	<b>100</b>	<b>100</b>	-	-

**Table 5: Comparison of QTc interval (seconds) in farmers exposed and controls**

QTc	Farmers	Controls	Total
Normal	83	98	181
Abnormal	17	2	19
<b>Total</b>	<b>100</b>	<b>100</b>	<b>200</b>

Chi Square Test P<0.01, Significant

**Table 6: Comparison of mean values of clinical parameters in farmers exposed and controls: Heart rate (bpm), PR interval (sec), QTc interval (sec) and QRS axis (degrees):**

Groups		N	Mean	Std. Deviation	P Value	Significance
HR	Farmers	100	88.45	9.59	p<0.000	HS
	Controls	100	82.70	9.80		
PR	Farmers	100	0.14734	0.1123	P<0.870	NS
	Controls	100	0.14762	0.128		
QTc	Farmers	100	0.42241	0.142	P<0.000	HS
	Controls	100	0.38860	0.298		
QRS axis	Farmers	100	48.57	12.57	P<0.96	NS
	Controls	100	48.66	12.89		

## Discussion

Cardiovascular diseases (CVD) are associated with increased morbidity and mortality rates and reduced life expectancy<sup>[7]</sup>. Apart from the fundamental risk factors, environmental toxic substance including pesticides may affect cardiovascular system<sup>[8]</sup>. ECG is influenced by these changes due to the exposure to pesticides. A combination of these changes and continued exposure predisposes to various cardiovascular diseases which can turn out fatal. It is essential to understand the repercussions of these occupational exposures to

pesticides and its associated future risk of cardiovascular diseases.

The current study showed the following results which is analysed as follows:

### ECG Report:

**Heart Rate:** The incidence of occurrence of changes in Heart rate was significantly higher in the farmers exposed compared to the control group (P<0.000) (Table 2). There was a highly significant increase in the heart

rate in exposed farmers group as compared to control to the control group ( $P < 0.000$ )

Our finding is in consistent with other studies.

Saaddeh AM and his coworkers in their study reported increase in heart rate compared to controls<sup>[9]</sup>.

Karki P and his colleagues found significant increase in heart rate as an electrocardiographical manifestation in pesticide exposed subjects<sup>[10]</sup>.

This increase in HR could be related to the nicotinic effects of OP compounds and other agrochemicals and increased sympathetic tone<sup>[11]</sup>. These compounds act by irreversibly inhibiting the enzyme cholinesterase, resulting in accumulation of acetylcholine at synapses and myoneural junctions leading to cholinergic over-activity<sup>[9][10]</sup>.

**PR interval:** In this study There was no significant change in the mean PR interval (seconds) in exposed farmers group as compared to the control group ( $P < 0.870$ ) (Table 3).

**QTc interval:** The mean QTc interval (seconds) in exposed farmers group was significantly prolonged compared to the control group ( $P < 0.000$ ) (Table 6) The QTc interval tended towards the upper end of the normal range.

Wahab A and his coworkers in their study showed increased QTc interval as an effect of pesticides on cardiovascular system<sup>[12]</sup>.

Mladenka P et al reported prolonged QTc interval in pesticide exposed subjects. Since QTc interval is more important parameter than QT interval since it is corrected with heart rate, here in this study special interest is given to QTc interval<sup>[13]</sup>.

The QT Interval is an element of ECG that is a representation of ventricular function which includes depolarization and re-polarization. Measurement of QT and QTc can be used as a simple indicator for cardiovascular diseases<sup>[14]</sup>.

In this study there was prolongation of QTc interval in fertilizer and pesticide-exposed farmers group which is highly significant compared to controls. The QTc interval tended towards the upper end of the range and significant number showed abnormal QTc prolongation. The QTc interval represents duration of activation

of ventricular myocardium and its recovery. The QT interval corrected for heart rate (QTc) which is longer than 0.44 seconds is generally considered to be abnormal. The QT interval represents the duration of activation and recovery of the ventricular myocardium. Prolonged QTc interval here suggests prolonged recovery from electrical excitation which increases the likelihood of dispersing refractoriness, when some parts of myocardium might be refractory to subsequent depolarization<sup>[12]</sup>.

Prolonged QTc interval can be attributed to the due to direct cardiac toxicity (myocardial necrosis) along with the associated structural myocardial damage caused by pesticides<sup>[13]</sup>.

Studies done in animals reported that prolongation of the QT interval is a direct myocardial pesticide effect and is independent of cholinergic effects.<sup>[9]</sup> The cardiovascular effects of organophosphorus compounds are unpredictable and often change and adverse effects increase with increase over the time course of exposure. ECG changes like QTc prolongation is potentially dangerous and indicates the requirements of continuous cardiac monitoring<sup>[11]</sup>.

