American Journal of Biomedical Sciences
ISSN: 1937-9080
nwpii.com/ajbms

Towards Corroborating the Arterial Stiffness with Lyfas Optical Biomarkers: A Novel Study

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Received:07 December 2021; | Revised:14 January 2022; | Accepted:07 March 2022

Abstract

Background: Vascular aging (VA) increases the risk of Cardiovascular Disease (CVD) and premature deaths. Increased VA is attributed to the extent of the Arterial stiffness index (ASI), which is evident in smokers, diabetics, hypertensives, etc. The authors of this paper have discussed the usage of a smartphone-based healthcare instrument, called Lyfas that works with the principle of arterial photoplethysmography (APPG) and estimates the Pulse rate variability (PRV), which surrogates for the Heart rate variability (HRV) and its correlated digital cardiovascular biomarkers (CVb). PRV reflects cardiovascular autonomic modulation, which helps obtain the ASI by Lyfas.

Objective: To establish the inter-relationships of the CVb and ASI using Lyfas.

Methods: (i) Lyfas assess the ASI from the 2nd derivative of the photoplethysmography (SDPPG) waveform of the capillary arteriole at the tip of index fingers using the camera sensor. From SDPPG, ASI is computed. (ii) Lyfas simultaneously captures the CVb such as SDNN, RMSSD, HR, HRV score, pNN50, SD1/SD2, and LF/HF. (iii) Linear regressions (LR) and One-way ANOVA, and Pearson's interclass correlations ('r') are applied to check the interrelationships of (a) ASI with that of (b) CVb in 116 adults (58 females and males, each) within the age group of 30-60 years and above. Hypersensitivity of Lyfas has been tested by taking the test thrice a day at a gap of 8 hours. The variance and the ranges are measured for hypersensitivity analysis.

Results: The study found that the ranges and variances fall within the normal limits. For female hypertensives, SDNN and LF/HF are statistically significant to predict for ASI with correlation (r) values of 0.79 in the age group >60 years and -0.98 in the age group 41-50 years and 0.99 in the age group 41-50 years, respectively. In the case of smokers, SD1/SD2 is negatively correlated (-0.77) with ASI and is statistically significant. male smokers, RMSSD, and HRV are statistically significant to predict for ASI with correlation (r) values of 0.59 in the age group 30-40 years and 0.96 in the age group 41-50 years and for HRV 0.96 in

the age group 41-50 years, respectively. In the case of hypertensives, SDNN is negatively correlated (-0.89) with ASI in the age group of 41-50 years and is statistically significant. No biomarker showed a significant correlation in diabetics in this sample.

Conclusions: Lyfas is a robust clinical-grade instrument and the CVb captured by it can predict the ASI and the vascular age in a personalized manner.

Keywords: Lyfas; Cardiovascular biomarkers; Arterial Photoplethysmography; Arterial Stiffness Index; Second Derivative Photoplethysmography; Vascular age

1. Introduction

Increased risk of cardiovascular diseases (CVD) is directly proportional to the Arterial stiffness index (ASI), often causing premature demise as the biological age of the arteries supersede the chronological age [1]. Tobacco smoking, hypertension, diabetes, exposure to pollutants, mental stress such as anxiety disorders, uncontrolled anger, maladjusted lifestyles, etc. pose to be high-risk factors for early ASI [1].

The arterial wall is a highly organized three-layered structure (refer to Fig. 1) - (i) Intima (the inner-most layer composed of an internal elastic lamina and endothelial tissue, (ii) Media (the middle layer made of smooth muscle cells), and (iii) Adventitia (the outer coating comprised of an external elastic lamina). As the arteries branch (arteries arterioles meta-arterioles), the media becomes smaller in size. Smooth muscles in the media are the most extensively studies visceral structure in medical physiology as it regulates the blood pressure of the body [2]. The membranes of the smooth muscle cells contain various types of potassium, calcium, and chloride channels [2]. The superexcited calcium channel causes vasoconstriction [2], which is multifold by an uncontrolled continuous adrenaline rush due to sympathetic overactivity, known as autonomic dysregulation [3]. The resultant prolonged vascular contraction determines its tone [4]. ASI typically happens when the tonicity is lost due to the disappearance of the elastin (half-life is 70 years) in the internal and external elastic lamina as can be seen in Fig. 1 wherein the layers between the red colored external and internal elastic lamina represent the disappeared elastin in case of a stiff arteriole [5-6]. Loss of elastin happens due to the above factors, leading to premature arterial stiffness in the susceptible population and is one of the key causes of early CVD and sudden cardiac death.

