

4Dimensional XStrain Echocardiography: M-Mode and Tissue Doppler Estimation of Age and Gender Specific Normative Values of Aortic Stiffness in Healthy Adults During Covid-19 Pandemic

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Received date: April 21, 2023; Accepted date: May 05, 2023; Published date: May 12, 2023

Citation: Akhil Mehrotra, (2023), 4Dimensional XStrain Echocardiography: M-mode and Tissue Doppler Estimation of Age and Gender Specific Normative Values of Aortic Stiffness in Healthy Adults During Covid-19 Pandemic, *Cardiology Research and Reports*. 5(3); DOI:10.31579/2692-9759/098

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Abstract

Background: The elastic properties of the aorta are modified in numerous cardiovascular and non-cardiovascular diseases. Multiple studies have evaluated aortic stiffness in myriads of disease state, albeit only few Indian studies have estimated the normal values of aortic stiffness in healthy population.

Objective: To the best of our knowledge, till date no research has been undertaken to determine the age and gender specific value ranges of aortic stiffness parameters in healthy subjects. Hence, in the present study we endeavored to estimate these values in our distinctive study groups of healthy adults.

Methodology: This was a prospective observational study in which 58 healthy adults were enrolled during the turbulent corona pandemic. Study group was of the age group 18-60 years of either sex and was arbitrarily divided into six groups. Exhaustive M-mode and Tissue doppler Imaging was performed by 4Dimensional XStrain echocardiography system for extensive evaluation of multiple M-mode and Tissue Doppler imaging derived parameters of Aortic stiffness and superior wall velocities of ascending aorta.

Result: AOS, AOD, Aortic strain, and elasticity modulus were greater in males. On the contrary Aortic superior wall velocities (SAO, EAO, and AAO) were higher in females. Increasing age lead to a decline in majority of stiffness parameters derived by M-mode echocardiography. Correspondingly EAO determined by TDI of superior wall of aorta, showed a deterioration with advancing age.

Conclusion: The authors report a normal range of M-mode and TDI derived values of Aortic stiffness of ascending aorta, in healthy Indian adults. Difference in magnitude of aortic elasticity indices has been demonstrated in men and women, as well as in different subsets of the study group.

Keywords: aortic stiffness; aortic elasticity; 4d x strain echocardiography; TDI of aorta; healthy adults; Covid-19 pandemic

Introduction

Functional properties of the aorta are major determinants of normal cardiovascular (CV) function [1]. Increments in aortic stiffness and reduction in aortic distensibility (indicators of elastic properties of aorta) are associated with coronary artery disease [2, 3]. Aortic elasticity is an established methodology for risk stratification of atherosclerotic heart disease, myocardial infarction, stroke and heart failure [3].

Numerous methods have been employed for the evaluation of aortic elasticity, namely magnetic resonance imaging (MRI), aortic angiography

applanation tonometry, velocity vector imaging, and gated radionuclide angiography [4-8]. Moreover M-mode and Tissue Doppler Imaging (TDI) of the ascending aorta is also used to estimate its elastic properties [9-14].

The elastic properties of the aorta are modified in numerous CV and non-cv diseases. Hypertension, mitral valve prolapses, aortic aneurysms, coronary artery disease and heart failure, being the major cv disease and cystic fibrosis, pregnancy, chronic kidney disease, hypothyroidism, sarcoidosis, al-

antitrypsin deficiency and diabetes, being the non-cv diseases, altering the aortic stiffness properties [15-24].

Even though aortic stiffness has been evaluated in myriads of disease states, in contrast only few Indian studies have assessed the normal values of aortic stiffness parameters in healthy population [25, 26]. Till date, no research has been undertaken to determine the age and gender specific value ranges of aortic stiffness parameters in healthy subjects. Hence, in the present study we endeavored, to determine the above-mentioned normative values in our distinctive study groups of healthy population.

Material and Methods

This study was carried out at Prakash Heart Station and Diagnostic centre, Lucknow, India. This was prospective, observational study in which 258 healthy Indian adults were recruited, and later on, 200 cases were omitted due to inferior image quality. Finally, 58 participants were enrolled during a period of 9 months from September 2021 to May 2022. Study group was of the age group 18-60 years of either sex, and was arbitrarily divided into six groups:

- ✓ **Group A-** Overall study population subjects from 18-30 years of age.
- ✓ **Group B-** Overall study population subjects from 31-60 years of age.
- ✓ **Group C-** Male subjects from 18-30 years of age
- ✓ **Group D-** Female subjects from 18-30 years of age
- ✓ **Group E-** Male subjects from 31-60 years of age
- ✓ **Group F-** Female subjects from 31-60 years of age

Those participant were included, if they were asymptomatic with a normal physical examination BMI-23 or less, waist size 85 cm² or less in men and 80 cm² or less in women, free from overt cardiovascular disease, not receiving any drugs, nonsmoker, nontobacco chewer, nondiabetic, non-hypertensive according to JNC-8 guidelines, having normal thyroid and lipid profile, normal resting electrocardiogram (ECG) in sinus rhythm with a normal two-dimensional echocardiography and Treadmill Stress ECG. Those individuals were excluded if there was presence of diabetes mellitus, neurological or psychiatric illness, malignancy, CAD, Aortic root abnormalities and aortic dilatation thyroid disease, valvular heart disease, history of cardiac rhythm abnormalities, heart failure, systemic hypertension, and significant pulmonary hypertension.

The study procedure was approved by the Institutional Ethics Board of Prakash Heart Station and Diagnostic, Lucknow, India. All subjects or their guardians gave their written informed consent prior to data collection and furthermore confidentiality of patient information was maintained.

Data collection and study procedure

All patients underwent full history taking, clinical examination, and a standard resting 12-lead ECG. A negative Covid-19 reverse transcription polymerase chain reaction report conducted within 72 h prior to the data of enrollment and echocardiography, was the essential requirement because the study was conducted during the raging Covid-19 pandemic.

Biochemical and Hormonal Assessment

After 12 h of overnight fasting, blood samples were withdrawn for HBAIC, T3T4TSH, Serum creatinine, Total cholesterol, Triglycerides, low-density cholesterol and high-density cholesterol. These estimations were done to rule out the presence of diabetes mellitus, hypothyroid or hyperthyroid state, renal failure, and dyslipidemia.

Blood pressure measurement

Blood pressure (BP) levels were measured from the right brachial artery at the level of the heart with a mercury sphygmomanometer after resting for at least 5 minutes in the supine position. Three measurements, at least 2-minute apart, were performed, and the average of the closet two readings was recorded. A pressure drop rate of approximately 2 mm Hg/S was applied, and Korotkoff's phases I and V were used for systolic and diastolic BP (SBP and DBP, respectively) levels. All BP measurements were made by a cardiologist. Pulse pressure (PP) was calculated as systolic minus diastolic BP.

