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RESEARCH ARTICLE

Serum levels of Nitric Oxide metabolites in relation to Body Mass Index and Arterial stiffness in young individuals

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ABSTRACT:

Introduction: Obesity is a multi-factorial disorder defined as abnormally high levels of body fat accumulation. It is a major emerging pandemic of this century which can be attributed to alterations in eating habits, lifestyle choices and rise in sedentary behaviour. The activation of pro-inflammatory signalling pathways linked to obesity can result in vascular endothelial dysfunction and changed levels of Nitric oxide (NO). Hyperlipidaemia may exacerbate arterial stiffness which is a precursor to atherosclerosis. In this study, we tried to evaluate the changes in levels of nitric oxide in obese individuals and its association with arterial stiffness.

Objectives: To identify the association of serum levels of Nitric oxide metabolites with Body Mass Index (BMI) in apparently healthy subjects. 2. To evaluate any correlation between serum nitric oxide metabolites, BMI and arterial stiffness in obese individuals.

Result: The results showed a significant increase in systolic blood pressure ($p<0.001$) and no change in diastolic blood pressure and significant increase in pulse pressure($p<0.001$) with increase in visceral adiposity (Waist Circumference) more than BMI. Results of Pearson correlation indicated that there is a significant small negative relationship between BMI and Serum NO levels, (r =- 0.534, p <.001). There was significant increase in Brachial ankle Pulse Wave Velocity (Ba PWV) ($p=0.001$) and Carotid Femoral Pulse Wave Velocity (CF PWV) ($p=0.01$) in obese subjects when compared to controls.

Conclusion: Our study results showed that abdominal obesity more than overall obesity is more strongly associated with decreased serum NO levels and increased arterial stiffness. In obese individuals, increased arterial stiffness as reflected by increased pulse wave velocity may contribute to predicting cardiovascular disease in both genders in addition to development of hypertension.

Keywords: BMI, Obesity, Arterial stiffness (AS), serum NOx (Nitric oxide metabolites) levels, Brachial ankle Pulse Wave Velocity (baPWV), Carotid Femoral PWV (CFPWV)

Abbreviations:

AS: Arterial Stiffness
BMI: Body Mass Index
WC: Waist Circumference
SBP: Systolic Blood Pressure
DBP: Diastolic Blood Pressure
PP: Pulse Pressure
MAP: Mean Arterial Pressure
Ba PWV: Brachial ankle pulse wave velocity
CF PWV: Carotid Femoral Pulse wave velocity
ASI: Arterial Stiffness Index
ABI: Ankle Branchial Index

Introduction:

Obesity is a major emerging pandemic of this century which can be attributed to change in lifestyle, eating behaviours and increase in sedentary behaviour. Increase in digital screen time as well as decrease in physical activity are also associated with obesity. According to WHO, Obesity is a multi-factorial disorder which is defined as abnormal and excessive fat accumulation that presents a risk to health. Obesity and overweight are associated with metabolic changes that increase the risk for comorbidities such as diabetes, hypertension, hypercholesterolemia and cardiovascular disease[1]. It is reported that obese individuals are likely to develop atherosclerosis leading to increase in aortic stiffness independent of BP levels, ethnicity and age[2]. Although arterial stiffness increases with vascular ageing, the process appears to be accelerated and occurs earlier in presence of obesity.

Nitric oxide (NO) is a colourless inorganic radical gas produced mainly in endothelial cells from oxidation of L-arginine by constitutive calcium-calmodulin dependent three isozymes of Nitric Oxide Synthase (NOS) including eNOS (endothelial NOS), nNOS (neuronal NOS) and iNOS (inducible NOS). The short half-life and low concentration of NO in-vivo makes it difficult to measure it directly. Therefore, the stable end products(metabolites) of NO are measured which includes nitrates and nitrites = NOx. NOx measurement is most suitable method for assessment of NO synthesis in vivo and high correlation between endogenous NO production and serum NOx levels has been reported[3].