**ST segment:** In this study non-specific ST segment elevation less than 1 mm was found out in three subjects in farmers exposed to fertilizers & pesticides but it was found to be statistically not significant compared to the normal control groups.(Table 4)

These changes are significant seen in higher dose exposures and acute poisoning<sup>[13]</sup>.

**T wave changes:** In our studies, changes in T wave were found to statistically not significant. But certain non-specific changes were seen in the farmers group such as inversions and flattened waves in a few leads which were low amplitude waves. (Table 5)

These non-specific changes in ST segment and T wave changes can be explained by the fact that the inorganic compounds in pesticides cause direct myocardiotoxic damage and studies have the revealed that patchy necrosis, cardiac discoloration, patchy pericarditis, auricular thrombus, right ventricular hypertrophy, myocardial interstitial oedema, vascular congestion, patchy interstitial inflammation and mural thrombus occurs in acutely exposed cases<sup>[13]</sup>.

**QRS axis change:** In the current study there was no significant difference in the mean QRS axis in

exposed farmers group as compared to the control group ( $P < 0.960$ ) (Table 7)

### Conclusion

The present study concluded that Occupational exposure to pesticides is the major health concern of today's society, especially among farmers as they are mostly unaware of it and it can predispose them for the development of various morbid conditions over the years. The prevalence of unreported case of cardiovascular deaths is of serious concern. The number of farmers that use agrochemicals then retired due to ill health normally die without knowing the cause of their death. The banning of agrochemicals in the agricultural sectors will produce adverse economic effects and people will suffer across the world. As in low dose chronic exposure they may not manifest clinically, and pesticides exposed subjects for long time remain asymptomatic. However, it brings Electrocardiographic changes that are noticed early over a period of exposure and the general impression is that it is advisable to detect these changes as early as possible so that future risk of cardiovascular disease can be reduced by adapting precautionary measures during application of pesticides. Therefore, some form of continuous cardiac monitoring seems advisable. Considering the gravity of this situation, more work needs to be done in this aspect. Farmers can be advised to quit toxic chemical fertilizers & pesticides and opt Organic ones to be safe from the hazardous implications of exposure.

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# Comparative Study of Sympathetic Activity in Normotensive Obese and Nonobese Adults

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## Abstract

**Aim:** The aim of the study is to assess sympathetic activity in Obese Normotensive subjects

**Objectives:** To investigate any changes in sympathetic activity in Normotensive obese subjects using Cold Pressor test

**Method:** 50 Obese subjects of both genders between age group 18-25 years with BMI>25kg/m<sup>2</sup> were selected as study group and 50 age matched subjects of both the genders with BMI<25kg/m<sup>2</sup> were selected as control group. Sympathetic activity was assessed using Cold pressor test. In this test, After recording the resting blood pressure (BP), the subject was asked to immerse his hand in cold water (temperature maintained between 5°–9°C). BP measurement from other arm was done at 30 sec interval for two minutes using continuous ambulatory Blood pressure monitoring, after which the subject was asked to remove the hand from cold water. Maximum increase in Diastolic Blood Pressure (DBP) was taken as test response.

**Results:** Statistical analysis was done using unpaired t test. Increase in diastolic blood pressure with Cold pressor test in study group was significantly increased compared to control group.

**Conclusion:** Increased sympathetic activity is seen in obese normotensive subjects, which is a risk factor for future development of hypertension and other complications associated with increased sympathetic activity. Weight loss and maintenance of healthy lifestyle is suggested to the subjects to prevent the future complications.

**Keywords:** Normotensive Obese, Cold pressor test, sympathetic activity, Diastolic blood pressure.

## Introduction

Obesity, considered a worldwide epidemic, is characterized by the excessive accumulation of fat tissue in the body and its causes are multifactorial, such as genetic susceptibility, sex, age, occupation, diet and others<sup>1</sup>. It is well recognized that obesity is associated with reduced quality of life and increased risk

of premature death and predisposes individuals to the development of a number of chronic illnesses including cardiovascular disease, type 2 diabetes, dyslipidemia, insulin resistance, hyperglycemia, hypertension, degenerative joint diseases, obstructive sleep apnea (OSA), gastroesophageal reflux disease, nonalcoholic fatty liver, and various forms of cancer<sup>2</sup>.

Obesity and its early complications (i.e. insulin resistance and impaired fasting glucose) are associated with overstimulation of the sympathetic nervous system (SNS) and decreased tone of the parasympathetic nervous system (PNS)<sup>3</sup>

Studies using norepinephrine urinary excretion and plasma concentration measurements from hypothalamic

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models of obesity show that sympathetic nervous activity in obesity is low<sup>4</sup>. Young and Macdonald<sup>5</sup> found that there were numerous studies proposing that Sympathetic nervous system (SNS) in subjects with obesity was either low, normal, or elevated. The heterogeneity of the results most likely occurred because of inadequacy of the method used (in particular, venous or urinary norepinephrine concentrations) and because SNS activity is typically regionalized, where the efferent outflow throughout the body is not uniform.