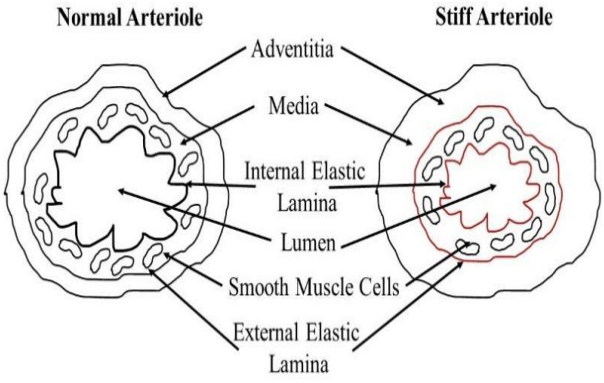


Figure 1: Cross-sectional diagram of a Normal and a Stiff Arteriole

Arterial blood flow in straight arteries is normally laminar in nature, i.e., the maximum velocity lies within the center of the blood column and reduces at the periphery. When the velocity attains or overshoots the 'critical velocity' (maximum cut-off value to remain laminar flow), the flow becomes turbulent, which is dependent on the diameter of the vessels and the viscosity of the blood [7]. Turbulence is more when the diameter is decreased and the viscosity is increased. Hence, ASI can be assessed by capturing the turbulence, reflected through the arterial Pulse wave velocity (PWV) to evaluate the risk of early CVD [8].

Since arterial stiffness is inversely related to baroreceptor sensitivity, changes in distensibility of vessels impact the mechanistic link of baroreceptors, which are highly stretch-sensitive with that of the NTS (nucleus of the solitary tract in the medulla) and the heart contractibility and hence, the HRV scores and its biomarker correlates come into play [8]. Therefore, PWV and hence Arterial stiffness (AS), is used for predictive analysis for disease

processes such as different types of hypertension, stroke, coronary artery disease, insulin resistance, anxiety, and many other.

Mental stress, lifestyle (smoking), and age have a profound impact on the structural properties of arterial vessels and decide on how much stretch-sensitive baroreceptors are to engage for the hemodynamic regulation and therefore, have an impact on the hemodynamic reactions to these [9]. On one hand, vascular anomalies disbalance the ANS interplay by increasing the sympathetic activity manifesting into vasoconstriction, hypertension, heart failure, and on the other hand, the dysfunction negatively influences the normal vascular tone, eventually leading to the formation of a cascading loop within the pathological process [4].

Therefore, ASI as a measure of vascular health is influenced by multiple factors.

Mobile health (mHealth) applications are on a surge in today's digital healthcare due to increasing smartphone users, widespread, and cheaper mobile data. The expected mHealth industry growth is about USD 156.82 billion by 2026 from 34.14 billion in 2020, i.e., a whopping 460% growth due to significant cost saving of healthcare accessibility, especially for chronic illness management [10]. Lyfas is an indigenous smartphone-based biomedical instrument (an optical biomarker) that utilizes the principle of APPG and photochromatography (PCG). From the index finger capillaries, it captures the PWV and Pulse rate variability (PRV) when the finger is pressed on the rear camera of the phone using the phone LED light 'on' [11]. PRV and its biomarkers (called cardiovascular biomarkers or CVb) are the surrogates for the cardiovascular autonomic modulation (CvAM) for the sympathovagal balance of the body [11]. The advantage of Lyfas is that it runs on android version 7 or more, a pervasive, economical, and non-invasive instrument. It can be taken at anytime from anywhere [11].

The objective of the study is to estimate ASI with Lyfas and correlate the ASI scores with that of the CVb as the screening factors.

The rest of the paper is organized as follows. Section 2 and 3 describe the methodology, results, and discussion of the experiments, performed. The paper is concluded in Section 4.