NO has a wide range of biological actions including regulation of elastic properties of conduit arteries, oxidation of Low-Density Lipoproteins (LDL), regulation of local cell growth, inhibition of smooth muscle proliferation and regulation of energy balance. Although the beneficial effects of NO in physiological ranges have been established, altered levels of NO indicate endothelial dysfunction. Many studies evidenced that high serum levels of NOx are positively associated with higher Body Mass Index (BMI) and waist circumference (WC)[4]. Increased NO metabolites in overweight and obese adolescents aged 14-19 yrs has been reported and is proposed that obesity leads to increase in NO production[5]. Obesity is associated with inflammation due to activation of pro-inflammatory signalling pathways and endothelial dysfunction promoting increased oxidative stress, which may affect NO production and activation. There is upregulation of NOS expression due to higher presence of NOS in adipose tissue in obese individuals [5]. Excessive NO production by iNOS is a mediator of non-specific tissue damage and endothelial dysfunction which may be involved in pathogenesis of metabolic disorders including obesity linked type -2 diabetes[6,7]. However, contradictory to this, Gruber et al reported a decrease in serum NOx in juveniles due to increased oxidative stress[8]. The vascular cells in hypercholesterolemia may synthesize greater quantities of nitric oxide than other cells but with rapid oxidative inactivation or conversion to toxic nitrogen oxides because of excess accumulation of superoxide anions and free radicals.

Arterial stiffness is a state of reduced arterial compliance with loss of elasticity which is measured by pulse wave velocity. Hyperlipidaemia in obesity has been associated with impaired function of endothelium and increased arterial stiffness which is a precursor for atherosclerosis independent of levels of blood pressure, ethnicity and age. There are studies indicating that the basal levels of nitric oxide activity is reduced in patients with coronary atherosclerosis. Endothelial dysfunction and arterial stiffness in obesity are considered to be independent and strong determinants of cardiovascular disease.
Hence in this study, we wanted to evaluate the changes in levels of NOx in obese and overweight individuals and its association with arterial stiffness.

OBJECTIVES OF THE STUDY:
1.To identify the association of serum levels of NOx with BMI levels in apparently healthy subjects
2. To Evaluate any correlation between serum NOx, obesity and arterial stiffness of large arteries in obese individuals

Methods:

STUDY DESIGN AND POPULATION: We conducted a cross sectional study on 120 adults in the age group of 20 -40 yrs, non-smokers having sedentary working habits who are apparently healthy who attended the out-patient department at AIIMS,

BIBINAGAR. Pregnant women, people suffering from chronic diarrhoea, renal dysfunction, subjects who have Diabetes mellitus, those using antihypertensive medications (includes nitrates, diuretics, beta-blockers, patients with cardiovascular disease, people with history of smoking and alcohol consumption were excluded from the study.

The study was conducted after obtaining Institutional Ethical committee (IEC) approval (AIIMS/BBN/IEC/SEP/2021/103A) dated 27-10-2021 at AIIMS BIBINAGAR between December 2021 to June 2023. A Written informed consent was taken from all the participants.

The weight and height of all the study participants were measured according to standard protocols. Body mass index (BMI) was calculated as weight divided by square of height(h2). Waist circumference (WC) was measured at midpoint between the lower rib margin and the iliac crest in the subjects. Hip circumference was measured at the maximum circumference of the buttocks. Waist -hip ratio was calculated.

The subjects were divided into three groups depending on their BMI as follows: **Group I: BMI 18.5 to 22.9 (controls)(n=40), GROUP II: BMI 23 to 29.9 (overweight and pre-obese subjects) (n=40) and Group III: BMI 30 and above (obese subjects) (n=40) (Asian classification of BMI).** Peripheral venous sample was collected after overnight fasting to avoid the effect of influence of nitro-compounds from food and smoking. The blood samples were centrifuged for 35-45 minutes and separated serums stored at -20degree C. Serum NOx concentration were measured by the nitrite, nitrate assay kit which determines the levels of NO metabolites (total nitrite and nitrate) using GREISS reaction method and absorbance read at 540nm. Serum lipids and triglycerides were measured using enzymatic colorimetric method with glycerol phosphate oxidase and serum cholesterol levels were estimated using standard kits.