Studies have also shown that SNS activation may be responsible for development of obesity. Increased plasma norepinephrine concentration and a hyperkinetic circulation in young adulthood have been shown to predict future weight gain and the development of insulin resistance<sup>6</sup>.

The present study is carried out to evaluate the sympathetic activity in Obese subjects.

### Materials and Method

The study was carried out at Great Eastern Medical school, Srikakulam, Andhra Pradesh

**Study Group:** 50 Obese subjects of both genders between age group 18-25 years with BMI>25kg/m<sup>2</sup> were selected as study group

**Control Group:** 50 Nonobese subjects of both genders between age group 18-25 years with BMI<25kg/m<sup>2</sup> were selected as control group

#### Inclusion criteria for study group:

1. BMI>25kg/m<sup>2</sup>
2. Normotensive subjects

#### Inclusion criteria for control group:

1. BMI<25Kg/m<sup>2</sup>
2. Normotensive subjects

#### Exclusion criteria (common for study and control group):

1. Hypertensive subjects
2. Diabetic subjects
3. Any history of chronic illness

All the subjects were explained about the test to assess sympathetic function and an informed consent was taken.

Sympathetic activity was assessed by cold pressor test.

**Cold pressor test (CPT):** After recording the resting blood pressure (BP), the subject was asked to immerse his hand in cold water (temperature maintained between 5°–9°C). BP measurement from other arm was done at 30 sec interval for two minutes using continuous ambulatory Blood pressure monitoring, after which the subject was asked to remove the hand from cold water. Maximum increase in Diastolic Blood Pressure (DBP) was taken as test response.

Somatosensory stimulation induced by the cold stimulus increases blood pressure; impulses from receptors in the skin relay via afferent pathways to C1 cells in the rostral ventrolateral (RVL) reticular nucleus and are transmitted via efferent sympathetic neurons to peripheral blood vessels from thoracic spinal cord. Thus, somatosensory stimulation produces the pressor response during the cold pressor test.

#### Statistical analysis:

Statistical analysis was done using unpaired t test

P value < 0.05 was considered as statistically significant

The results were expressed as Mean ± standard deviation

#### Finding:

Variable	Study Group		Control Group		P Value
	Mean	SD	Mean	SD	
Increase in DBP (mmHg) with Cold Pressor Test	15.7	3.1	13.2	2.3	<0.0001*

DBP-Diastolic blood pressure

On Analysis,

Increase in diastolic blood pressure with cold pressor test in study group was significantly increased compared to control group.

### Discussion

In our study, we found that increase in Diastolic blood pressure with cold pressure in obese normotensive subjects was significantly increased compared to Nonobese normotensive subjects.

The findings of our study were in accordance with previous studies<sup>7</sup> which showed increased sympathetic activity in obese normotensive subjects.

However some studies reported no change and even reduction in sympathetic activity in obese subjects<sup>8</sup>.

Obesity is characterized by excessive accumulation of fat, a highly dynamic endocrine and paracrine organ that releases many cytokines and bioactive mediators which may influence sympathetic nervous system activity<sup>9</sup>. While there exists a large body of evidence indicating that sympathetic nervous activity is evident in obesity, it is important to recognize that many factors may be involved in the genesis of obesity-related sympathetic activation<sup>10</sup>.

It is well established that obesity is one of the major determinants in the development of hypertension in the general population<sup>11</sup>. The mechanisms contributing to the development of higher blood pressure in humans with obesity include many factors such as hyperinsulinemia, activation of the renin-angiotensin-aldosterone system, abnormal levels of certain adipokines such as leptin, and an altered spectrum of cytokines acting at the vascular endothelial level<sup>12</sup>.

Sympathetic nervous stimulation is certainly a key factor in the development of hypertension. It was demonstrated that when weight gain develops in young men, increased Muscle Sympathetic Nerve Activity (MSNA) occurs early, together with increased blood pressure<sup>13</sup>. It was shown that sympathetic activation to the kidneys occurs as early as 1 wk after exposure to a high-fat diet in rabbits<sup>14</sup>.

### Conclusion

Increased sympathetic activity is seen in obese

normotensive subjects, which is a risk factor for future development of hypertension and other complications associated with increased sympathetic activity. Weight loss and maintenance of healthy lifestyle is suggested to the subjects to prevent the future complications.

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**Ethical Clearance:** Taken from Scientific Ethical committee, Great Eastern Medical school, Srikakulam, Andhra Pradesh.