Echocardiography

All echocardiographic evaluations were performed by the author, using- My Lab X7 4D X Strain echocardiography machine, Esaote, Italy. The images were acquired using a harmonic variable frequency (1-5 MHz) electronic single-crystal array transducer with the subject lying in left lateral decubitus position.

Conventional Echocardiography

M-mode, 2-Dimensional and pulsed wave doppler (PWD) echocardiography was performed from parasternal long-axis, short-axis and apical 3 chamber, 4 chamber and 2 chamber views and the following data were derived: Interventricular septum thickness in diastolic and systolic (IVSD and IVSs respectively), left ventricular posterior wall thickness in diastole and systole (LVPWD and LVPWs, respectively), left ventricular end-diastole and end systole volumes (LVEDV and LVESV, respectively). Moreover, 2-Dimensional ejection fraction (2D-EF %) by biplane Simpson's method, LV mass in diastole (LV Mass d) and cardiac out-put (CO) were also determined. Cardiac Index was calculated by dividing the CO by body surface area (BSA). By using PWD, early diastolic velocity (E), late diastolic velocity (A), and E/A ratio was measured.

Aortic stiffness assessment by M-mode echocardiography of Ascending Aorta

Systolic and diastolic inner diameter of ascending aorta were recorded by M-Mode echocardiography 3 cm above the aorta valve in a parasternal long-axis image. Aorta systolic diameter (AOS) was measured at the maximum anterior motion of the aorta, and aorta diastolic diameter (AOD) was measured at the peak of QRS complex on the recorded ECG. (Figure 1, 2). All the parameters were computed and the average of 5 consecutive cycles were calculated.



Figure 1: Measurement of aortic diameter obtained at 3 cm above the aortic cusps.

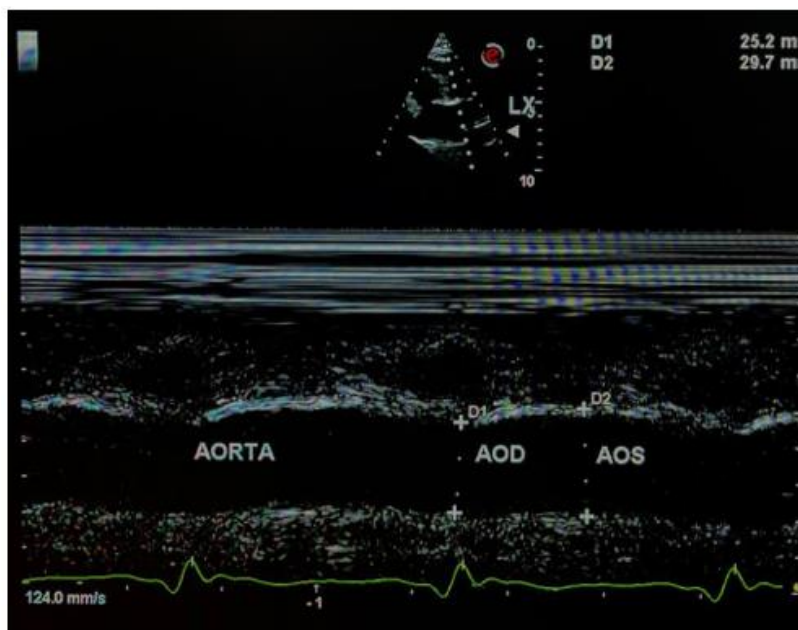


Figure 2: Aorta visualized on M- mode. The movement of aortic wall appears as two wavy lines. The space between the two lines is the aortic lumen. Systolic and diastolic diameters are measured on M- mode.

Aortic distensibility (D), aortic stiffness index (SI) and other elasticity parameters were determined by using the following formulas [27, 28].

1. aortic Distensibility = $2x \text{ AOS} - \text{AOD} / [(\text{SBP} - \text{DBP}) \times \text{AOD}]$ (106. cm² dyn⁻¹)
2. Aortic Stiffness Index = $\ln (\text{SBP} / \text{DBP}) / [\text{AOS} - \text{AOD}] / \text{AOD}$ (pure number), ln = natural logarithm
3. Aortic Pulsatile Change (APC) = $\text{AOS} - \text{AOD}$ (cm)
4. Aortic Systolic index (ASysI)
5. Aortic Diastolic Index (ADI)
6. Aortic pulsatile index (API)

ASysI, ADI and API were calculated by dividing AOS, AOD and APC by body surface area (BSA), respectively

1. Aortic compliance (AS) = $(\text{AOS} - \text{AOD}) / (\text{SBP} - \text{DBP})$ (cm/mmHg)
2. Elasticity Modulus (EM) = $(\text{SBP} - \text{DBP}) / [(\text{AOS} - \text{AOD}) / \text{AOD}]$ (Pa)
3. Aortic strain (AS) = $(\text{SAO} - \text{AOD}) \times 100 / \text{AOD}$ (%)

Tissue Doppler imaging of Ascending Aorta

Aortic upper-wall velocities were measured by Tissue Doppler Imaging (TDI) at the same point as in the M-mode measurements (Figure 3) gain and filter were adjusted to optimize the image. High temporal resolution (>100 frames/s) and a sweep speed set to 100 mm/s were used. The TDI of

expansion peak velocity during systole (SAO) and early (EAO) and late (AAO) contraction peak velocities during diastole were obtained with a 1-mm sample volume size.

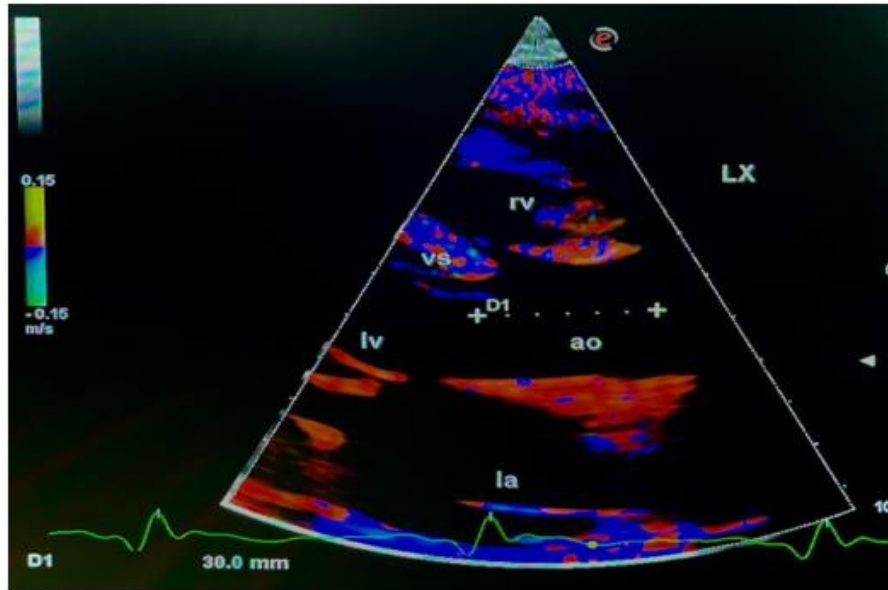


Figure 3: Tissue Doppler Imaging of the ascending aorta. The measurements were made at a level of 3 cm above the aortic cusps, at the same point as that for M-mode echocardiography.

