ORIGINAL ARTICLE

Ageing and Pulse Wave Velocity in Relation to Serum Nitric Oxide

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Abstract:

Background: The Pulse Wave Velocity (PWV) is an important marker of arterial stiffness. Age related changes of arterial stiffness in relation to PWV and endothelial derived Nitric Oxide (NOx) are least explored. *Aim and Objectives:* The present study was aimed to assess a relationship between age associated vascular stiffness and endothelial derived nitric oxide in both males and females. *Materials and Methods:* One hundred twenty healthy subjects male (n= 60) and female (n=60) subjects (20 to 95 years) were randomly selected among general population of Vijayapur city, Karnataka. Subjects were divided into group I (20-29 years), II (30-39 years), III (40-49 years), IV (50-59 years), V (60-69 years) and VI (>70 years). Physiological parameters like blood pressure and endothelial derived NOx were assessed. Vascular stiffness parameter like brachial-ankle PWV (b-aPWV) and carotid femoral PWV (c-fPWV) were also evaluated. Statistical analysis was done by using one way ANOVA and post hoc t test by using SPSS software. *Results:* Group I to group VI showed significant steady increase of b-a PWV and c-f PWV with concomitant significant decrease of serum NOx in both male and female subjects. Further a significant negative correlation between b-aPWV and c-f PWV with NOx in both male and female subjects were also observed. *Conclusion:* Results suggested possible influences of ageing on vascular stiffness which may be due to alteration of endothelial derived NOx.

Keywords: Pulse Wave Velocity, Vascular Stiffness, Nitric Oxide, Gender, Ageing.

Introduction:

Achievement of ageing is a privilege, at the same time it is also a challenge which will impact on all aspects of 21st century society [1]. In 2000, there were 600 million people aged 60 years and above and it will be 1.2 billion by 2025 or 2 billion by 2050 [2].

Age is one of the most powerful determinants of cardiovascular risk and is associated with a number of deleterious changes in the cardiovascular system [3]. Large arteries stiffening and dilatation are the more prominent changes with ageing which has been documented worldwide.

Arterial stiffness is an independent marker of Cardiovascular (CV) risk that increases with age [4]. Pulse Wave Velocity (PWV) and Arterial Stiffness Index (ASI) are widely accepted and recommended for measure of arterial stiffness [5-6]. High PWV indicates either decrease in vascular compliance or an increase in arterial stiffness.

Measurement of PWV and wave reflection have now recognized as an important prognostic indicator than Blood Pressure (BP) to assess the CV risk [7-8]. Brachial-ankle PWV (b-a PWV) and carotid-femoral PWV (c-f PWV) are considered as index of arterial stiffness [4,9]. PWV reflects the stiffness of both the aorta and peripheral arteries in an arm and a leg, and would be more applicable to general practice since its measurement, which uses a separate cuff for each

limb, is automated and easier to perform [10-12]. Several cross-sectional studies showed PWV could be a good predictor of Cardiovascular Events (CVE) including coronary artery disease and myocardial injury [13-16]. Further to note that central elastic artery and peripheral muscular arteries functions are assessed by c-f-PWV. It is an independent predictor of carotid atherosclerosis in the elderly and simple measure of arterial stiffness [17].

As Nitric Oxide (NOx) is in gaseous form so it acts as an ideal paracrine and autocrine signaling molecule to diffuses freely across membranes [18]. NOx along with its anti-atherogenic property it also influences vascular tone. Decreased bioavailability of NOx, in resistance and conduit arteries is characterized as an endothelial dysfunction, is a predictor of cardiovascular risk and outcome [19-21].

The present study was aimed to assess the vascular health through ageing and PWV in relation to endothelial functions among the general healthy population (age from 20-70+ years) of Vijayapur city, Karnataka, India.

Material and Methods:

The present cross sectional study was conducted in Laboratory of Vascular Physiology and Medicine, Department of Physiology, BLDE University's, Shri. B. M. Patil Medical College and Research Centre, Vijayapur, Karnataka, India. This study was approved by the Institutional Ethics Committee (IEC Ref No-141/2015-16 dated July 20, 2015) of Sri B.M. Patil Medical College, Hospital and Research Centre, BLDE University as per the ICMR guidelines 2006. The study was conducted on 120 apparently healthy subjects of age ranging from 20 to 95 years. Subjects from both sexes were included in the study.

Sample size calculation:

A total sample size of 120 subjects included in the study. The probability is 80% (power) that the study detected a relationship between dependent and independent variables at a two sided 0.05 significant level, if the true change in the dependent variable (NOx) is 0.5µmole/L per 1 standard deviation change in independent variable. Calculated sample size by using following formula.

$$n= \frac{[(Z_{\alpha/2}+Z\beta)^2*\{2(\sigma)^2]}{(\mu)^2}$$

Where N=No of sample, Z=Standard normal variate, α=type I error (level of significance)=1.96 β=type II error (1-β=power of test)=0.842, σ=Standard deviation=1, µ=mean difference=0.5 After calculation we got 60 subjects, so we selected 60 subjects in each gender so total 120 subjects we have included.