**Conflict of Interest:** Nil

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# Evaluation of Efficacy of Long-term Yogic Training on Vascular Endothelial Function and Cardiovascular Responses in Healthy Individuals

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## Abstract

There are numerous studies confirming the positive effects of physical exercise in improving the functional status of vascular endothelium and cardiovascular parameters. However, there is very less scientific data available showing the efficacy of yoga training on vascular endothelium functions and various cardiovascular parameters with large sample size. Hence the study was intended to measure the change in plasma nitric oxide level (NO<sub>x</sub>), resting heart rate (HR) and resting systolic (SBP) and diastolic blood pressure (DBP). Yoga intervention was given on age matched 200 healthy individuals of both the gender. NO<sub>x</sub>, Resting HR, SBP and DBP were measured at Baseline and after six months. Data collected was analyzed statistically showed significant elevation in NO<sub>x</sub>, reduction in HR, SBP and DBP. Positive findings are the suggestive of improved vascular endothelial health and cardiovascular responses.

**Keywords:** *Yoga, Vascular Endothelium, Nitric Oxide, Heart Rate, Blood Pressure.*

## Introduction

The endothelium is a single cell layer lining the luminal surface of entire vascular tree. It serve in wide vascular homeostatic processes.<sup>1,2</sup> Modulation of blood flow, vascular tone<sup>3,4</sup> and vascular wall protection<sup>5</sup> are some of the noted functions of vascular endothelium. Normally, it releases diverse vasoactive agents causing vasoconstrictions and vasodilation. Nitric Oxide (NO) is one of the potent endogenous vasodilatory agents released in response to physical stimuli i.e. sheer stress, hormones and platelet-derived substances. Through these agents, healthy endothelium is proactively involved in vascular tone regulation and protection.<sup>6-8</sup> The damaged endothelium is critical in a variety of

human disorders including peripheral vascular disease, stroke, heart diseases, venous thrombosis, disturbance in the physiological haemostatic processes, renal failure, diabetes, etc.<sup>1</sup>

Endothelial dysfunction primarily characterized by reduced NO production, bioavailability and vasomotor response which plays crucial role in pathogenesis of atherosclerosis.<sup>9,10</sup> Lack of regular exercise, high blood pressure, diabetes mellitus, and aging are some of the risk factors linked with vascular endothelial dysfunction.<sup>11</sup> Insufficient NO release may cause vasoconstriction in coronary arteries during exercise and mental stress which may result in myocardial infarction, ischemia and promote vascular inflammation.<sup>2</sup>

There is reduction in cardiovascular risks factors especially hypertension with improved vascular endothelial functions.<sup>12,13</sup> Scientific literature indicate the beneficial effects of regular aerobic exercise in improving vascular endothelial health<sup>14</sup>, reducing arterial stiffness<sup>15</sup> and blood pressure in healthy individuals.<sup>16</sup> Vascular endothelium-dependent vasodilatory response

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is principally depends on the NO release. Therefore, measurement of plasma NO and basal blood pressure could be reliable approach to recognize its functional status.<sup>17</sup>

Various studies reports yoga as a mind-body exercise modality which enhances cardiopulmonary fitness and mental wellbeing, if practiced regularly.<sup>18,19</sup>

Physiological role of NO is all apparent, however effect of yoga to assess the vascular endothelial functional status with large sample size in healthy individuals has been least attempted. Therefore, the present study was primarily intended to evaluate the effect of long term yoga training on Total Plasma Nitric Oxide (NOx). Secondly, to assess the cardiovascular responses at rest to long-term yoga practice.

## Material and Method

**Design and Sample size:** In this pre- and post yoga interventional study, healthy individuals (n=200; 120 Male and 80 Female) within 30-50 years of age range (mean age 39±0.95 years) were recruited.

**Inclusion and Exclusion Criteria:** Healthy individuals willing to practice yoga daily for six months were included. While, individuals with any systemic, mental disorders, pregnant women and receiving any physical and/or yoga training were excluded from the study.

**Ethics:** Written consent was obtained from all study participants. The study was approved by institutional ethical committee (Registration No. ECR/581/INST/MH/2014).

**Intervention:** Yoga (Asanas, Pranayama and Meditation) intervention was given for six months (1 hour per day, 6 days per week) by trained yoga instructor.

**Variables Studied:** Total plasma nitric oxide (NOx); resting heart rate (HR), resting SBP and DBP were measured at baseline i.e pre-yogic and post-intervention i.e post-yogic, respectively.

**Statistical Analysis:** For statistical data analysis, SPSS (24<sup>th</sup> Version) was used. Quantitative data was presented as Mean±Standard Deviation (SD). Mean±SD was calculated as Mean Difference. Paired t-test was used for data comparison. P-value at 5% was established for significance.

## Results

Pre-yogic and post-yogic Total Plasma Nitric Oxide (NOx); Resting HR, SBP and DBP were measured and are shown in Table I. There highly significant (p<0.0001) reduction in post-yogic resting HR, SBP and DBP compared to their respective baseline values was observed.