Arterial stiffness of the participants was measured using an oscillometric non-invasive arteriography for cardiovascular risk assessment. The device determines the Brachial ankle Pulse Wave Velocity (BaPWV), carotid femoral pulse wave velocity (CFPWV), Arterial Stiffness Index (ASI) and ankle brachial index (ABI). The device also records the systolic and diastolic blood pressure, pulse pressure and heart rate.

Statistical analysis: Data analysis was done by using SPSS software version 23. All the descriptive statistics was expressed as mean + standard error with 95% confidence interval.

The continuous data were analyzed by Mann Whitney U test and ANOVA test for comparison among the 3 groups. Pearson correlation analysis was done to determine probable correlation relationships among continuous variables. A 2-sided P values <0.05 were considered statistically significant

Results:

A total of 114 apparently healthy subjects in the age group of 20-40 yrs were recruited in the study which included group I (n=40) (BMI**18.5 to 22.9; controls**) and group II (**BMI 23 to 29.9; overweight and pre-obese) (n=40)** and group III (n=34) (BMI >30; obese) (6 subjects from group III were excluded since their DBP was > 90 mmHg).

The median age of the study participants was 33.35 ±6.66. Baseline demographic, BMI and blood pressure characteristics of the participants is presented in **table 1**. There was a significant increase in WC (p<0.001) with increase in BMI (21.28±1.11-group I, 25.77±2.16 -GROUP II and 33.63±2.54 -Group III) among the groups. The results of the study showed a significant increase in Systolic Blood Pressure (p <0.05) with increase in WC and BMI. There was not much change in diastolic blood pressure and there was a significant increase in Pulse Pressure (<0.001) with increase in WC and BMI.

The triglycerides levels, total cholesterol (TC) and LDL were higher significantly in group III subjects compared to the other 2 groups. No significant difference was found in levels of HDL and VLDL among the groups (Table 2).

A downward trend in serum NO levels was observed with increase in BMI (Fig 1) and it was observed that the serum levels of NO were significantly lower in overweight and obese subjects (group III) (p<0.001). Results of Pearson correlation indicated that there is a significant small negative relationship between BMI and Serum NO levels (r =- 0.534, p <.001) (Fig 2) and there was no significant difference with gender.

A significant increase was observed in mean Ba PWV (**1553.05**±340.97) (p<0.001) and mean CF PWV (**976.23**±132.87)) among obese (group III) subjects compared to other groups (Table 3). The normal baPWV defined (<60 yrs) as **<1400 cm/s** and elevated Arterial Stiffness defined as baPWV

>1400 cm/s. The reference values for normal CFPWV **<760cm/sec** and elevated arterial stiffness defined as >760cm/sec. A significant positive correlation was observed between increase in baPWV with increase in BMI by Pearson correlation (r = 0.536, p <.001) (Fig 3). There was no significant difference in the mean ASI(Arterial Stiffness Index) and ABI(Ankle Brachial Index) values among the groups. Results of multiple linear regression analysis show a strong significant increase in baPWV and CF PWV associated with increase in waist circumference (r= 0.112; p<0.001) and BMI (r= 0.085, p<0.05) after controlling for age and sex.

Table 1: Demographic characteristics and BP levels of study participants (p value < 0.05 significant)

Variables	Group I(n= 40)	GROUP II(n= 40)	Group III(N= 34)	P value
Gender - Males	27(67%)	18(45%)	14(35%)	
Females	13(32%)	22(55%)	22(55%)	
Age (years)	30.87 ± 6.21	31.62 ± 6.54	32.56± 7.24	
BMI (kg/m2)	**21.28 ± 1.11**	**25.77 ± 2.16**	**33.63 ± 2.54**	**0.05**
WC (cm)	**78.67± 1.56**	**89.65± 1.85**	**106.26± 2.68**	**<0.001**
W/H ratio	0.91± 0.01	1.02±0.01	1.04±0.04	<0.05
SBP(mmHg)	**114.28 ± 8.38**	**118.675 ± 9.64**	**123.67± 11.31**	**<0.001**
DBP (mmHg)	80.80 ± 5.82	81.07 ± 5.36	82.92 ± 6.71	0.182
PP(mmHg)	**34.7±6.43**	**37.60±6. 95**	**40.75±9.22**	**0.001**
MAP (mmHg)	93.9 ± 13.12	100.12± 13.7	104.80 ± 15.21	<0.01