The inclusion and exclusion criteria are as follows:

Inclusion criteria:

1. Apparently healthy subject age ranging from 20 to 95 years.
2. Subjects with BMI < 30kg/m^2
3. Subjects with resting blood pressure <140/90mmHg
4. Non smokers
5. Subjects not taking medications or dietary supplements

Exclusion criteria:

1. Subjects with hypercholesterolemia
2. Evidence of hypertension (systolic blood pressure more than 140 and diastolic blood pressure more than 90 mm Hg).
3. Subjects with diabetes mellitus
4. Subjects taking medications like statins, antidiabetics, diuretics, antihypertensives,

beta blockers, sympathomimetic drugs and vasodilators

5. Subjects with history of tobacco consumption in any form.
6. Subjects with history of alcohol intake.

Informed consent was obtained for participation in the study. A detailed history was taken from all the subjects. All the recordings were done in the morning between 9 am to 11 am at room temperature following supine rest for 10 minutes. The entire sample is divided into six groups by age decades [22].

I. Measurement of anthropometric and physiological parameters:

All subjects underwent recording of anthropometric parameters like height (cms), weight (kg), Body Mass Index (BMI) (kg/m^2) and Body Surface Area (BSA) (m^2) and physiological parameters like pulse rate in (beats/min), Systolic Blood Pressure (SBP) (mmHg), Diastolic Blood Pressure (DBP) (mmHg), Pulse Pressure (PP) (mmHg) and Mean Arterial Pressure (MAP) (mmHg) by using standard procedures.

II. Vascular function parameters: Arterial stiffness was assessed by using a non-invasive automatic device based on Oscillometric method (Periscope, Genesis Medical Systems, India). Periscope uses two channel Electrocardiography (ECG) leads to record ECG and four BP cuffs to record arterial pressure waveforms [23]. This device is a validated 8 channel real time based simultaneous acquisition and analysis system while acquisition rate was 200 samples/ second. All recordings were made in supine position while BP cuffs were wrapped on both upper arms and above ankles and ECG electrodes applied on ventral surface of both wrists and medial side of ankles. BP volume waveforms were measured by an oscillometric pressure sensor connected by BP cuffs. Volume pulse form were determined from brachial and tibial arteries by plethysmographic sensor. The data was recorded for 10 seconds. For further analysis the data was stored in computer. The procedure is devoid of any operator bias because the device is fully automated and does not require any operator. As the periscope is automatic the recording completes itself by displaying the results. PWV and ASI are calculated by periscope as follows:

Pulse wave velocity:

a. Brachial-ankle PWV (b-a PWV): This reflects stiffness of central elastic artery & peripheral semi-muscular arteries. Periscope uses brachial and tibial artery pressure waveforms and ECG recordings (Lead I & II) to estimates b-a PWV. Pulse Transit Time (PTT) between brachium and respective ankle was calculated as the time difference between the feet of respective pulse wave which originates from R-wave (QRS complex) of ECG. The device calculated automatically the distance between the sampling points of b-a PWV according to the height of the subject. The formula is used to calculate b-a PWV.

$$ba\,PWV = \frac{Lba}{PTTba}$$

Where b-a PWV= Brachial ankle pulse wave velocity.

Lba = Distance between respective brachium and ankle.

PTTba = PTT between brachium and respective ankle was calculated as the time difference between the feet of respective pulse wave originated from R-wave (QRS complex) of ECG.

a. **The carotid-femoral PWV (c-f PWV):** A measure of aortic stiffness was calculated by the composite b-a PWV found out by averaging left and right b-a PWV. Studies conducted elsewhere [11] estimate the c-f PWV on the basis of equation (0.8333*Avg. b-aPWV-233.33) derived by regression analysis between b-a PWV and c-f PWV by using periscope.

I. **Serum Nitric oxide (NOx) level:** Total serum NOx concentration was measured as an index of endothelial function. Serum NOx was estimated by improved Griess method using vanadium chloride as a reducing agent for reduction of nitrate to nitrite (QuantiChrom™ Nitric Oxide Assay Kit: D2NO-100, BioAssay Systems, USA).

Statistical Analysis:

Statistical analysis was carried out using SPSS version 16.0. Results are expressed as mean ± standard deviation. The data have expressed in the form of tables and graphs. Differences between mean values of parameters between Group I, Group II, Group III, Group IV, Group V and Group VI were evaluated by one way ANOVA followed by Post-Hoc test (LSD). We compared mean values for men and women in each age group using the unpaired t- test. Correlation b-a PWV, c-f PWV and NOx was done by Pearson's correlation. Further correlation between aging and b-a PWV, c-f PWV in both male and female were also done. P-value <0.05 was taken as significant.