The resulting velocities were recorded for 5 consecutive cardiac cycles and stored for later playback and analysis.

Following data were estimated by TDI of the superior wall of ascending aorta (Figure 4).

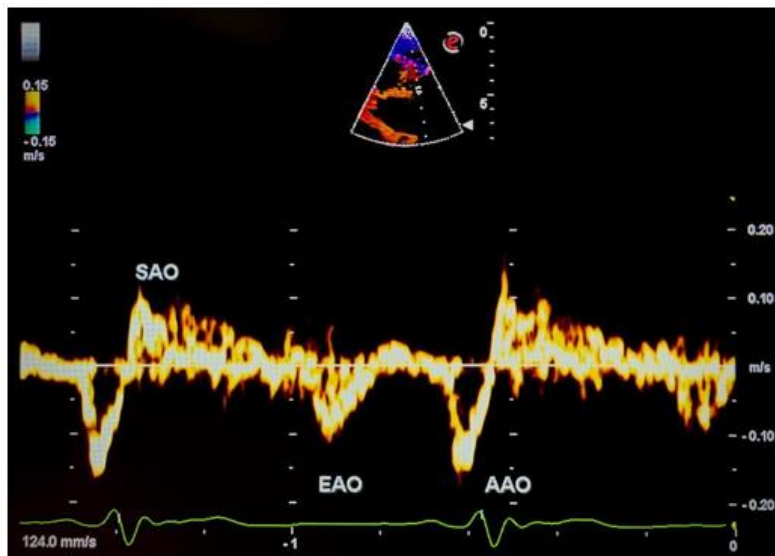


Figure 4: Aortic superior wall velocity measurements with tissue doppler imaging. SAO, systolic superior wall velocity, EAO, early diastolic superior wall velocity, AAO, late diastolic superior wall velocity.

1. SAO- Aortic superior wall velocity in systole was calculated at the same point used in M-mode measurement.
2. EAO- Early diastolic velocity
3. AAO- Late diastole velocity

TDI of LV was conducted by placing the PWD sample volume at the lateral mitral annulus in apical four-chamber view, and early diastolic velocity (E') and E/E' ratio was determined in the TDI mode.

Four-dimensional XStrain speckle-tracking echocardiography

From the apical position, two-dimensional cine loops were acquired from two-chamber, three-chamber, and four-chamber views. High-quality ECG signal was must for proper gating, and a minimum of three cardiac cycles

Tissue Doppler Echocardiography of left ventricle

were acquired of each cine loop. The study was performed with a frame rate between 40 and 75 fps and then stored digitally on a hard disk for offline analysis by software package XStrain™ advanced technology TOMTEC

GMGH 3D/4D rendering Beutel™ computation capabilities (Figure 5) [29].

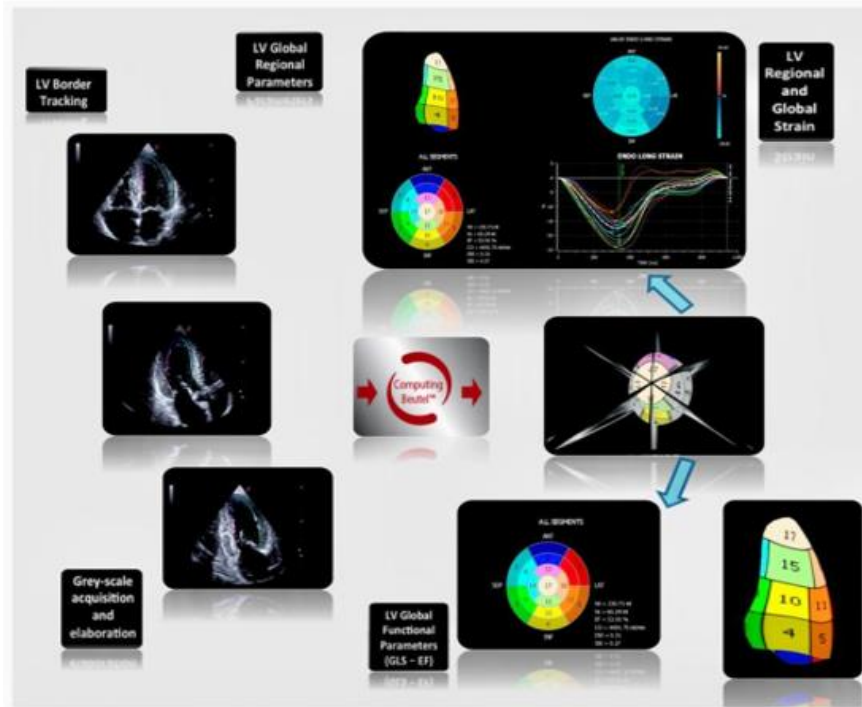


Figure 5: X Strain 4D global LV analysis. At the end of each scanning section, the three apical views are acquired. Then, after left ventricular (LV) endocardial border tracking, the software analyzes LV regional deformation parameters. Finally, the Beutel 3D reconstruction allows quantification of global LV function (global longitudinal strain (GLS)—ejection fraction). X Strain™ 4D.

The LV endocardial and epicardial borders were identified, tracked, and highlighted by a semiautomatic tool-AHS Aided Heart segmentation Esaote, for border segmentation. Thirteen equidistant tracking points were automatically incorporated along the LV endocardial border and where necessary manual adjustment of endocardial tracing was done. The software automatically divided the LV wall into 6 segments and then the acquired cine loop of each apical view was tracked frame by frame throughout the

entire cardiac cycle. The cine loops with inadequate tracing quality and with any signs of arrhythmia were excluded.

The LV bull’s eye depiction according to 17-segment model was generated by XStrain 4D software, by integrating the results of each set of cine loops [30, 31]. XStrain-4D software created a 3D reconstruction for calculating LV volumes and EF [32], and XStrain 4D-EF by the “Beutel Mode” method (TOMTEC, Germany) (Figure 6) [33].