BMI- Body Mass Index; WC- Waist circumference; SBP- Systolic blood pressure; DBP-Diastolic Blood pressure; PP- pulse pressure; MAP –Mean Arterial pressure

Values of age, BMI, WC, SBP, DBP and MAP are expressed as Mean± SD

Table 2: Serum Lipid profile of study participants (p value < 0.05 significant)

Variables	Group I(n= 40)	GROUP II(n= 40)	Group III(n= 34)	P value
Cholesterol (mg/dl)	**158.3± 31.6**	**173.2± 31.05**	**189.76± 32.32**	**0.05**
TG(mg/dl)	**89.9± 41.0**	**101.64± 39.08**	**139.45± 35.80**	**<0.001**
LDL(mg/dl)	71.32± 27.1	97.43 ± 26.9	107.64± 28.05	0.05
HDL(mg/dl)	40.71± 11.08	43.21± 12.62	49.20± 13.21	0.12
VLDL(mg/dl)	24.86± 22.08	28.32± 23.07	35.37± 25.32	0.012

TG: Triglycerides; LDL: Low density lipoproteins; HDL: High density lipoproteins; VLDL: Very Low density lipoproteins. Values of cholesterol, TG, LDL, HDL and VLDL are expressed as Mean± SD

Table 3: Comparison of parameters of Arterial stiffness among the different groups (p value < 0.05

Group I -Controls (N=40)				Group II OVER WEIGHT AND PRE-OBESE SUBJECTS(N=40)			Group III OBESE (N=34) SUBJECTS			
Parameter	Mean	SD	SE	MEAN	SD	SE	MEAN	SD	SE	P value
Ba PWV (cm/s)	1093.45	76.12	23.13	1497.15	315.82	33.84	1553.05	340.97	22.12	0.001★
CF PWV (cm/s)	727.25	77.23	11.84	847.72	119.54	26.93	976.23	232.87	20.99	0.01★
ASI (mmHg)	36.67	10.90	1.22	43.09	13.27	2.34	51.15	8.65	3.07	0.257
ABI (ratio)	1.007	0.10	0.01	1.13	0.11	0.00	1.16	0.12	0.01	0.184

Ba PWV: Brachial ankle pulse wave velocity, CF PWV: Carotid Femoral Pulse wave velocity
ASI: Arterial Stiffness Index, ABI: Ankle Branchial Index

Figure 1: Bar diagrams representing Serum levels of NOx among different groups

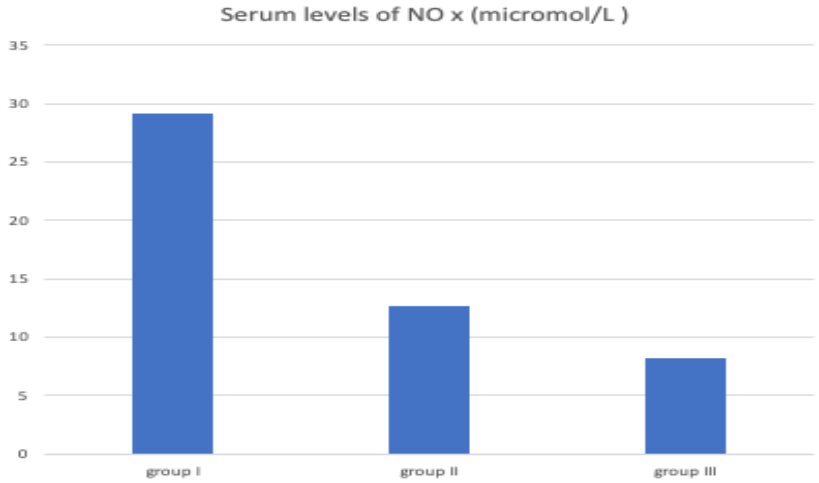


Fig 2: Correlation of BMI with serum levels of NOx (Nitric Oxide Metabolites).