Results:

Among males, there was no significant difference in means of weight, height, BMI, BSA, DBP and PP between different age groups observed. However, mean SBP (p=0.005) and mean MAP (p=0.021) differed significantly among the different age groups (Table 1).

Among female participants, there was no significant difference in means of height, BMI and DBP between different groups. However means of weight (p=0.000), BSA (p=0.008), SBP (p=0.019), PP (p=0.001) and MAP (0.002) differed significantly among the different age groups (Table 2).

It was found that both b-a PWV and c-f PWV increased with age among both male and female subjects. Our results showed both b-a PWV and c-f PWV in females were significantly lower as compared to males in all the respective age groups except group V (60-69 yrs) and VI (70 yrs plus). Further it was observed that a greater magnitude of steady rise in b-a PWV and c-f PWV in females from age forty onwards (group III) as compared to males. Further it was observed that from 60 years age (group V) onwards there were hardly any differences between male and female b-a PWV and c-f PWV (Fig.1 & 2).

It was found that NOx was decreased with age among both male and female subjects in our study. There was no significant difference in serum NOx levels between male and female subjects in all the age groups. It was observed that from 60 yrs (group V) onwards decrease of NOx concentration between male and female subjects remained near similar (Fig. 3).

Results showed a significant negative correlation between NOx and b-a PWV in males (r = -0.344, P= 0.032) (Fig. 4) and in females (r = -0.322, P= 0.031) (Fig. 5). In case of c-f PWV a similar negative correlation (r = -0.402, P= 0.011) in male (Fig. 6) and (r = -0.344, P= 0.021) in female were noticed (Fig. 7).

Results showed a significant positive correlation between age and b-a PWV in males (r=0.665, P=0.000) (Fig. 8) and in females (r = 0.552, P= 0.000) (Fig. 9). In case of c-f PWV and aging a similar positive correlation (r = 0.548, P= 0.000) in male (Fig. 10) and (r = 0.620, P= 0.000) in female were noticed (Fig. 11).

Table 1: Anthropometric and Physiological Characteristics of Male Subjects

Parameters	Age groups (years)							
	Group I 20-29 years (n=10)	Group II 30-39 years (n=10)	Group III 40-49 years (n=10)	Group IV 50-59years (n=10)	Group V 60-69years (n=10)	Group VI 70 years plus (n=10)	ANOVA	
							F' value	p' value
Weight (Kg)	68.25±9.75	65±4.94	72.37±9.72	65±4.3	68±15.87	58.44±10.15	2.04	0.099
Height (cm)	168.5±4.89	166.6±3.20	167.63±8.73	164.2±5.71	169±1.73	162.78±7.03	1.045	0.409
BMI (kg/m^2)	24.06±2.8	23.33±1.16	25.76±2.44	24.84±0.85	23.87±4.54	21.89±2.31	2.38	0.061
BSA (m^2)	1.77±0.13	1.74±0.73	1.83±0.15	1.71±0.1	1.79±0.17	1.64±0.16	1.867	0.128
SBP (mmHg)	120±7.7^{V,VI}	118.2±10.2^{V,VI}	122.5±5.1^{V,VI}	122+14.6^{V,VI}	132.67±13.6^{I,II,III,IV}	136.88±10.2^{I,II,III,IV}	4.075	0.005
DBP (mmHg)	71.5±7.76	69±7.61	73.25±6.58	76.8±9.23	82±14.4	79.33±5.19	2.103	0.09
PP (mmHg)	48.5±6.11	49.2±11.6	49.25±7.99	45.2±5.76	50.67±7.15	57.55±6.8	2.333	0.06
MAP (mmHg)	87.66±7.2^{V,VI}	85.4±6.59^{V,VI}	89.67±4.83^{VI}	91.86±10.9	98.88±14.1^{I,II,III}	98.52±6.5^{I,II,III}	3.111	0.021

Data are Mean ± S.D. Values in the final column represent results of one-way analysis (ANOVA) among different age groups. Post-hoc comparisons were made between each group with LSD method. Superscripts I, II, III, IV, V and VI on each of the group are significantly differ from that group at p<0.05 level. BMI: body mass index, BSA: body surface area, SBP: systolic blood pressure, DBP: diastolic blood pressure, PP: pulse pressure, MAP: mean arterial pressure