2. Materials and Methods

In this section, the techniques and approaches of the study have been discussed. The study was conducted from May 2021 to July 2021 in Bangalore, India.

2.1 Ethical compliance

a) An ethical committee clearance (No. ECR/1181/Inst/K.A., 2019, dated 30th January 2020) was obtained before the study.

b) Informed consent of all participants was obtained as per the Helsinki protocol before the study as well as before beginning the Lyfas test.

2.2 Recruitment of the subjects

Adult males and females, 58 each were recruited. The mean duration of smoking was 10.34 years in males and 8.94 years in females in all age groups, 10.76 years of hypertension in males and 9.91 years in females, and 6.65 years of diabetes history in males and 5.89 years in females. All hypertensives and diabetics were under medications. The number of females in the age group of 30-40 years, 41-50 years, 51-60 years, and above 60 years is 19, 9, 11, and 19, respectively. The number of smokers, hypertensive, and diabetic females according to the above age groups is 10, 6, 3; 3, 3, 3; 3, 4, 4; and 0, 7, 12, respectively. In the case of males, 19, 8, 12, and 19 subjects fall under the age group of 30-40 years, 41-50 years, 51-60 years, and above 60 years. The number of smokers, hypertensive, and diabetic males according to the above age groups is 10, 5, 4; 4, 4, 0; 4, 5, 3; and 4, 10, 5, respectively. In the total sample, the average cigarette smoking per day is 10-15 in males and 6-8 in females. The average blood pressure is 130/92 mm Hg in males and 128/86 mmHg in females. Glycosylated Hb (HbA1c) levels for males and females were 6.12 and 6, respectively. In this study, all samples were examined for any evidence of dyslipidemia and the values of critical lipid components, such as LDL, VLDL, LDL/HDL, triglyceride, total cholesterol was found within the normal limit.

2.3 Analysis of hypersensitivity of Lyfas scores

Testing the hypersensitivity of a novel instrument is an important step before it is applied

in the real-world scenario [12]. In this work, the authors tested the hypersensitivity, if any, existing in Lyfas by allowing each participant to take Lyfas tests thrice a day-7 am, 2 pm, and 10 pm. The CVb and ASI scores are noted at each time for all participants. Finally, variations in the mean score are computed and visualized (see Fig. 3 and Table 2).

2.4 ASI calculation from the SDPPG with Lyfas

PPG is a non-invasive optical method to measure the changes in the peripheral subcutaneous capillary blood circulation. The camera of the smartphone possesses a finger sensor consisting of a light-emitting diode (LED), which is usually Red or Infrared, and a photodetector (PD), and both the LED and PD remain on the other side of the finger. LED emits light and a small fraction of it is sensed by the PD according to the blood volume, flow, vascular wall movement, and the axial orientation of the RBCs within the vessels. Typically, a PPG signal consists of DC, AC, and noise components. The DC denotes the venous flow, while the AC is proportional to the deviation with the pulsatile BP and blood volume, synchronous to the HR. From the PPG signals, the noise is filtered and ASI is estimated. SDPPG is a method of estimation of ASI, proposed by Takazawa et al [13]. The SDPPG is analyzed by using the amplitudes of the distinctive waves 'a', 'b', 'c', 'd', and 'e', which are found in the cardiac systole in the cardiac cycle. The amplitudes or heights of the pulse waves are then normalized as 'b/a', 'c/a', 'd/a', and 'e/a'. The researchers observed that normalized amplitude 'b/a' increases and 'c/a', 'd/a', and 'e/a' are decreased in proportion to the increase in the subject's age. Based on such an observation, they formulated the 'arterial aging index' (AGI) parameter as the outcome of the equation '(b-c-d-e)/a' as a ratio. Using this principle, Lyfas can compute the AGI, which is synonymous with the ASI (range -0.2 to 0.5, i.e. the difference of 0.7 between the minimum and maximum values, mean difference of 0.35, any value beyond the range warrants the arterial investigation) as arterial 'aging' surrogates for the arterial 'stiffness'. The authors of this work have proposed that any value below and above the mentioned range warrants the arterial investigation. It is important to note that the authors followed the work of Chung, et al., where Framingham risk score (FRS) has been used for estimating the ASI [14]. Fig. 2 presents the schematic diagram of ASI estimation with Lyfas.