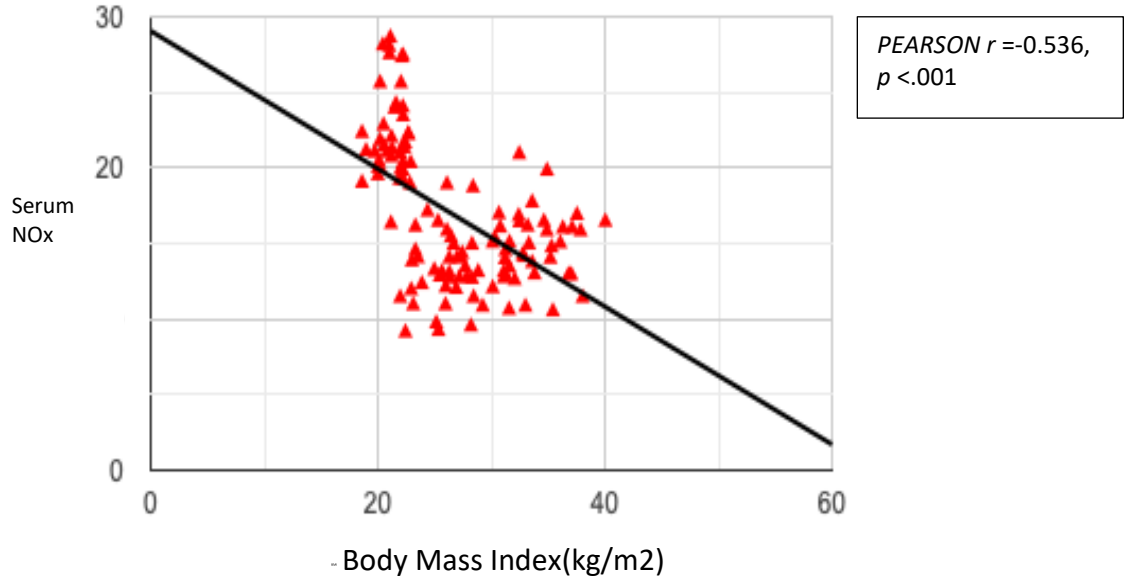


Figure 3: Bar diagrams representing BaPWV and CFPWV among Different groups

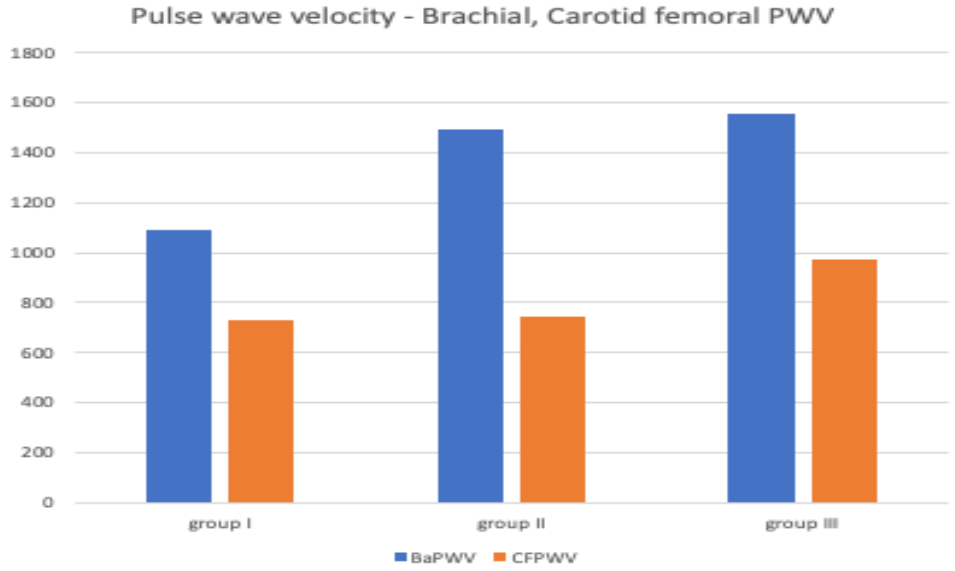
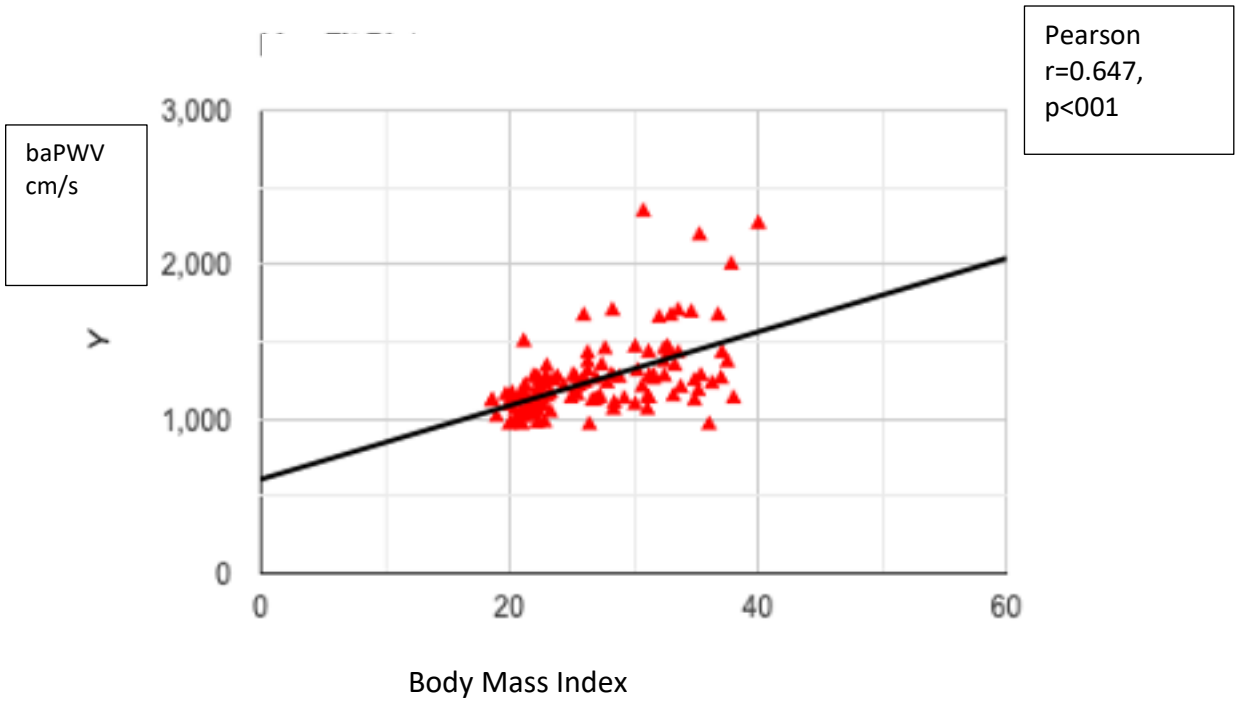


Fig 4: Fig showing Correlation between baPWV(Brachial ankle Pulse wave velocity) with BMI



Discussion:

This study ought to expand the literature on association of serum levels of Nitric Oxide metabolites in relation to BMI and arterial stiffness in apparently healthy subjects.

The results of the study illustrate considerable linear association between abdominal obesity (as measured by WC) with increase in BMI among the groups. In this sense, Ahmed et al [9] have showed a strong and positive correlation of WC with BMI and concluded WC as best indicator for abdominal obesity which are similar to the results reported in our study. Inclusion of waist circumference to denote the visceral obesity in routine clinical practice along with BMI would help in better prediction of cardiovascular morbidity.