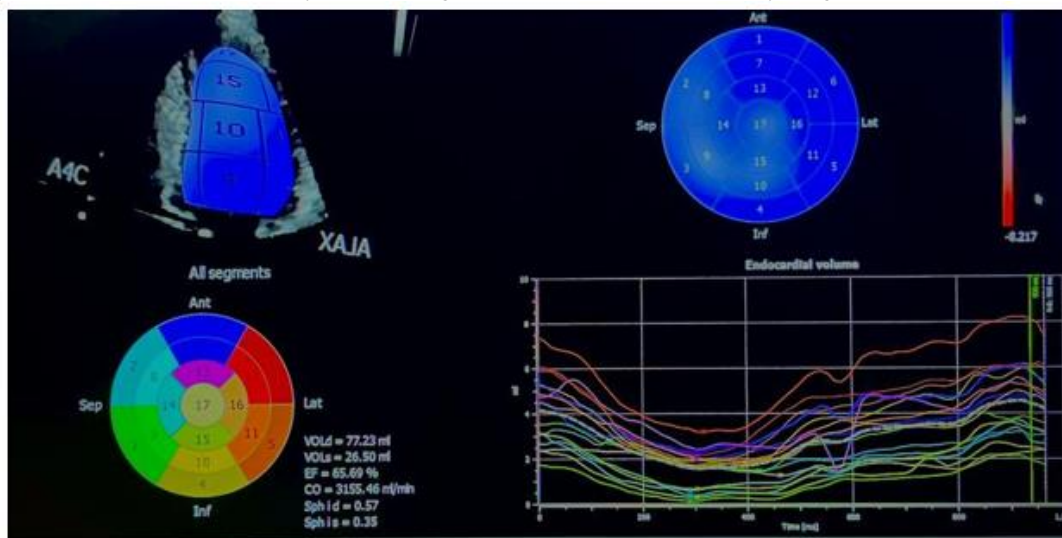


Figure 6: XStrain 4D software created a 3D reconstruction for calculating LV volumes and XStrain 4D-EF by the “Beutel Mode” method (TOMTEC, Germany).

XStain 4D-EF by the “Beutel Mode” method (TOMTEC, Germany).

The following 4D XStrain estimated values of volumetric values were statistically analyzed.

- Volumetric data: Sphericity index in diastole and systole, LVEDV, LVESV, 4D-EF%, and CO.

Statistical Analysis

Statistical analysis was performed with the Microsoft excel® (Excel 2019. Microsoft corp. Seattle Washington. USA). The continuous variables are expressed as mean ± SD. The 95% confidence interval of mean was also calculated. Enrolled participants were stratified according to Groups A-F, age: < 30 years and > 31 years and gender: male and female. Comparison of various

datasets between men and women and between different age groups was performed by Students t-test for independent groups.

The level of significance used was <0.05. A higher t value having a probability <0.05 was marked significant. A p value <0.01 was marked highly significant.

Result

We performed Aortic stiffness assessment of ascending aorta in 58 healthy Indian adults of age 18-60 years mean 32.16±11.82 years, free from overt cardiovascular disease (Table 1). The study population was arbitrarily divided into six groups: Group A from 18-30 years of age, Group B from 31-60 years of age, Group C, male subjects of 18-30 years, Group D, female subjects of 18-30 years, Group E, male subjects of 31-60 years and Group F, female participants of 31-60 years.

TABLE 1: Demographic Data (n=58)			
VARIABLES	Study Population(n:58)	MALE(N-38)	FEMALE(N-20)
		MEAN ± SD	MEAN ± SD
AGE (yrs)	32.16 ± 11.82	30.53 ± 12.22	35.25 ± 10.61
WEIGHT s(kg)	61.45 ± 11.08	64.18 ± 10.44	56.25 ± 10.62
HT (cm)	164.45 ± 8.62	167.42 ± 6.84	158.80 ± 8.99
BSA (m²)	1.67 ± 0.18	1.72 ± 0.16	1.57 ± 0.18
BMI	22.56 ± 2.66	22.77 ± 2.52	22.17 ± 2.93
SBP (mmhg)	118.28 ± 10.97	118.26 ± 10.58	118.30 ± 11.95
DBP (mmhg)	76.66 ± 6.58	76.74 ± 6.60	76.50 ± 6.71
HEART RATE (bpm)	80.45 ± 14.52	77.89 ± 13.06	85.30 ± 16.21

NS=Not Significant(p>0.05), ** Highly Significant=(p<0.01), * Significant=(p<0.05)

Demographic data

The mean BSA of the participants was 1.67±0.18 sq. meter. There were 38 males and 20 females with a mean age of 30.53±12.22 years and 35.25±10.61 years respectively, and a mean BSA of 1.72±0.16 sq. meter and 1.57±0.18

sq. meter respectively (Table 1). The mean age in Group A-E was 23.13±4.33 years, 42.52±8.71 years, 21.68±3.95 years, 26.66±3.08 years, 42.68±8.61 years and 42.27±9.25 years respectively and mean BSA was 1.64±0.17m², 1.40±0.2m², 1.67±0.14m², 1.56±0.18m², 1.78±0.16m², 1.56±0.17m², respectively (Table 2).

TABLE 2: Demographic Data (n=58)						
VARIABLES	GROUPS					
	A(n=31)	B(n=27)	C(n=22)	D(n=9)	E(n=16)	F(n=11)
	MEAN±SD	MEAN±SD	MEAN±SD	MEAN±SD	MEAN±SD	MEAN±SD
AGE(yrs)	23.13 ± 4.33	42.52 ± 8.71	21.68±3.95	26.66±3.08	42.68±8.61	42.27±9.25
WEIGHT(kg)	59.58 ± 10.71	63.59 ± 11.31	61.22±10.00	55.55±11.88	68.25±9.90	56.81±10.02
HT(cm)	163.87 ± 6.51	165.11 ± 10.65	165.54±5.40	159.77±7.44	170±7.88	158±10.37
BSA(m²)	1.64 ± 0.17	1.40 ± 0.20	1.67±0.14	1.56±0.18	1.78±0.16	1.56±0.17
BMI	22.03 ± 2.71	23.17 ± 2.50	22.22±2.58	21.56±3.11	23.52±2.28	22.65±2.81
SBP(mmhg)	115.35 ± 11.33	121.63 ± 9.69	115.90±11.78	114±10.67	121.5±7.91	121.81±12.24
DBP(mmhg)	74.71 ± 6.19	78.89 ± 6.41	74.36±5.84	75.55±7.26	80±6.32	77.27±6.46
HEART RATE(bpm)	78.84 ± 13.29	82.30 ± 15.87	77.09±13.22	83.11±13.22	79±13.19	87.09±18.74

NS=Not Significant(p>0.05), ** Highly Significant=(p<0.01), * Significant=(p<0.05)

Group A: overall subjects (age 18-30 years), **Group B:** overall subjects (age 30-60 years) **Group C:** Male Subjects (age 18-30yrs),

Group D: Female Subjects-(age 18-30yrs), **Group E:** Male Subject-(age 31-60yrs), **Group F:** Female Subjects-(age 31-60yrs)

Conventional Echocardiography Data

LA size, E/A ratio, Lateral TDI E' and Lateral TDI E/E' ratio are surrogate measurements for assessment of diastolic function of LV and LVIDd, LVEDV, EPSS and EF% are representative of systolic function. In our study

LA size, E/A ratio, lateral TDI E', LVIDd and LVEDV were significantly higher in males (p<0.01) even though CO & 2D-EF% was higher in females (p<0.01) (Table 3). Additionally E/A ratio and 2D-EF% were lower in Group B when compared with Group A (p<0.01), suggesting a reduction in diastolic & systolic function of LV, with increasing age.