**Table I: Comparison between Mean Pre- and Post-yogic NOx, Resting HR, SBP and DBP**

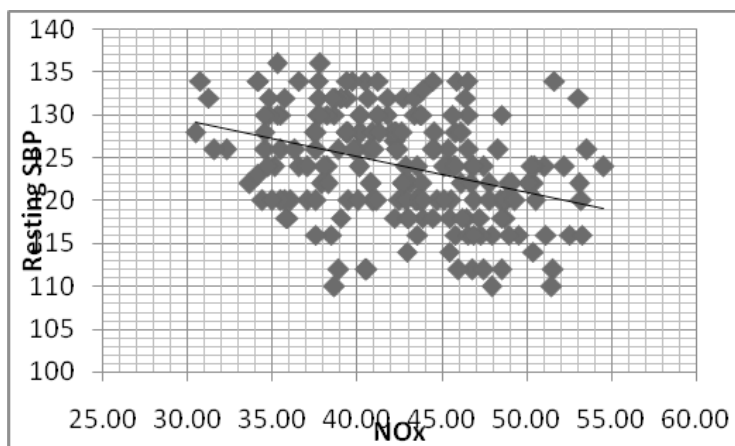
Variables	Measure-ments	Mean±SD (n=200)	Mean Difference	p-value
NOx (µMole/L)	Pre-yogic	36.25±4.76	6.27	p<0.0001**
	Post-yogic	42.52±5.32		
Resting HR (bpm)	Pre-yogic	79.97±8.13	5.15	p<0.0001**
	Post-yogic	74.82±4.99		
Resting SBP (mmHg)	Pre-yogic	128.41±7.64	4.36	p<0.0001**
	Post-yogic	124.04±6.21		
Resting DBP (mmHg)	Pre-yogic	79.63±4.70	1.08	p<0.0001**
	Post-yogic	78.55±3.61		

NOx: Plasma Nitric Oxide; SD=Standard Deviation; HR=Heart Rate; SBP=Systolic Blood Pressure; DBP= Diastolic Blood Pressure, p<0.0001\*\*=Highly Significant.

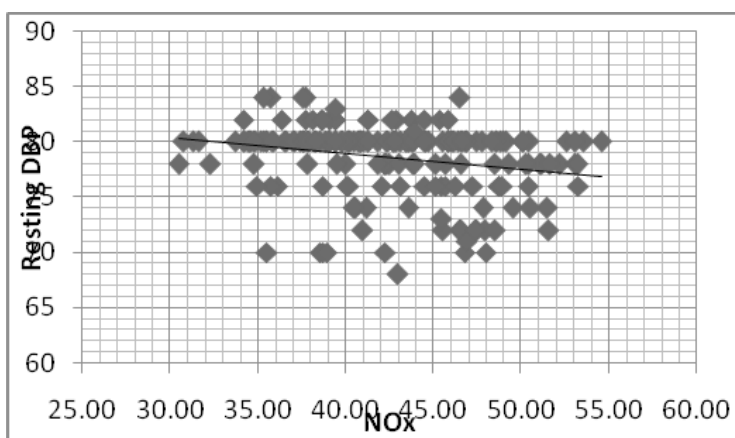
Post-yogic Resting SBP and DBP were correlated with post-yogic NOx level and are shown in Fig. 1 and Fig. 2 respectively. There significant negative

correlation between post-yogic resting SBP and DBP (p<0.0001) with post-yogic NOx was observed and is shown in Table II.

**Fig. 1: Correlation between post-yogic Resting SBP and post-yogic NOx level**



**Fig. 2 :Correlation between post-yogic Resting DBP and post-yogic NOx level**



**Table II: Correlation between post-yogic NOx level with post-yogic Resting SBP and DBP**

Variables	r-value	p-value
Resting SBP (mmHg) vs NOx (µMole/L)	-0.359	p<0.0001**
Resting DBP (mmHg) vs NOx (µMole/L)	-0.245	p<0.0001**

NOx = Total Plasma Nitric Oxide level; SBP = Systolic Blood Pressure; DBP = Diastolic Blood Pressure; S = Significant; p<0.0001\*\* = Highly Significant.

### Discussion

The present study was intended to find efficacy of long term yoga training on vascular endothelial functional status by measuring total plasma nitric oxide level and cardiovascular responses by measuring heart rate and blood pressures at rest.

We found, highly significant (p<0.0001) increase in post-yogic NOx level and reduction in resting HR, SBP & DBP compared to their respective baseline values (Table I). Further, correlation showed significant

(p<0.0001) reduction in resting SBP (Fig. 1) & DBP (Fig. 2) with post-yogic NOx (Table II).