In our study, that there was a significant Increase in systolic pressure more than the diastolic pressure with increase in WC and BMI and also there was increased pulse pressure. These results are similar to results of study done by Cox BD et al[10] and Fisher et al [11] where prevalence of elevated blood pressure was associated with quantiles of BMI and WC. An increase in systolic pressure increases cardiac overload and oxygen demand whereas decrease in diastolic pressure impairs coronary

blood flow. These changes can result in left ventricular remodeling and ischemia contributing to left ventricular dysfunction and coronary artery disease[12].

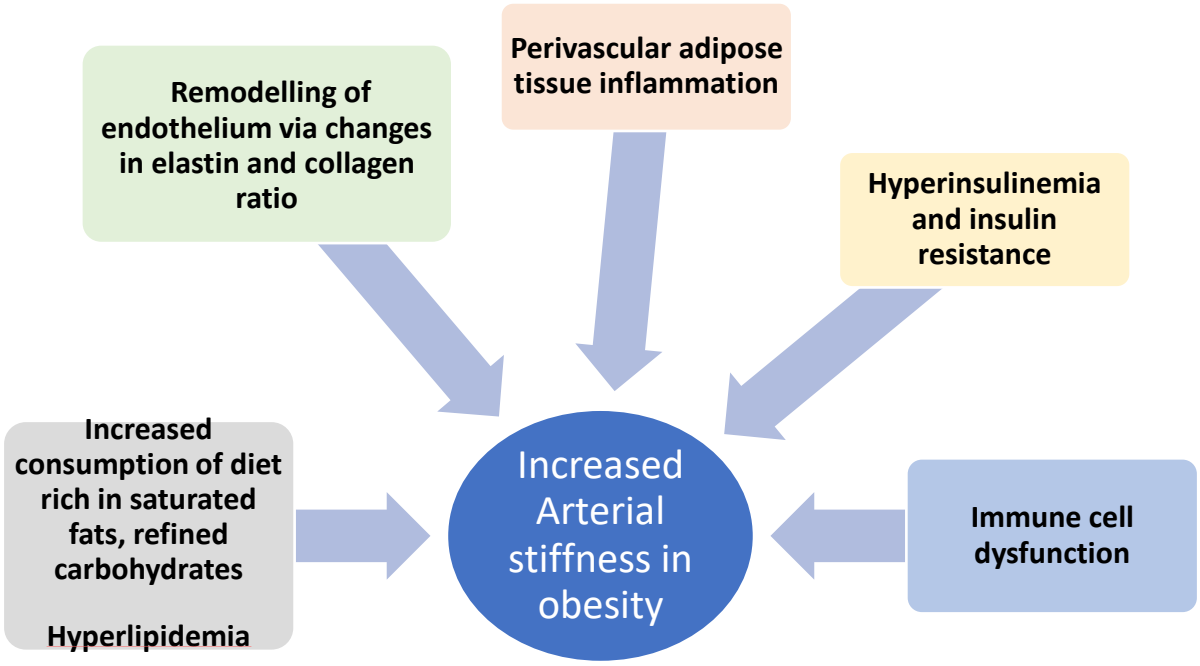
Our study results showed significant higher levels of fasting triglycerides, total cholesterol and LDL in obese individuals. Hence, presence of these lipid abnormalities may be associated with pro-inflammatory gradient affecting the endothelium increases cardiovascular risk. The results of study done by Yoichiro et al[13] revealed the risk of CHD increased independently with elevation of BMI or total cholesterol levels and decreasing risk of CHD with increasing HDL across all BMI categories.