Table 2: Anthropometric and Physiological Characteristics among Female Subjects

Parameters	Group I 20-29 years (n=10)	Group II 30-39 years (n=10)	Group III 40-49 years (n=10)	Group IV 50-59 years (n=10)	Group V 60-69 years (n=10)	Group VI 70 years plus (n=10)	ANOVA	
							F' value	P' value
Weight (Kg)	57.42±8.3^{VI}	57.33±6.13^{VI}	59.16±6.5^{VI}	61.4±7.8^{VI}	56.6±11.54^{VI}	45±5^{I,II,III,IV,V}	2.04	0
Height (cm)	158.64±2.76	150.25±5.81	152.83±3.9	156.2±3.56	149.3±5.16	148.33±3.7	1.045	0.173
BMI (kg/m^2)	22.9±3.33	25.00±2.03	24.9±2.9	24.7±3.15	25.07±3.8	24.43±2.3	2.38	0.084
BSA (m^2)	1.59±0.10^{VI}	1.53±0.10^{VI}	1.57±0.08^{VI}	1.62±0.09^{VI}	1.51±0.16^{VI}	1.31±0.1^{I,II,III,IV,V}	1.867	0.008
SBP (mmHg)	112.78±10.8^{V,VI}	110.58±11.07^{V,VI}	118.33±17.08	118.8+9.01	132.67±21.14^{I,II}	130.6±25.48^{I,II}	4.075	0.019
DBP (mmHg)	68.64±5.63	70.08±9.6	75.33±14.58	72±6	75±9.7	67.33±11	2.103	0.463
PP (mmHg)	44.14±9.2^{V,VI}	40.5±3.08^{V,VI}	43±4.73^{V,VI}	46.8±7.56^{VI}	57±14.8^{I,II,III}	62±15.6^{I,II,III,IV}	2.333	0.001
MAP (mmHg)	83.35±6.43^{V,VI}	83.58±10.01^{V,VI}	89.66±15.3^{VI}	87.59±15.3^{VI}	93.99±12.78^{I,II,VI}	113±22.6^{I,II,III,IV,V}	3.111	0.002

Data are Mean ± S.D. Values in the final column represent results of one-way analysis (ANOVA) among different age groups. Post-hoc comparisons were made between each group with LSD method. Superscripts I, II, III, IV, V and VI on each of the group are significantly differ from that group at p<0.05 level. BMI: body mass index, BSA: body surface area, SBP: systolic blood pressure, DBP: diastolic blood pressure, PP: pulse pressure, MAP: mean arterial pressure

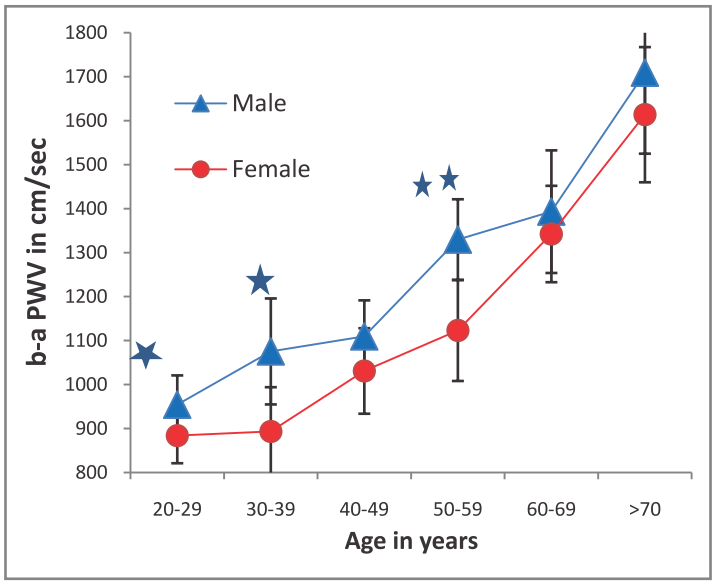

Fig. 1: Brachial-ankle Pulse Wave Velocity (b-a PWV) between Males and Females from Different Age Groups. Values are mean ± SD of each age group. *p<0.05, ** p<0.01 while Comparing Male and Female Values

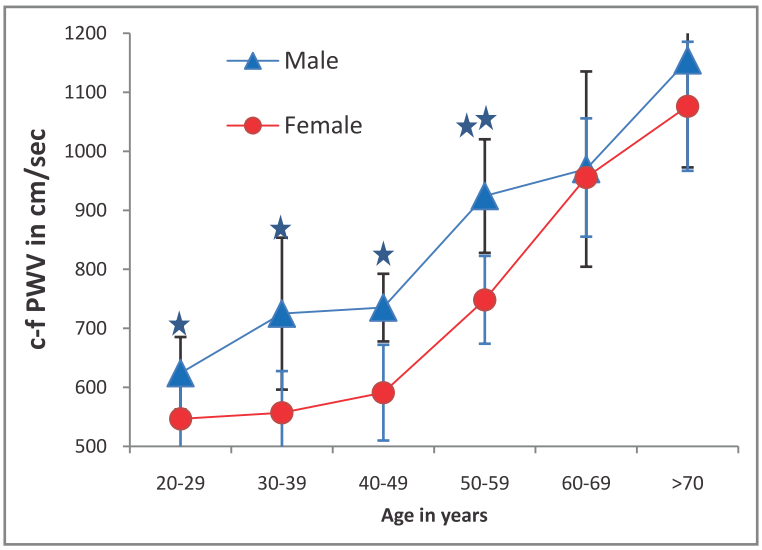

Fig. 2: Carotid-Femoral Pulse Wave Velocity (C-F PWV) Between Males and Females from Different Age Groups. Values are Mean ± SD of Each Age Group. *P<0.05, ** P<0.01 While Comparing Male and Female Values