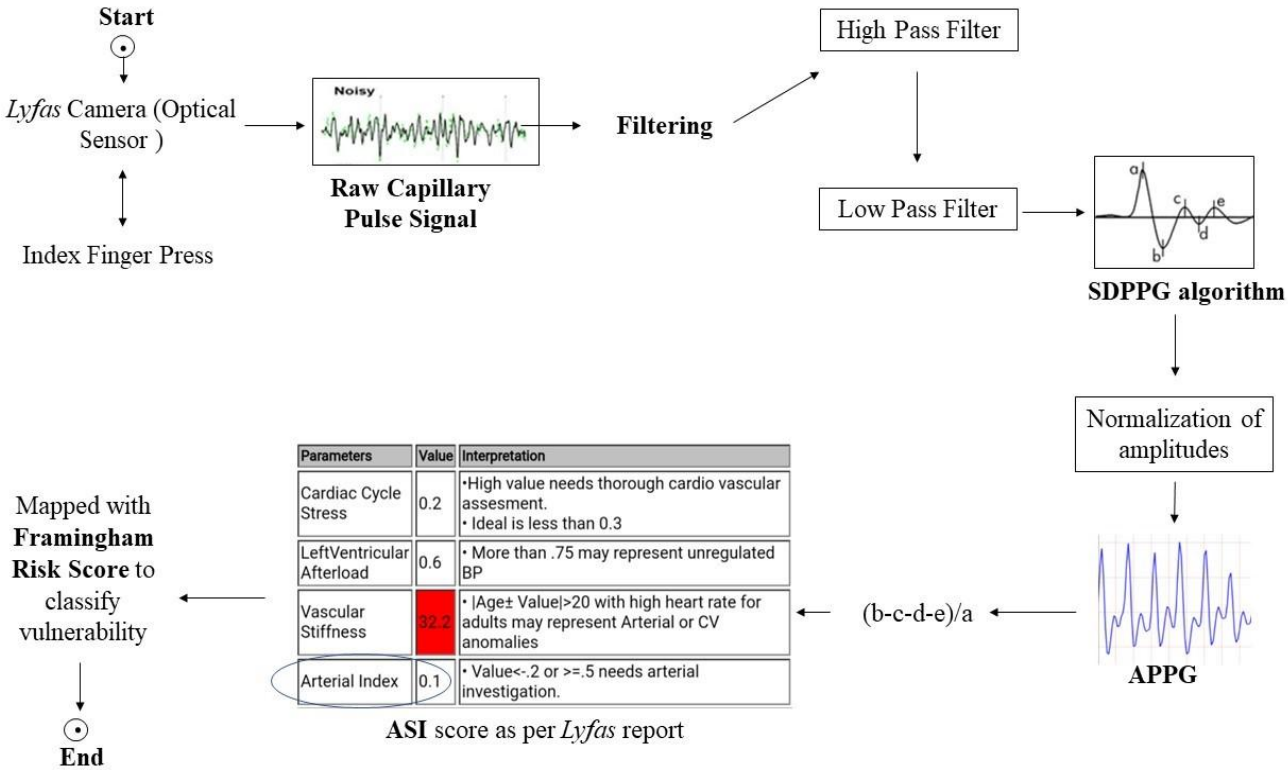


Figure 2: The schematic diagram of ASI calculation with Lyfas

2.5 HR, Age, and CVb measured with Lyfas

Heart Rate (HR, measured in beats per minute or bpm) is the most important vital sign of the body. The normal range is 60-100 bpm. A value <60 refers to bradycardia and indicates of high vagal tone, while values >100 denote tachycardia and are indicative of sympathetic drive [15]. Using the PPG technique, from the peripheral capillary arterioles, Lyfas can measure the pulse rate that reflects the HR of the individual.