TABLE 3: Conventional Echocardiography Data (n=58)

VARIABLES	Study Population (n:58)	MALE(N-38)	FEMALE(N-20)	P		Age wise Group (Years)		P	
		MEAN ± SD	MEAN ± SD	P-Val.	Sig	Group A(Overall)	Group B(Overall)	P-Val.	Sig
						(n=31)	(n=27)		
EPSS(mm)	0.60 ± 0.32	0.57 ± 0.26	0.66 ± 0.41	<0.01	*	0.58 ± 0.37	0.63 ± 0.26	0.71	NS
Left Atrium (cm)	2.80 ± 0.53	2.83 ± 0.55	2.75 ± 0.49	<0.01	*	2.68 ± 0.40	2.94 ± 0.63	0.59	NS
IVS d (cm)	0.73 ± 0.16	0.73 ± 0.17	0.73 ± 0.13	<0.01	*	0.70 ± 0.16	0.76 ± 0.14	0.50	NS
LVID d (cm)	4.70 ± 0.47	4.84 ± 0.40	4.42 ± 0.48	<0.01	*	4.68 ± 0.46	4.72 ± 0.48	0.07	NS
LVPW d (cm)	0.77 ± 0.13	0.79 ± 0.13	0.73 ± 0.12	<0.01	*	0.73 ± 0.13	0.82 ± 0.12	0.69	NS
LVEDV(ml)	63.38 ± 42.32	74.90 ± 43.46	41.49 ± 30.40	<0.01	*	79.61 ± 43.10	44.75 ± 33.28	0.22	NS
LV MASS d (gm)	114.40 ± 31.20	122.00 ± 29.74	99.95 ± 29.37	<0.01	*	108.10 ± 26.82	121.63 ± 34.68	0.84	NS
CO.(L/min)	5.43 ± 1.67	5.43 ± 1.46	5.43 ± 2.07	<0.01	*	5.14 ± 1.37	5.75 ± 1.94	0.81	NS
E/A RATIO	1.41 ± 0.54	1.44 ± 0.53	1.33 ± 0.56	<0.01	*	1.73 ± 0.48	1.03 ± 0.32	<0.01	**
Lateral TDI E'	6.92 ± 7.34	8.38 ± 7.56	4.15 ± 6.17	<0.01	*	10.71 ± 7.57	2.56 ± 3.90	<0.01	**
Lateral TDI E/E' RATIO	0.40 ± 0.40	0.32 ± 0.33	0.55 ± 0.48	0.70	NS	0.25 ± 0.38	0.57 ± 0.35	<0.01	**
2D-EF(%)	0.65 ± 0.07	0.62 ± 0.07	0.69 ± 0.06	<0.01	*	0.65 ± 0.07	0.64 ± 0.07	<0.05	*

EPSS, Epoint septal separation, IVSd, interventricular septum in diastole, LVPWd, Left ventricular posterior wall in diastole, LVID, left ventricular, internal dimension LVEDV, Left ventricular end-diastole volume, CO, cardiac output, TDI, Tissue doppler imaging, EF, ejection fraction

NS=Not Significant(p>0.05),** Highly Significant=(p<0.01),* Significant=(p<0.05)

Group A: overall subjects (age 18-30 years), **Group B:** overall subjects (age 30-60 years)

4 Dimensional Volumetric Data

The sphericity index in diastole and systole, LVEDV and LVESV were higher in males (p<0.01). Nevertheless, 4D-EF% was more in female

(p<0.01) (Table 4). We noticed a decline in sphericity indices in Group B as compared to Group A (p<0.05), suggesting a significant change in LV geometry with increasing age.

TABLE 4: 4-Dimensional volumetric data (n=58)

VARIABLES	Study Population (n:58)	MALE(N-38)	FEMALE(N-20)	P		Age wise Group (Years)		P	
		MEAN ± SD	MEAN ± SD	P-Val.	Sig	Group A(Overall)	Group B(Overall)	P-Val.	Sig
						(n=31)	(n=27)		
Sphericity Index d	0.44 ± 0.12	0.45 ± 0.13	0.42 ± 0.10	<0.01	**	0.46 ± 0.14	0.42 ± 0.09	<0.05	*

Sphericity Index s	0.37 ± 0.12	0.38 ± 0.13	0.35 ± 0.12	<0.01	**	0.39 ± 0.14	0.34 ± 0.11	<0.05	*
LVEDV (ml)	78.47 ± 17.12	81.07 ± 17.14	73.52 ± 16.39	<0.01	**	78.18 ± 17.99	78.80 ± 16.40	0.14	NS
LVESV (ml)	35.06 ± 9.30	36.27 ± 8.71	32.74 ± 10.16	<0.01	**	35.50 ± 9.21	34.55 ± 9.56	0.09	NS
EF(%)	55.81 ± 5.24	55.50 ± 4.52	56.40 ± 6.48	<0.01	**	55.10 ± 4.59	56.63 ± 5.88	0.13	NS
CO(L/min)	3.37 ± 0.83	3.43 ± 0.78	3.26 ± 0.94	<0.01	**	3.29 ± 0.76	3.45 ± 0.92	0.34	NS
Cardiac Index(L/mm/m2)	2.02 ± 0.47	1.99 ± 0.45	2.07 ± 0.52	<0.01	**	2.01 ± 0.45	2.03 ± 0.51	0.17	NS

LVEDV, Left ventricular end-diastolic volume,LVESV,Left ventricular end-systolic volume,EF,ejection fraction,CO,cardiac output
NS=Not Significant(p>0.05),** Highly Significant=(p<0.01),* Significant=(p<0.05)

Group A: overall subjects (age 18-30 years), **Group B:** overall subjects (age 30-60 years)

M-Mode data of Aortic stiffness

AOS, AOD, Pulsatile change, Pulsatile index, Aortic Strain and Elasticity Modulus were greater in males (p<0.01), and Aortic distensibility was insignificant elevated (p=NS). On the contrary, Aortic Systolic index, Aortic diastolic index was higher in females (p<0.01) (Table 5). s

Furthermore, Pulsatile change, Pulsatile index, Aortic Strain were lower in Group B as compared to Group A (P<0.01), demonstrating a decline of these stiffness parameters with increasing age.