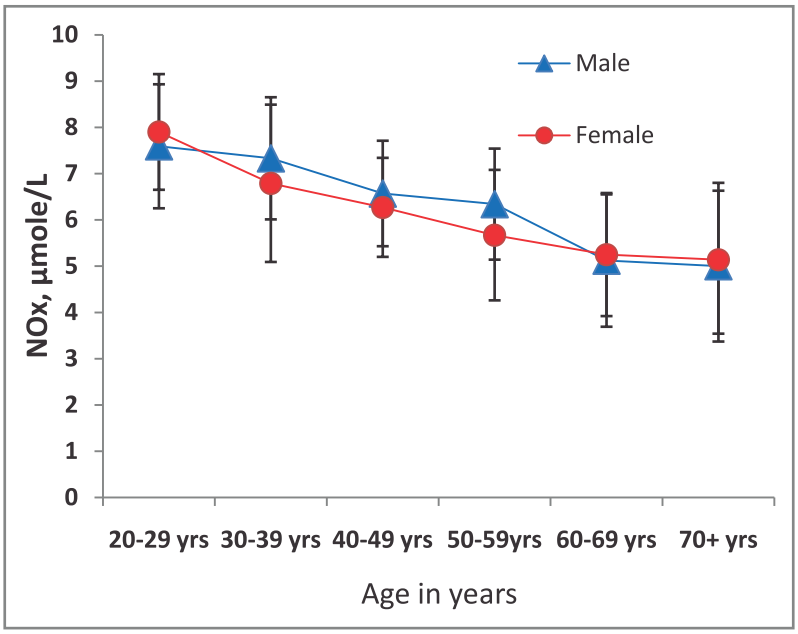

Fig. 3: Serum Nitric Oxide (NOx) Level between Males and Females from Different Age Groups. Values are Mean ± SD of Each Age Group.

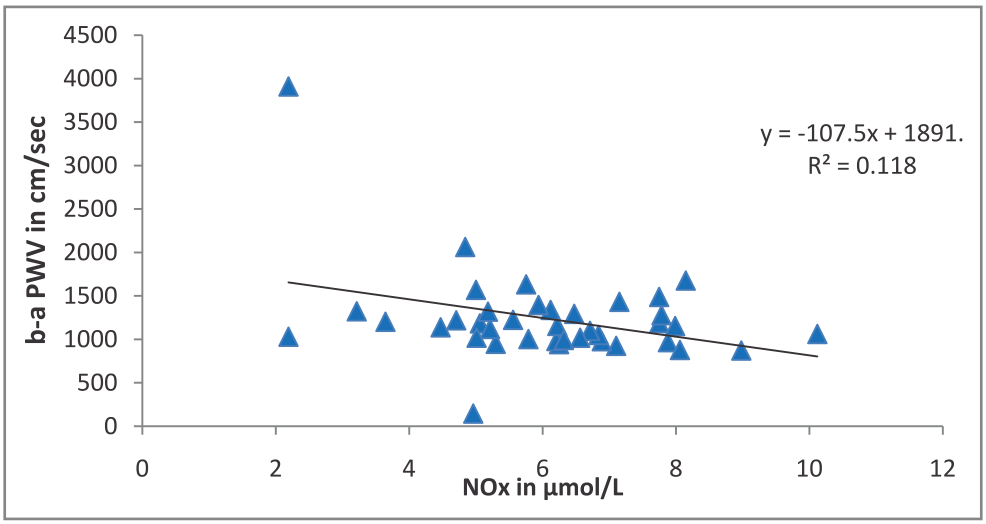

Fig. 4: Pearson's Correlation between B-A PWV and Nox among Male Subjects in Different Age Groups. Correlation (R) =-0.344; P=0.032