Significant increased level of total plasma NO level after long term yoga practice was one of the novel finding of our study. The outcome of this study is in accordance with the finding of Preethi Bangalore Lakshmgowda, et.al.<sup>20</sup> Patil SG et.al<sup>21</sup> had reported the significant increase in NO level in yoga practicing elderly subjects which resulted in reduction in arterial stiffness. Another study has reported the NO elevating effect of Bhramari Pranayama.<sup>22</sup> A range of studies showed exercise

stimulates NO release from the endothelial cells.<sup>23-25</sup> Increased blood flow through vessels is identified as the root cause of endothelial release of NO.<sup>26,27</sup> The exact mechanism behind the elevated post-yogic NOx remained unclear. However, we presume that it is most likely the stimulation to increased blood flow due to yoga practice have caused the sheer force over the endothelium. As a consequence, it releases more NO than normal.

Our finding regarding significant reduction of Resting HR was consistent with the findings of various researchers.<sup>28-32</sup> According to theme, that was probably due to stronger vagal activation and balance in autonomic activities caused by yoga practice. On that context, we attribute the reduction in post-yogic Resting HR to increased vagal tone, deep psychosomatic relaxation and reduction in exercise induced stress on cardiovascular system over the period of yoga training.

The Our findings i.e. significant reduction in post-yogic Resting SBP & DBP compared to their respective baseline values were similar with the outcomes of various studies.<sup>28,29,33-35</sup> It is suggested that the arterial stiffness causes not only elevation of resting BP but also influence BP responses to exercise.<sup>36</sup> Arterial stiffness not only decided by the structural components but also by vascular tone and endothelial functional status. It is implied that NO and vascular tone are the key components in the regulation of arterial stiffness therefore they are vital determinants for exercise induced BP response. A study reveals that, pharmacological inhibition of NO synthase increases BP during sub-maximal level of exercise.<sup>37</sup> Hence, it may be assumed that, reduction in Resting SBP & Resting DBP might be due to increased parasympathetic activity, reduced vascular tone; increased cardiopulmonary endurance, blood flow to the muscles at rest; elevated post-yogic NOx might have increased vasodilatory effect, decreased arterial stiffness which collectively have caused significant reduction Resting SBP & DBP after long term yoga training.

### Conclusion

Increased NOx level due to long term yoga may reflect the improved functional status of the vascular endothelial layer. Reduced resting HR, SBP & DBP also indicate the cardiovascular response modulating efficacy of regular yoga training. This also suggestive of improved cardiac endurance and fitness. Elevated NO level is the indicator of improved endothelial functional

status. Therefore, it may be concluded that, yoga as a complementary exercise modality to reduce the risk of CVDs in healthy adult individuals.

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# Effect of Family History of Hypertension on Left Ventricular Mass in Normotensive Individuals

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## Abstract

**Introduction:** Presence of family history of hypertension is a strong predictor of development of hypertension in normotensive subjects. Increased left ventricular mass might antedate the development of hypertension in the individuals destined to develop systemic hypertension in life.

**Aim:** To study the left ventricular mass in offspring of normotensive parents (ONP) and offspring of hypertensive parents (OHP) and to find the variations in between the two groups.

**Materials and Method:** 75 healthy normotensive individuals with family history of hypertension and 75 healthy normotensive individuals without family history of hypertension were included in the study. LV mass was calculated by using 2D M-mode echocardiography by the formula given by American society of Echocardiography,  $LV\ mass = 0.8 \times [1.04 \times (LVIDd + IVSd + PWd)^3 - (LVIDd)^3] + 0.6\ grams$ . [LVIDd – Left ventricular internal diameter during diastole; IVSd- Interventricular septal thickness during diastole; PWd – Posterior wall thickness during diastole].

**Observation and Results:** Left ventricular mass was significantly higher in the normotensive individuals with family history of hypertension ( $64.33 \pm 20.22g$ ) when compared to those without family history ( $56.12 \pm 16.13g$ ).

**Conclusion:** The present study reveals increased left ventricular mass in normotensive individuals with family history of hypertension. Early evaluation of left ventricular mass and life style modification are recommended in healthy normotensive individuals with family history of hypertension to prevent and delay the clinical symptomatology of hypertension.

**Keywords:** Left ventricular mass, echocardiography, hypertension.

## Introduction

Hypertensive heart disease is a complex entity involving cardiovascular changes due to arterial hypertension. Heart as a pump of circulatory system is effected by the increased blood pressure from the early stages of hypertension and actually suffers the common

hypertension related organ damage<sup>8</sup>. Current approach to the management of systemic hypertension is early diagnosis and identification of individuals at risk through detection of early markers of the disease<sup>9</sup>. Left ventricular hypertrophy is one of the pathological hallmarks of systemic hypertension. Increased left ventricular mass might antedate the development of hypertension in the individuals destined to develop systemic hypertension in life.<sup>1</sup> Since essential hypertension is thought to have an important genetic component in its causation, offspring of hypertensive parents represent an excellent opportunity to study the early subclinical phases of syndrome of systemic hypertension. The main purpose of the present

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study was to evaluate left ventricular structure in normotensive Offspring of hypertensive parents (OHP) with the aim of detecting early abnormalities preceding the onset of systemic hypertension .