TABLE 5: Comparison of M-Mode data of Ascending Aorta Data (n=58)

VARIABLES	Study Population (n:58)	MALE(N-38)	FEMALE(N-20)	P		Age wise Group (Years)		P	
		MEAN ± SD	MEAN ± SD	P-Val.	Sig	Group A(Overall)	Group B(Overall)	P-Val.	Sig
						(n=31)	(n=27)		
AOS	2.68 ± 0.40	2.73 ± 0.38	2.59 ± 0.42	<0.01	**	2.53 ± 0.36	2.86 ± 0.37	0.85	NS
AOD	2.22 ± 0.40	2.23 ± 0.38	2.18 ± 0.45	<0.01	**	2.02 ± 0.33	2.44 ± 0.35	0.52	NS
Pulsatile Change	0.47 ± 0.14	0.49 ± 0.14	0.42 ± 0.14	<0.01	**	0.51 ± 0.12	0.42 ± 0.15	<0.01	**
Ao syst. Index	1.62 ± 0.26	1.59 ± 0.22	1.67 ± 0.32	<0.01	**	1.55 ± 0.22	1.70 ± 0.27	0.59	NS
Ao diast. Index	1.34 ± 0.24	1.30 ± 0.20	1.40 ± 0.31	<0.01	**	1.23 ± 0.19	1.45 ± 0.24	0.76	NS
Pulsatile Index	0.28 ± 0.09	0.29 ± 0.09	0.27 ± 0.10	<0.01	**	0.31 ± 0.08	0.25 ± 0.10	<0.01	**
Ao Distensibility (10⁻⁶ cm² dyn⁻¹)	0.01 ± 0.02	0.02 ± 0.03	0.01 ± 0.01	0.09	NS	0.02 ± 0.03	0.01 ± 0.01	0.07	NS
Ao Stiffness Index	1.99 ± 0.47	1.95 ± 0.38	2.08 ± 0.61	<0.01	**	1.76 ± 0.30	2.26 ± 0.49	0.22	NS
Ao Strain(%)	22.78 ± 8.86	23.58 ± 8.55	21.25 ± 9.45	<0.01	**	27.06 ± 7.70	17.85 ± 7.51	<0.01	**
Elasticity Modulus(Pa)	204.16 ± 102.32	200.42 ± 80.95	211.26 ± 136.27	<0.01	**	159.29 ± 61.37	255.67 ± 115.98	<0.05	*

AOS,Aortic dimension in systole,AOD,Aortic dimension in diastole,Ao,Aorta

NS=Not Significant(p>0.05),** Highly Significant=(p<0.01),* Significant=(p<0.05)

Group A: overall subjects (age 18-30 years), **Group B:** overall subjects (age 30-60 years)

Tissue Doppler imaging data of Aortic stiffness

SAO, EAO and AAO were higher in females (p<0.01) (Table 6). It was also observed that EAO was lower in Group B, indicating a deterioration of in

early diastolic upper wall velocity with aging. Contrarily SAO and AAO showed insignificant (p=NS) increment with increasing age.

TABLE 6: Comparison of TDI data of Ascending Aorta Data (n=58)

VARIABLES	Study Population (n:58)	MALE(N-38)	FEMALE(N-20)	P		Age wise Group (Years)		P	
		MEAN ± SD	MEAN ± SD	P-Val.	Sig	Group A(Overall)	Group B(Overall)	P-Val.	Sig
						(n=31)	(n=27)		

						(n=31)	(n=27)		
SAO (cm/sec)	1.10 ± 0.35	1.08 ± 0.35	1.14 ± 0.36	<0.01	**	1.06 ± 0.29	1.14 ± 0.41	0.57	NS
EAO (cm/sec)	0.94 ± 0.30	0.93 ± 0.32	0.96 ± 0.27	<0.01	**	0.98 ± 0.28	0.89 ± 0.33	<0.05	*
AAO (cm/sec)	1.14 ± 0.47	1.13 ± 0.44	1.15 ± 0.53	<0.01	**	1.07 ± 0.42	1.21 ± 0.51	0.89	NS

TDI, Tissue doppler imaging, SAO, Systolic upper velocity, EAO, Early Diastolic aortic upper wall velocity, AAO, late diastolic upper wall velocity
 NS=Not Significant(p>0.05),** Highly Significant=(p<0.01),* Significant=(p<0.05)

Group A: overall subjects (age 18-30 years), Group B: overall subjects (age 30-60 years)

Comparison of Aortic stiffness data in various subjects

On comparing Group C and D (male and female subjects of age 18-30 years), it was shown that AOS, AOD, and Aortic Stiffness Index and Elasticity modulus were greater in males of 18-30 years of age (p<0.01), even though

Aortic Strain was higher in females (p<0.01) (Table 7). Similarly, SAO, AAO and EAO reflected a lower value in Group D than Group C, indicating diminished aortic superior wall velocities in female subjects of age 18-30 years of age.

Variables	Groups		P		Groups		P	
	C(n=22)	D(n=9)	P-Value	Sig	E(n=16)	F(n=11)	P-Value	Sig
Aortic Stiffnes Parameters								
AOS	2.59 ± 0.38	2.38 ± 0.26	0.000003	**	2.92 ± 0.29	2.77 ± 0.46	0.01	*
AOD	2.06 ± 0.37	1.90 ± 0.20	0.000003	**	2.47 ± 0.25	2.41 ± 0.48	0.02	*
Ao Distensibility (10 ⁻⁶ cm ² dyn ⁻¹)	0.02 ± 0.04	0.02 ± 0.01	0.11	NS	0.01 ± 0.004	0.01 ± 0.01	0.62	NS
Ao Stiffness Index	1.79 ± 0.31	1.67 ± 0.27	0.000005	**	2.16 ± 0.38	2.41 ± 0.61	0.14	NS
Ao Strain (%)	26.77 ± 8.23	27.78 ± 6.63	0.0001	**	19.19 ± 7.08	15.91 ± 8.04	0.01	*
Elasticity Modulus (Pa)	167.84 ± 64.25	138.40 ± 50.97	0.00001	**	245.22 ± 81.77	270.88 ± 156.58	0.25	NS
Tissue Doppler Imaging Parameters								
SAO (cm/sec)	1.10 ± 0.25	0.95 ± 0.35	0.000003	**	1.04 ± 0.46	1.29 ± 0.30	0.44	NS
EAO (cm/sec)	1.03 ± 0.28	0.88 ± 0.26	0.000002	**	0.79 ± 0.33	1.03 ± 0.27	0.61	NS
AAO (cm/sec)	1.10 ± 0.38	0.99 ± 0.54	0.00005	**	1.16 ± 0.53	1.29 ± 0.50	0.24	NS