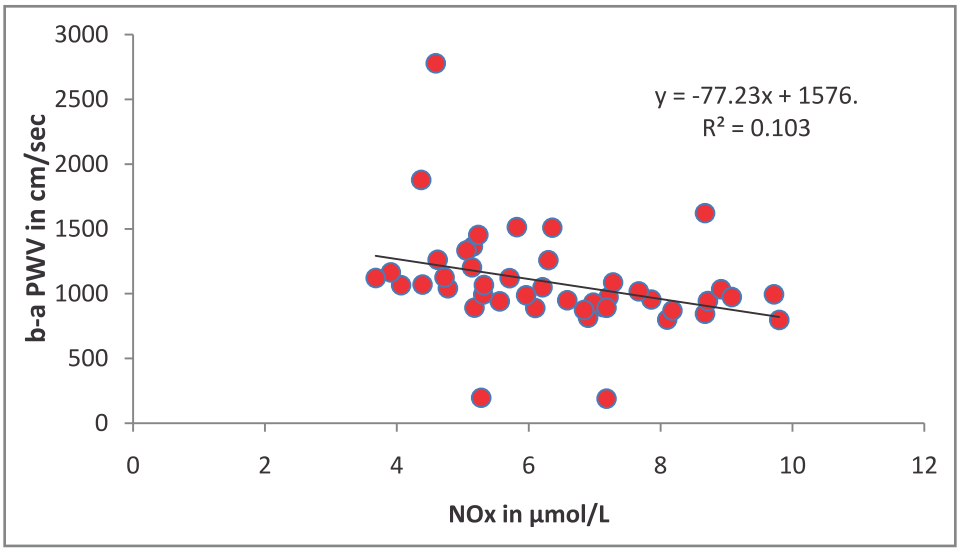

Fig. 5: Pearson's Correlation between b-a PWV and NOx among Female Subjects in Different Age Groups. Correlation (r) = -0.322; P= 0.031

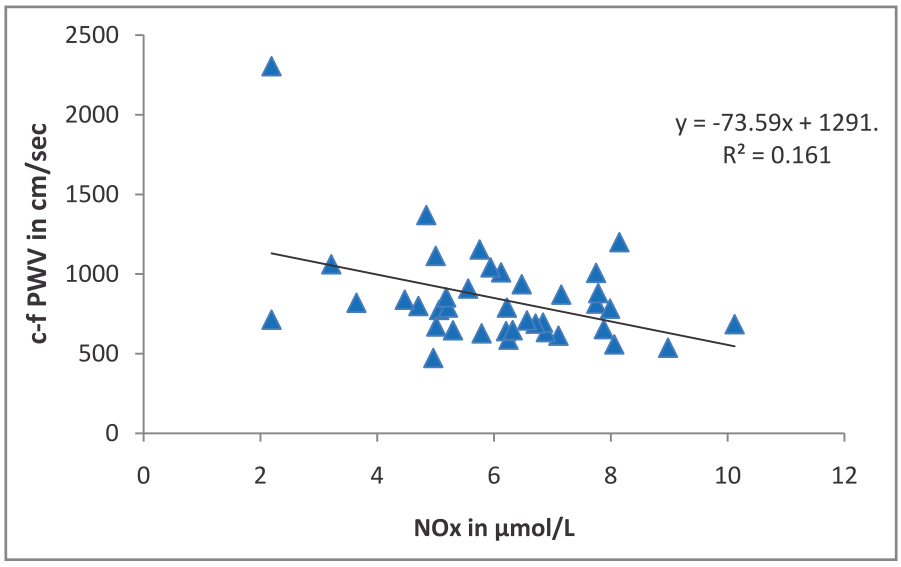

Fig. 6: Pearson's Correlation between C-F PWV and Nox among Male Subjects in Different Age Groups. Correlation (R) = -0.402; P= 0.011

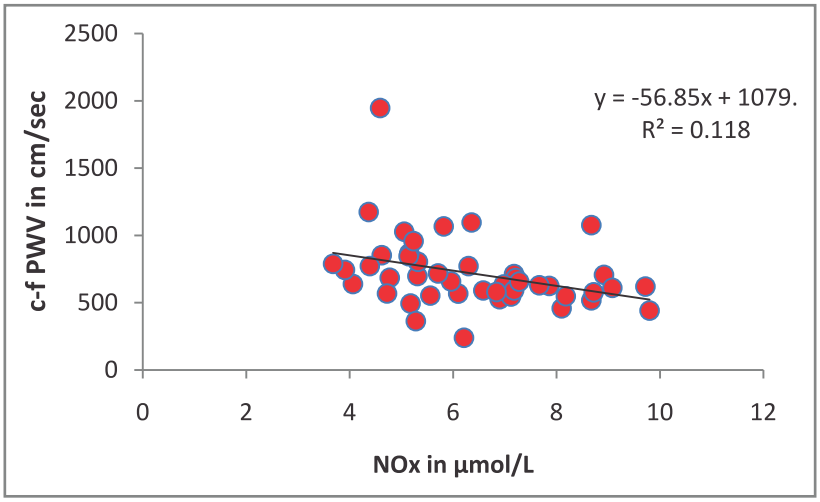

Fig. 7: Pearson's Correlation between c-f PWV and NOx among Female Subjects in Different Age Groups. Correlation (r) = -0.344; P= 0.021