Moreover, our findings document a significant negative correlation between serum levels of NO with abdominal obesity and BMI. In line to our results, the study by Rostamzadeh F et al[14]. revealed significant lower levels of NO in obese groups with COVID 19. Another study done by Viera et al[15] also indicated a decrease in in NO metabolites in smoker as well as non-smoker obese women. The primary proposed mechanism by which serum NO levels is decreased in obesity is through decreased expression of eNOS especially NOS3[16]. It appears that TNF-alpha in obese individuals downregulates the expression of eNOS by decreasing the stability of eNOS mRNA in adipose tissue and skeletal muscle[17,18]. Conditions of nutrient excess in obesity were also shown to upregulate caveolin -1, a negative regulator of eNOS. Critical changes in eNOS phosphorylation which is decreased in obesity is largely attributed to insulin resistance mediated by free fatty acid induced activation of TLR2, TLR4, and NF-kBD[7,19]. Increased oxidative stress characterized by lipid and protein oxidation in vascular wall leading to RAAS- induced activation of NADPH oxidase, xanthine oxidase result in increased destruction of NO[20]. Other factors associated with obesity like vascular hemodynamic forces, oxidized phospholipids, oxidized LDL and lack of exercise can also downregulate NOS3 expression[21] Hence, obesity can be causally linked with decreased vascular NO bioavailability. On the other hand, in contrast to our results, a study done by Foroumandi et al[22] presented a strong positive correlation of serum levels of NO with increasing BMI in both males and females and suggested that obesity induced pro-inflammatory status may upregulate NOS expression in adipose tissue. Few other studies [23-26] showed a positive correlation between higher serum NO levels, BMI and body visceral fat in obese and overweight subjects. Another study done by Vitale et al[27] also showed a positive correlation between BMI, inactivity and salivary NO concentrations in overweight and obese subjects.

Our study findings highlight the facts that obese individuals have significant higher baPWV and CFPWV with any level of systolic blood pressure indicating an increased arterial stiffness in both central and peripheral arteries. Our findings also confirm that the circulation in lower limbs is normal in obese individuals as there was no significant increase in ABI with increase in baPWV. Increased arterial stiffness expressed as increased PWV often precedes development of hypertension suggesting that AS is one of the earliest biomarkers for CVD risk[28-31]. A meta-analysis of cohort studies conducted in general population with hypertension and other high-risk individuals have shown that a 1m/s increase in baPWV is associated with 12% increase in risk of cardiovascular events[32]. Several factors like endothelial and vascular smooth cells, extracellular matrix remodeling, perivascular adipose tissue inflammation and immune cell dysfunction contribute to increased arterial stiffness observed in individuals with obesity (Fig 5)[33-36]. Sympathetic neural activation is another mechanism contributing to arterial stiffness[37]. Non-enzymatic glycosylation of the matrix proteins of arterial vessels may enhance the production of cross-links between collagen fibres which in turn are responsible for increased arterial stiffness[38]. Several key steps in the process of atherosclerosis may be inhibited by altered NO levels production in vascular endothelium which may contribute to its pathogenesis.

Hence, abdominal adiposity measured by waist circumference and increased arterial stiffness in obesity can thus contribute to development of cardiac hypertrophy in addition to hypertension. More aggressive management of abdominal obesity in young individuals is required to reduce the cardiovascular morbidity.

Fig5: Mechanisms causing Increase in Arterial stiffness in Obesity: Complex Interaction of endocrine factors, cytokines, vascular, cellular components and immune cells in vasculature



Limitations of the Study:

As the study is a cross sectional study, the causal relationship of obesity and arterial stiffness could not be established. The study population was restricted to limited area in Telangana. Of, course, more studies should be done to apply these results to general population. Availability of normative and reference arterial stiffness values derived from populations in a particular region would add value.

Conclusion:

From the findings of the study, abdominal obesity more than overall obesity is more strongly associated with decreased serum NO and Increased arterial stiffness. In obese individuals, increased arterial stiffness as reflected by increased pulse wave velocity may contribute to predicting cardiovascular disease in addition to development of hypertension.

Due to the ease and convenience of measurement of Arterial Stiffness, it may be used clinically as a routine screening tool for risk prediction of cardiovascular diseases in individuals with comorbidities. Further studies on Arterial stiffness and its correlation with anthropometric parameters in a larger population are required and whether reduction of abdominal obesity would reduce the arterial stiffness need to be studied through prospective studies.

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Conflict of Interest: None found

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