Age in years, when the Lyfas test has been performed and the CVb is captured utilizing the principle of APPG are as follows [16]:

a.Time-domain components-

i.SDNN (estimated in milliseconds) denotes the standard deviation of NN intervals. It refers to the grade of cardiac risk as <40 high risks, 40-100 moderate risks, >100 no risks. The risk attributes to sympathetic dominance.

ii.pNN50 (estimated in %) denotes the percent of NN intervals >50 milliseconds. A value <20% indicates cardiac risk due to sympathetic dominance.

iii.RMSSD (estimated in milliseconds) denotes the root mean square of RR or NN interval difference. A value <54 indicates health risk and refers to sympathetic dominance.

b.Frequency-domain components-

i.LF/HF (estimated as a ratio) denotes the sympathovagal balance, respectively. A value >2 refers to cardiac risk due to sympathetic dominance.

c.Non-linear measure-

i.SD1/SD2 (estimated as a ratio) denotes the ratio of Poincare plot standard deviation perpendicular to the line of identity and denotes the sympathovagal balance. In other words, it is the ellipse fitted around the Poincare scatter plot. Standard deviation 1 (SD1) denotes short-term HRV, while Standard deviation 2 (SD2) refers to the long-term HRV. The Lyfas normal range is 1-2.5. Value >2.5 poses to be risky for the heart owing to sympathetic dominance.

It is important to note that

A. CVb or factors: SDNN, pNN50, RMSSD, HRV score, LF/HF, and SD1/SD2

B. Attributes: Smokers, Hypertensives, and Diabetics as per gender and age groups.

It is important to note that the *hypersensitivity* or *robustness* of Lyfas has been tested by taking three readings of CVb at 7 am, 2 pm, and 10 pm daily in both the genders to note any diurnal change in the CVb scores.

2.6 Statistical analysis

2.6.1 Descriptive statistics

It provides information on the dispersion of the data by calculating the mean, median, and standard deviations, and data count (refer to Table 1).

2.6.2 Linear regressions (LR) and One-way ANOVA

LR attempts to model the relationship between two variables by fitting a linear equation (Y = a+bX, where 'X' is the explanatory variable, 'b' is the coefficient value, 'a' is the constant, and 'Y' is the dependent variable) to the observed data.

One-way ANOVA tests whether there are any 'mean' differences between two or more unrelated groups and whether the differences are statistically significant. No mean difference confirms the null hypothesis, alternate hypothesis, otherwise. Significant CVb has been engineered to predict the ASI in the sample (refer to Fig. 4). The objective is to evaluate the significant biomarkers/factors according to the age groups, gender, and attributes.

2.6.3 Pearson's Interclass Correlations ('r', refer to Table 3)

It is a measure of association between ordered pairs of continuous measurement of any two significant groups or factors, obtained from the LR and is expressed in 'r'. Value of 'r' close to '+1' refers to positive correlation, close to '-1' denotes the negative correlation, values close to '0' refers to non-correlation.

3. Results and discussion

In this section, the results of the above statistical analysis are shown.