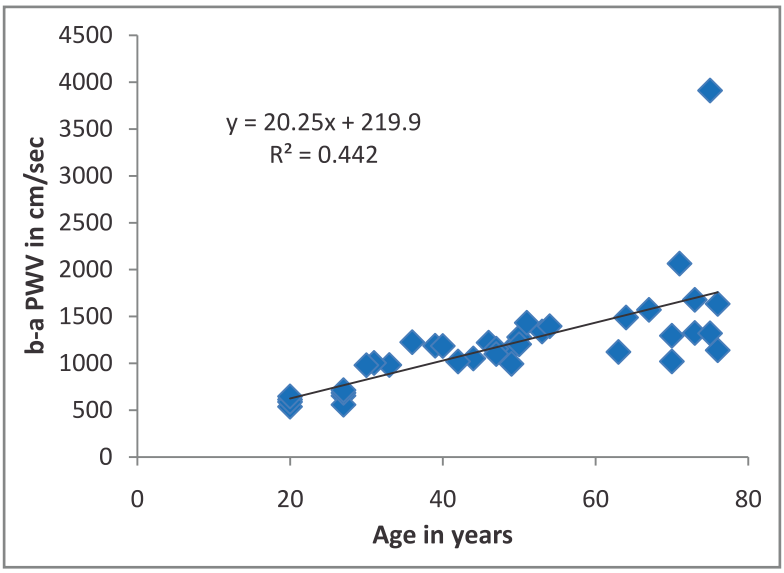

Fig. 8: Pearson's Correlation between b-a PWV and Aging among Male Subjects in Different Age Groups. Correlation (r) = 0.665; P= 0.000

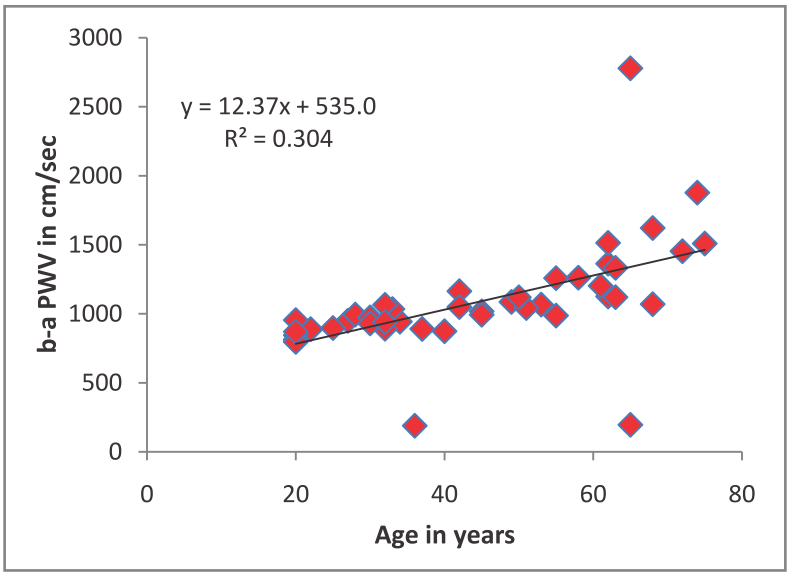

Fig. 9: Pearson's Correlation between b-a PWV and Aging among Female Subjects in Different Age Groups. Correlation (r) = 0.552; P= 0.000

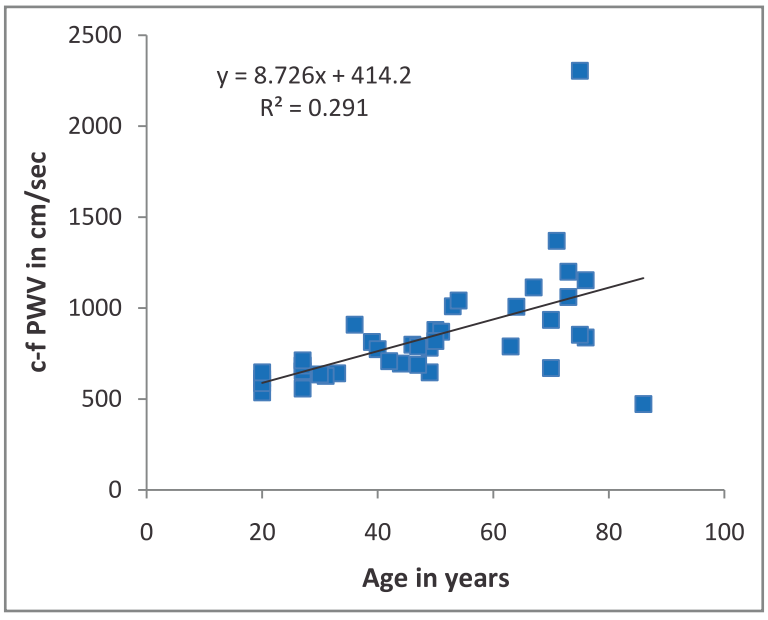

Fig.10: Pearson's Correlation between c-f PWV and Aging among Male Subjects in Different Age Groups. Correlation (r) = 0.548; P= 0.000