### Materials and Method

A cross sectional hospital based study including 75 normotensive individuals with family history of hypertension and 75 normotensive individuals without family history of hypertension between the age group of 30 – 40 years was conducted in Osmania general hospital. Subjects with blood pressure > 130/90 mmHg, with family history of – Diabetes mellitus, Thyroid disorders and subjects using cardioactive drugs were excluded from the study.

#### Materials:

1. Automatic blood pressure monitor
2. Height measuring stand
3. Digital weighing machine
4. 2D M-mode Echocardiographic machine

**Procedure:** Institutional ethics committee approval and informed consent was taken.

Height, weight and average of two blood pressure recordings with a time gap of 10 min was taken . BMI was calculated using Quetelet formula  $BMI = \text{weight in kg/height in m}^2$ . Echocardiography was done in the partial left lateral decubitus position after a resting period of 3 – 4 minutes. A 2D guided M- mode recording of the left ventricle was obtained in the parasternal long axis view and measurements of wall thickness and chamber diameter were determined in diastole in accordance with method outlined by American society of echocardiography. The mean value of the parameters were taken from three consecutive beats. LVM was calculated using the formula given by American society of echocardiography

$$LVM = 0.8 \times [ 1.04 \times (LVIDd + IVSd + PWD)^3 - (LVIDd)^3 ] + 0.6 \text{ grams}$$

### Observations and Results

Statistical analysis was done using unpaired students t test and P value < 0.05 was considered as statistically significant.

**Table 1: Age and gender distribution in OHP and ONP**

	OHP	ONP	P Value
Age	37.32 ± 3.81	37.63 ± 2.98	0.584NS
Males	37 (49.5%)	37(49.5%)	0.47NS
Females	38 (50.5%)	38(50.5%)	0.47NS

**Table 2: Comparison of anthropometric measurements in OHP and ONP**

	OHP	ONP	P Value
Height (Inch)	5.1 ± 1.0	4.9 ± 1.3	0.22NS
Weight (kg)	64 ± 12	68 ± 9	0.61NS
BMI	34.88 ± 8.68	32.67 ± 5.66	0.03 S

**Table 3: Comparison of systolic and diastolic blood pressure in OHP and ONP**

	OHP	ONP	P Value
SYSTOLIC BP	116.0 ± 8.85	118.27 ± 8.75	0.117NS
DIASTOLIC BP	75.20 ± 7.94	77.47 ± 8.39	0.092NS

**Table 4: Comparison of left ventricular geometric parameters in OHP and ONP**

	<b>OHP</b>	<b>ONP</b>	<b>P Value</b>
IVS	1.13 ± 0.12	1.01 ± 0.02	0.12NS
PWT	0.95 ± 0.12	0.89 ± 0.52	0.47NS
LVIDd	4.29 ± 0.32	4.12 ± 0.57	0.53NS
LVIDs	2.93 ± 0.43	2.57 ± 0.34	0.32NS
LVM	126.15 ± 28.04	104.56 ± 28.01	0.01S

## Discussion

The results of this study showed that left ventricular mass was significantly higher in OHP. The presence study suggests that alterations in left ventricular structure may also have genetic basis as there is abnormal left ventricular geometry in normotensive offspring of hypertensive parents. This is supported by a recent study by Lam et al which showed familial aggregation of left ventricular geometry in a two generation community-based sample.<sup>2</sup>

At equal blood pressure levels, some individuals develop LV hypertrophy, whereas others do not, indicating a genetic susceptibility of the development of LV hypertrophy. Because the major causes of morbidity and mortality among hypertensive patients are due to the cardiovascular manifestations of hypertension and not the level of blood pressure per se, understanding the genetic susceptibility of the LV hypertrophy to the effects of hypertension remains a matter of intense interest<sup>5</sup>.

Left ventricular mass (LVM) is a significant risk factor for cardiovascular disease and hence it is an important clinical measure in both healthy individuals and patients with disease, LVM is determined by a combination of genetic factors, environmental and mechanical factors. During embryogenesis, heart development is under the control of a series of transcription and growth factors acting on cell differentiation and on heart morphogenesis<sup>6</sup>.