3.1 Descriptive statistics

Table 1: Age, gender, CVb, and attribute-wise Descriptive statistics

FEMALES (N=58)			30-40 years	41-50 years	51-60 years	>60 years
SDNN	Mean	Smoker (N=16)	44.54	40.57	53.3	-
		Hypertensive (N=20)	50.82	49.83	46.4	51.99
		Diabetic (N=22)	47.97	51.03	53.7	52.48
	Median	Smoker (N=16)	49.8	40.2	53.9	-
		Hypertensive (N=20)	51.3	49.8	48	52.4
		Diabetic (N=22)	49.7	52.7	53	55.15
	SD	Smoker (N=16)	12.44	1.19	6.42	-
		Hypertensive (N=20)	6.54	3.15	5.54	3.14
		Diabetic (N=22)	4.09	7.05	4.26	6.24
RMSSD	Mean	Smoker (N=16)	55.62	48.17	70.8	-
		Hypertensive (N=20)	67.07	66	59.2	76.81
		Diabetic (N=22)	63.63	62.63	66.15	65.66
	Median	Smoker (N=16)	56.05	46.9	71.1	-
		Hypertensive (N=20)	66.2	61.2	58.9	73
		Diabetic (N=22)	61.9	64.6	58.7	68.85
	SD	Smoker (N=16)	17.71	5.61	1.67	-
		Hypertensive (N=20)	15.79	10.46	10.92	10.18
		Diabetic (N=22)	14.18	4.76	18.39	14.65
HRV Score	Mean	Smoker (N=16)	79.24	77.43	85.2	-
		Hypertensive (N=20)	83.65	83.63	81.38	86.67
		Diabetic (N=22)	82.73	82.7	83.33	83.2
	Median	Smoker (N=16)	80.5	77	85.3	-
		Hypertensive (N=20)	83.8	82.3	81.4	85.8
		Diabetic (N=22)	82.5	83.4	81.4	84.6
	SD	Smoker (N=16)	7.51	2.28	0.46	-
		Hypertensive (N=20)	4.79	3.03	3.75	2.69
		Diabetic (N=22)	4.45	1.57	5.07	4.77
SD1/SD2	Mean	Smoker (N=16)	1.43	11.38	1.71	-
		Hypertensive (N=20)	1.21	2.1	2.86	1.34
		Diabetic (N=22)	2.14	1.73	2.2	2.17
	Median	Smoker (N=16)	1.335	3.2	1.19	-
		Hypertensive (N=20)	1.1	1.61	2.26	1.52
		Diabetic (N=22)	2.25	1.58	1.73	1.45
	SD	Smoker (N=16)	0.77	16	0.99	-
		Hypertensive (N=20)	0.40	1.12	2.31	0.49
		Diabetic (N=22)	0.30	0.62	1.48	1.51
LF/HF	Mean	Smoker (N=16)	1.29	1.4	1.4	-
		Hypertensive (N=20)	1.47	1.37	1.4	1.34
		Diabetic (N=22)	1.73	1.17	1.38	1.21
	Median	Smoker (N=16)	1.25	1.5	1.4	-
		Hypertensive (N=20)	1.5	1.3	1.45	1.2
		Diabetic (N=22)	1.8	0.8	1.05	1.3
	SD	Smoker (N=16)	0.30	0.26	0.4	-
		Hypertensive (N=20)	0.47	0.21	0.316228	0.42
		Diabetic (N=22)	0.40	0.72	0.83	0.42
HR	Mean	Smoker (N=16)	113.3	107.33	109.67	-
		Hypertensive (N=20)	111.83	114	111.25	110.43
		Diabetic (N=22)	108	121.33	104.5	117.5

	Median	Smoker (N=16)	108	108	106	-
		Hypertensive (N=20)	109.5	111	106	109
		Diabetic (N=22)	109	125	103.5	113
	SD	Smoker (N=16)	18.99	3.06	10.02	-
		Hypertensive (N=20)	9.35	7.94	13.33	9.24
		Diabetic (N=22)	6.56	12.90	2.38	15.28
ASI	Mean	Smoker (N=16)	***0.53***	0.1	0.57	-
		Hypertensive (N=20)	-0.67	***0.33***	0.13	***-0.01***
		Diabetic (N=22)	0.73	0.53	0.28	0.52
	Median	Smoker (N=16)	0.7	0.1	0.6	-
		Hypertensive (N=20)	0.35	0.3	0.35	0.1
		Diabetic (N=22)	0.6	0.7	0.5	0.5
	SD	Smoker (N=16)	0.76	0.7	0.06	-
		Hypertensive (N=20)	2.88	0.15	0.93	0.98
		Diabetic (N=22)	0.23	0.47	0.59	0.29
MALES (N=58)			**30-40 years**	**41-50 years**	**51-60 years**	**>60 years**
SDNN	Mean	Smoker (N=22)	52.2	51.93	46.3	44.18
		Hypertensive (N=24)	48.58	38	47.08	49.89
		Diabetic (N=12)	48.73	-	54.37	50.12
	Median	Smoker (N=22)	55.55	52.15	47.2	46.05
		Hypertensive (N=24)	52	35.4	51.8	48.25
		Diabetic (N=12)	52.6	-	51.9	48.9
	SD	Smoker (N=22)	10.56	10.42	10.8	5.93
		Hypertensive (N=24)	9.42	7.98	12.91	9.67
		Diabetic (N=12)	12.41	-	4.53	2.97
RMSSD	Mean	Smoker (N=22)	72.14	58.03	59.55	56.28
		Hypertensive (N=24)	62.78	47.5	58.32	60.41
		Diabetic (N=12)	63.43	-	72.43	71.02
	Median	Smoker (N=22)	76.15	55.5	58.75	53.5
		Hypertensive (N=24)	68.8	38.7	69.9	55.7
		Diabetic (N=12)	65.35	-	70.7	71.3
	SD	Smoker (N=22)	19.36	6.89	21.89	15.67
		Hypertensive (N=24)	15.88	21.21	22.57	17.55
		Diabetic (N=12)	21.46	-	4.65	2.24
HRV Score	Mean	Smoker (N=22)	84.63	81.1	80.68	80.05
		Hypertensive (N=24)	82.22	75.95	79.9	81.27
		Diabetic (N=12)	82.03	-	85.67	85.24
	Median	Smoker (N=22)	86.6	80.3	81.1	79.45
		Hypertensive (N=24)	84.6	73.05	84.9	80.35
		Diabetic (N=12)	83.45	-	85.2	85.3
	SD	Smoker (N=22)	7.18	2.24	7.66	5.48
		Hypertensive (N=24)	5.35	7.85	8.64	5.86
		Diabetic (N=12)	7.48	-	1.27	0.61
SD1/SD2	Mean	Smoker (N=22)	1.60	1.58	1.26	1.35
		Hypertensive (N=24)	1.73	1.68	1.23	2.08
		Diabetic (N=12)	1.04	-	3.50	2.3
	Median	Smoker (N=22)	1.23	1.61	1.26	1.31
		Hypertensive (N=24)	1.27	1.26	1.23	1.15
		Diabetic (N=12)	0.88	-	3.94	2.06