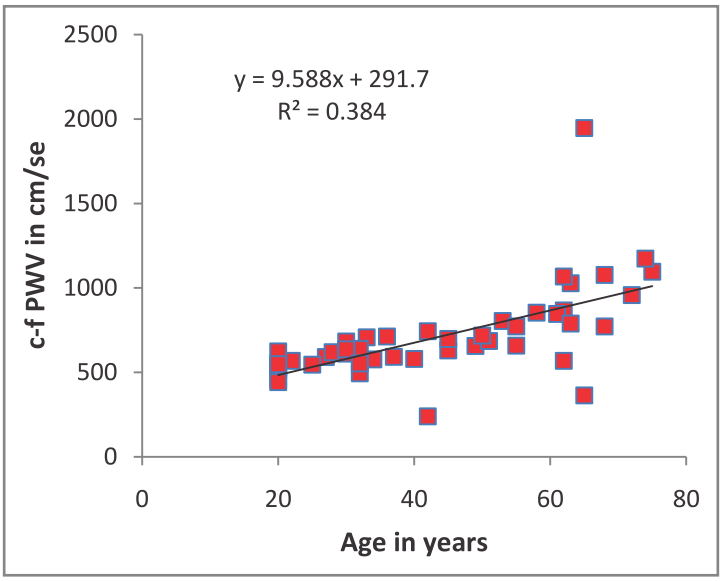

Fig.11: Pearson's Correlation between b-a PWV and Aging among Female Subjects in Different Age Groups. Correlation (r) = 0.552; P= 0.000

Discussion:

In the present study, we assessed arterial stiffness (PWV) and serum NOx in relation to ageing in apparently healthy males and females among different age groups (20-95 years). In this study involving six age groups among both male and female healthy subjects did not show any significant difference ($p<0.05$) between age groups in anthropometric, physiological parameters except SBP and MAP. Our study showed statistically significant ($p<0.05$) increase in SBP and MAP after the age of sixty years i.e. in group V (60-69 years) and VI (70 plus years) in both male and female subjects. Our study also showed significant increase ($p<0.05$) in PP after the age of 60 years in females i.e. in group V (60-69 years) and VI (70 plus years). A linear rise in SBP from age 30 to 84 years with initial increase in DBP were also reported earlier [24]. Study further reported a decline of DBP after age of 50 years with concomitant increase of PP and MAP [24]. Our results from BP in all the age groups in both male and female corroborate with this study [24].

To evaluate arterial stiffness, PWV is considered useful marker. The PWV indicates the speed at which the arterial pulsation produced due to ejection of blood from the heart propagates to the periphery. The PWV is also known to be proportional to the rigidity of the arterial wall through which it propagates and inversely proportional to the vessel diameter [25]. Age dependent increase in b-a PWV and c-f PWV of males and females in our study corroborate with the study of McEniery and Hall (2005) [26]. There are no any cut off value of Brachial-Ankle and Carotid-Femoral Pulse Wave Velocity Index to confirm arterial stiffness among normal individuals with aging in India. PWV increased linearly with aging with 6–8% with each decade of life; this tendency is more pronounced after 50 years. A significant increase of PWV over 60 years

in our study is supported by Diaz *et al.* (2014) [27]. Kawai *et al.* (2013) showed 1750.0 cm/sec could be a useful cut-off value for baPWV to predict cardiovascular prognosis in hypertensive individuals [28]. Some studies indicated differential changes of PWV in females due to post menopausal physiology and the results from our study in females support these observations [29-30].

The results from the present study on age associated gradual decrease of serum NOx in both male and female subjects indicate a reduction of bioavailability of NOx as age advances [31]. Interestingly our results differ from another observation where increase of serum NOx was found as age advances from 50 years onwards in both male and female subjects [32]. A negative correlation between NOx with b-a PWV and c-f PWV in both male and female subjects in our studies reflect that the changes of PWV are dependent on NOx in any age group. In our study significant difference in PWV in both male and females with age but there is no significant difference in sNOx level in males and females may be due to hormonal influence in female subjects and our results corroborates with study by Ahimastos *et al.* (2003) [33].

Age associated increased PWV and MAP in both male and females in the present study clearly showed altered vascular functions in ageing. PWV may be considered as more reliable marker than MAP to evaluate age associated arterial stiffness. Decreased serum NOx level in both males and females in association with age shows possible functional alterations of vascular homeostasis. Conditions associated with endothelial dysfunction may also be associated with increased arterial stiffness which may be partially counteracted through improved Nitric Oxide Synthase 3 (NOS3) pathways by generating greater NOx that improve endothelial stability and reduce arterial stiffness [34-37].

Conclusion:

Arterial stiffness is a major indicator of altered vascular functions and it is age dependent. PWV may be considered as potential marker in age associated alteration of vascular stiffness. Serum NOx probably plays as an important endothelial derived mediator to influence vascular stiffness in both males and females in the process of ageing. Understanding of these mechanisms in depth may help to explore new avenues on our knowledge in vascular sciences.

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