Left ventricular structural adaptation is highly dependent on oxidation of glucose and fatty acids by mitochondria. A plausible working hypothesis is therefore that the close concordance between mothers and offspring might be explained by mitochondrial DNA that sons and daughters inherit from them. The mendelian hypothesis that offspring derived their genetic makeup in equal proportions from their mothers and

fathers is known to be incorrect. Genomic imprinting is the differential modification of the maternal and paternal contributions to the zygote. Thus, during development and growth into adulthood offspring are under influences of distinct maternal and paternal “imprints” that result in divergent expression of parental alleles. The intrauterine environment plays a pivotal role in the development of the fetus and is predominantly determined by genetic and environmental factors linked to the mother<sup>7</sup>.

In the Dutch Hypertension and Offspring Study, the thickness of LV posterior wall and inter-ventricular septum were increased in the offspring of hypertensive parents but the difference from the offspring of normotensive parents was not significant. However, LV mass and LV end-diastolic diameter were significantly increased in the former group. This is in agreement with our study which also showed significant increase in LVM in OHP when compared to ONP.

Family history of cardiovascular diseases are predictors of cardiovascular risk in adulthood and the relationship between them starts a symptomatically from the first years of life. High blood pressure (HBP) is considered one of the major risk factors for cardiovascular disease among them. Normotensive children of hypertensive parents are exposed to greater risk of developing hypertension in adulthood as they have shown early cardiovascular changes.

There are many reports on left ventricular mass heritability with estimates from different populations. Most studies showed significant heritability and sibling correlations, especially among African Americans. The heritability decreased after matching the blood pressure. It implied possible pleiotropic effects of genes on controlling blood pressure and LV mass. Among young adults and adolescents, Hemodynamic load, such as stroke volume, has an influence on LV mass, and this impact is more important than body size. However,

high proportions of LV mass variations still remain unexplained. Genetic components played important role in residual LV mass variations<sup>4</sup>.

In this study the relation between LVM measured by echocardiography with BMI and blood pressure was studied which showed that there was significant increase in LVM in subjects with increased BMI. This is in agreement with the study done by Chad Garner et al. which showed that the strongest predictor of LVM was BMI and they both are strongly correlated.

Such a correlation implies that at least some of the familial factors that affect weight also affect LVM. Thus, if the familial factors are genetic, this would suggest that at least some of the genes acting on the traits are pleiotropic, that is influence both traits. Thus, LVM and weight are determined in large part by common genetic and/or familial environmental factors in both adults and children, whereas the impact of correlated nonfamilial environmental factors appears to be much larger in children compared with adults<sup>6</sup>.

Left ventricular (LV) hypertrophy profoundly affects morbidity and mortality from cardiovascular diseases, including myocardial infarction, congestive heart failure, and stroke. Therefore, it is important to measure LV mass and manage LV hypertrophy among the hypertensive population in clinical practice. The understanding of natural history of LV hypertrophy has been enhanced by noninvasive imaging method of echocardiography that have greatly increased the capability of determining the increased LV mass. Blood pressure and volume overload are considered as a strong determinant of LV hypertrophy. However, inter-individual variations in LV mass can be explained, only to a limited extent, by hemodynamic load. Moreover, LV hypertrophy may occur in the absence of hypertension. Genetic components are considered to be important factors for LV mass, and it has been proved by twin studies which showed that heritability accounts for up to 20–70%. Significant heritability and evidence of strong familial aggregation in LV mass have been reported in different populations, such as in Caucasian, African Americans, American Indians and Caribbean Hispanic families. Previous segregation study showed the mode of inheritance of LV mass was compatible to polygenic model, but the study did not prove major gene effects controlling LV mass. The existence of major gene effects can be investigated by segregation analysis besides the polygenic background effects, and help to facilitate the

further genomic study.

Our results are contrast to that of Jalal et al who found no significant difference in LVM between the two groups. This may be because our patients were older than those evaluated by Jalal et al. and hence age does appear to be an important factor in the expression of LVM phenotypes in OHP. It is possible and seems likely that their subjects might have been examined at an earlier stage in the natural history before the full expression of the morphological cardiovascular changes of the preclinical hypertension syndrome

Graettinger, et al reported the absence of any significant difference in LV mass index or wall thickness between normotensive healthy volunteers with or without genetic risk for hypertension<sup>5</sup>. However, their contention that such a lack of difference could be due to a meaningful difference in diastolic blood pressure between the two groups cannot be offered as a similar explanation for our observations since our subject groups were properly matched for the blood pressure level

## Conclusions

### The present study reveals:

**IN OHP** - 1. ↑ BMI is associated with ↑ LVM

2. Even with normal BMI there is ↑ LVM

**IN ONP** - 1. ↑ BMI is associated with ↑ LVM

2. With normal BMI, LVM is in normal range

This clearly points that not only genetic basis but also BMI is an individual risk factor for developing cardiovascular morbidity.

Life style modifications would protect and prevent these complications.

**Conflict of Interest:** None

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**Ethical Clearance:** Obtained

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