AOS, Aortic dimension in systole, AOD, Aortic dimension in diastole, Ao, Aorta TDI, Tissue doppler imaging, SAO, Systolic upper velocity, EAO, Early diastolic, aortic upper wall velocity, AAO, late diastolic upper wall velocity, Ao, Aortic

NS=Not Significant(p>0.05),** Highly Significant=(p<0.01),* Significant=(p<0.05)

Group C: Male Subjects (age 18-30yrs), Group D: Female Subjects-(age 18-30yrs), Group E: Male Subjects-(age 31-60yrs), Group F: Female Subjects-(age 31-60yrs)

In addition, when we analysed the data of Group E and F (male and female subjects of age 31-60 years), it was noted that AOS, AOD and Aortic strain values were higher in Group E than Group F. Conversely, the SAO, AAO and EAO values were more in Group F, even though insignificantly (p=NS).

Interestingly, only Aortic strain was lower in Group E when compared to Group C (P<0.01), implying that aortic strain was deteriorating with increasing age in male subjects (Table 8). On the contrary AOS, AOD, Aortic stiffness index and Elasticity modulus were insignificantly higher in Group E (p=NS). We also observed that SAO values were higher and EAO values were lower in Group E (p<0.01), on comparing with Group C.

Variables	Groups		P		Groups		P	
	C(n=22)	E(n=16)	P-Value	Sig	D(n=9)	F(n=11)	P-Value	Sig
Aortic Stiffnes Parameters								

AOS	2.59 ± 0.38	2.92 ± 0.29	0.14	NS	2.38 ± 0.26	2.77 ± 0.46	0.03	*
AOD	2.06 ± 0.37	2.47 ± 0.25	0.30	NS	1.90 ± 0.20	2.41 ± 0.48	0.01	*
Ao Distensibility (10 ⁻⁶ cm ² dyn ⁻¹)	0.02 ± 0.04	0.01 ± 0.004	0.10	NS	0.02 ± 0.01	0.01 ± 0.01	0.58	NS
Ao Stiffness Index	1.79 ± 0.31	2.16 ± 0.38	0.34	NS	1.67 ± 0.27	2.41 ± 0.61	0.002	**
Ao Strain(%)	26.77 ± 8.23	19.19 ± 7.08	0.0001	**	27.78 ± 6.63	15.91 ± 8.04	0.15	NS
Elasticity Modulus(Pa)	167.84 ± 64.25	245.22 ± 81.77	0.74	NS	138.40 ± 50.97	270.88 ± 156.58	0.01	*
Tissue Doppler Imaging Parameters								
SAO (cm/sec)	1.10 ± 0.25	1.04 ± 0.46	0.02	*	0.95 ± 0.35	1.29 ± 0.30	0.01	*
EAO (cm/sec)	1.03 ± 0.28	0.79 ± 0.33	0.0004	**	0.88 ± 0.26	1.03 ± 0.27	0.05	*
AAO (cm/sec)	1.10 ± 0.38	1.16 ± 0.53	0.13	NS	0.99 ± 0.54	1.29 ± 0.50	0.07	NS

AOS,Aortic dimension in systole,AOD,Aortic dimension in diastole,Ao,Aorta TDI,Tissue doppler imaging,SAO,Systolic upper velocity,EAO,Early diastolic aortic, upper wall velocity,AAO,late diastolic uper wall velocity,Ao,Aortic
 NS=Not Significant(p>0.05),** Highly Significant=(p<0.01),* Significant=(p<0.05)

Group C: Male Subjects (age 18-30yrs), **Group D:** Female Subjects-(age 18-30yrs), **Group E:** Male Subjects-(age 31-60yrs), **Group F:** Female Subjects-(age 31-60yrs)

Subsequently, on collating the Aortic stiffness data in female subjects (Group D and F) we found higher values of AOS, AOD, Aortic Stiffness Index and Elasticity modulus in Group F than D (p<0.05, p<0.05, p<0.01, p<0.05), suggesting that in female adults that there is decline in these stiffness parameters with advancing age. Simultaneously SAO and EAO were also higher in Group F (p<0.05).

We have extensively estimated the Age and Gender specific values of Aortic Stiffness in various subsets of our study population. Here we are furnishing a summarized values (Table 9) of the above-mentioned parameters discerned from the current study. This table is particularly meant for contemporary and prospective medical researchers to conceptualize further on these interesting original findings.

Table 9: Summary of Aortic Stiffness Parameter									
Variables	Study Population (n:58)	Male(n-38)	Female(n-20)	GROUPS					
		MEAN ± SD	MEAN ± SD	A(n=31)	B(n=27)	C(n=22)	D(n=9)	E(n=16)	F(n=11)
				MEAN ± SD	MEAN ± SD	MEAN ± SD	MEAN ± SD	MEAN ± SD	MEAN ± SD
M-mode parameter									
Ao Distensibility (10 ⁻⁶ cm ² dyn ⁻¹)	0.01 ± 0.02	0.02 ± 0.03	0.01 ± 0.01	0.02 ± 0.03	0.01 ± 0.01	0.02 ± 0.04	0.02 ± 0.01	0.01 ± 0.004	0.01 ± 0.01
Ao Stiffness Index	1.99 ± 0.47	1.95 ± 0.38	2.08 ± 0.61	1.76 ± 0.30	2.26 ± 0.49	1.79 ± 0.31	1.67 ± 0.27	2.16 ± 0.38	2.41 ± 0.61
Ao Strain(%)	22.78 ± 8.86	23.58 ± 8.55	21.25 ± 9.45	27.06 ± 7.70	17.85 ± 7.51	26.77 ± 8.23	27.78 ± 6.63	19.19 ± 7.08	15.91 ± 8.04
Elasticity Modulus(Pa)	204.16 ± 102.32	200.42 ± 80.95	211.26 ± 136.27	159.29 ± 61.37	255.67 ± 115.98	167.84 ± 64.25	138.40 ± 50.97	245.22 ± 81.77	270.88 ± 156.58
Tissue Doppler Imaging Parameters									
SAO (cm/sec)	1.10 ± 0.35	1.08 ± 0.35	1.14 ± 0.36	1.06 ± 0.29	1.14 ± 0.41	1.10 ± 0.25	0.95 ± 0.35	1.04 ± 0.46	1.29 ± 0.30
EAO (cm/sec)	0.94 ± 0.30	0.93 ± 0.32	0.96 ± 0.27	0.98 ± 0.28	0.89 ± 0.33	1.03 ± 0.28	0.88 ± 0.26	0.79 ± 0.33	1.03 ± 0.27
AAO (cm/sec)	1.14 ± 0.47	1.13 ± 0.44	1.15 ± 0.53	1.07 ± 0.42	1.21 ± 0.51	1.10 ± 0.38	0.99 ± 0.54	1.16 ± 0.53	1.29 ± 0.50

Discussion

It is well known that increased aortic stiffness has been associated with impaired LV systolic and diastolic functions. The association between increased stiffness and LV systolic dysfunction, has been demonstrated in a

previous study [34], particularly along the long axis. The relation is often attributed to increased hemodynamic load caused by stiffer arteries [35, 36]. An alternative explanation for the observed relation between aortic stiffness and LV systolic function, is the direct mechanical ventricular-vascular coupling.

Systolic contraction shortens the LV long axis by pulling the aortic annulus and Sino-tubular junction of the aorta towards the LV apex, which moves minimally during systole [37-40]. The combination of aortic annulus displacement along with sparse movement of the aortic arch implies that there is substantial longitudinal stretch of the ascending aorta during systole [40-42].

Abhayaratna et al [43] assessed the relationship of arterial stiffness with LV diastolic dysfunction in 188 elderly individuals and found a significant correlation between central pulse pressure and severity of diastolic dysfunction and concluded that increased arterial stiffness was associated with more severe left ventricular diastolic dysfunction.

Arterial stiffness index establishes the elastic properties of the arterial wall, in a manner relatively independent of blood pressure, and aortic distensibility evaluates the ability of the arteries to dilate during the cardiac cycle [44-51].

Aortic stiffness and aortic distensibility have been examined with VVI and pulse wave velocity (PWV) [46, 52]. However, VVI is a new and invasive method, requiring transesophageal echocardiography, which limits its routine use in clinical practice. Also, PWV is not the ideal procedure to evaluate aortic elasticity properties since it is affected by many factors including hematological and physiological characteristics, as well as heart rate and blood pressure variations [53-55].

Direct measurements of aortic elasticity by TDI, which is a practical method for the measurements of diameter changes related to wall movements, may provide further help than other methods described above, because it is not affected by hematological and cardiovascular physiology [56-58]. Multiple articles have shown a link between loss of elasticity in major arteries and cardiovascular adverse events [57, 58]. In the Framingham Cardiology study, over 20 years of monitoring, increased pulse pressure, which is an indication of large vessel wall stiffness has been shown to increase coronary artery disease risk in the middle and older age group, who had no clinical coronary artery disease [59].

Hence the, determination of normal value ranges of Aortic stiffness parameters is imperative, because then only the normal values can be compared to the values obtained in different disease states.

A considerable amount of literature is available on the adverse impact caused by various disease states on the aortic stiffness parameters, nevertheless, it is exceptionally rare to find a study depicting these values in healthy population. After a deep search of the literature, we could only come across a solitary study [25] which has recently endeavored to put forward the normal values ranges of Aortic stiffness properties in healthy population by 2Dimensional and 4Dimensional XStrain Echocardiography. There were 72 healthy participants in the 2Dimensional group and 30 individuals in the 4D XStrain group. The results are analogous to the current study, even though there were small number of subjects in 4D XStrain group.

In the study of elasticity properties of ascending aorta in healthy children and adolescents [60] 165 subjects were enrolled with a mean age of 11.92 ± 4.0 years. The mean age in our study group was 32.16 ± 11.82 years and to compare their data with the present study would not be feasible. Another research study investigated the effects of subclinical hypothyroidism on elastic properties of the ascending [17]. This study had a strict inclusion criterion and they recruited 48 healthy controls with a mean age of 42 ± 11 years. The values of their control group are incongruous with our study and the reason seems to be the disparity of mean age of the controls of their study and the healthy subjects of the present study. Correspondingly Vitarelli et al [14] reported in their 80 healthy controls, two-dimensional M-mode and TDI guided ascending aorta wall stiffness parameters. The mean age was 49 ± 17 years and the values of stiffness index (SI), Aortic distensibility (D), elastic modulus (EM), SEO, AAO and EAO reflected gross incongruity with our study group. The divergence of results may be because of dissimilarities in the mean age of our study group and their control group (mean age 49 ± 17 years).

Gungor et al [15] showed that aortic stiffness is increased in patients with premature coronary artery disease (CAD). In their study there were 50 patients of acute coronary (ACS) and 70 age sex matched controls. However, in their control groups there were 26 smokers and several were having hypertension, diabetes and hyperlipidemia controlled on medication. Nevertheless, the mean age in their study group was 34 ± 3.9 years which is similar to our study. Since this study included, in their control group volunteers who were current smokers, controlled hypertensives, diabetics and hyperlipidemic therefore to collate the results of aortic stiffness in their control group with our study would not be meaningful.

Earlier studies mentioned above are in some way or the other, inharmonious with the present research work. We have extensively compared our data in healthy population by constructing various subsets of groups and then collating the values amongst them, in a judicious manner. The main results of our study can be outlined as follows: (i) we provided exhaustive data on several parameters of Aortic stiffness determined by M-mode and TDI echocardiography. (ii) Our study group was arbitrarily divided into six groups A-E (iii) 4Dimensional volumetric data : sphericity index, LVEDV and LVESV were higher in males and importantly, 4D-EF was more in females (iv) AOS, AOD, Aortic strain, and elasticity modulus were greater in males (v) On the contrary Aortic superior wall velocities (SAO, EAO, AAO) were higher in females (vi) Increasing age lead to a decline in parameters of sphericity index, and majority of stiffness parameters derived by M-mode echocardiography (vii) correspondingly EAO determined by TDI of superior wall of aorta, showed a deterioration with advancing age.

Study Limitations

The echocardiographic method of determining the aortic stiffness using mathematical equations may have some limitations [56,57]. Firstly, blood pressure and pulse pressure measured at the level of brachial artery may not exactly reflect aortic pulse pressure and secondly, blood pressure measurement and aortic echocardiographic assessment cannot be carried out simultaneously. All the participants are of Indian ethnicity and the normal value ranges of the present study cannot be anticipated to be identical with other ethnic groups, particularly Caucasians.

Our study had modest number of subjects, because it was undertaken during the raging corona pandemic and to encounter a normal healthy subject during this period was an arduous task. Moreover, this is a single center experience.

Recommendations and future research directions

The authors recommend, in future large scale multiple centers randomized controlled trials for enrolling hundreds of healthy subjects to further investigate the important properties of Aortic stiffness.

Conclusion

The authors report normal range of M-mode and TDI derived values of Aortic stiffness of ascending aorta, in healthy Indian adults. Difference in magnitude of aortic elasticity indices has been demonstrated in men and women, as well as in different subsets of the study group.

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DOI:10.31579/2692-9759/098

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