	SD	Smoker (N=22)	1.1	0.87	0.27	0.27
		Hypertensive (N=24)	1.04	1.36	1.23	2.95
		Diabetic (N=12)	0.48	-	1.8	0.56
LF/HF	Mean	Smoker (N=22)	1.58	1.08	1.33	1
		Hypertensive (N=24)	1.24	1.58	1.18	1.22
		Diabetic (N=12)	1.45	-	1.17	2.26
	Median	Smoker (N=22)	1.35	0.9	1.3	1
		Hypertensive (N=24)	1.4	1.6	1.1	1.25
		Diabetic (N=12)	1.5	-	1	2.2
	SD	Smoker (N=22)	0.72	0.5	0.29	0.33
		Hypertensive (N=24)	0.38	0.22	0.41	0.51
		Diabetic (N=12)	0.3	-	0.38	0.90
HR	Mean	Smoker (N=22)	113.63	108.5	123	110.5
		Hypertensive (N=24)	111.6	105.75	113	112.7
		Diabetic (N=12)	122.75	-	116	123.4
	Median	Smoker (N=22)	110	108	116.5	109
		Hypertensive (N=24)	105	106	116	112
		Diabetic (N=12)	120	-	123	134
	SD	Smoker (N=22)	11.87	7	24.18	3.79
		Hypertensive (N=24)	13.05	4.03	6.25	11.58
		Diabetic (N=12)	17.73	-	13	16.04
ASI	Mean	Smoker (N=22)	***0.61***	***0.6***	0.3	0.25
		Hypertensive (N=24)	0.66	***0.3***	0.22	0.34
		Diabetic (N=12)	-1.1	-	0.33	0.28
	Median	Smoker (N=22)	0.6	0.55	0.35	0.35
		Hypertensive (N=24)	0.8	0.3	0.4	0.4
		Diabetic (N=12)	0.05	-	0.2	-0.1
	SD	Smoker (N=22)	0.24	0.14	0.65	0.24
		Hypertensive (N=24)	0.45	0.26	0.54	0.42
		Diabetic (N=12)	2.96	-	0.23	1.22

In the above table, female smokers in the age-group 30-40 years, hypertensives in the age-group 41-50 and >60 years are in high risk of vascular health. Male smokers in the age-group 30-40 and 41-50 years and hypertensives in the age-group 41-50 years are at risk of vascular health (shown in bold and italics font).

3.2 Hypersensitivity/Robustness analysis:

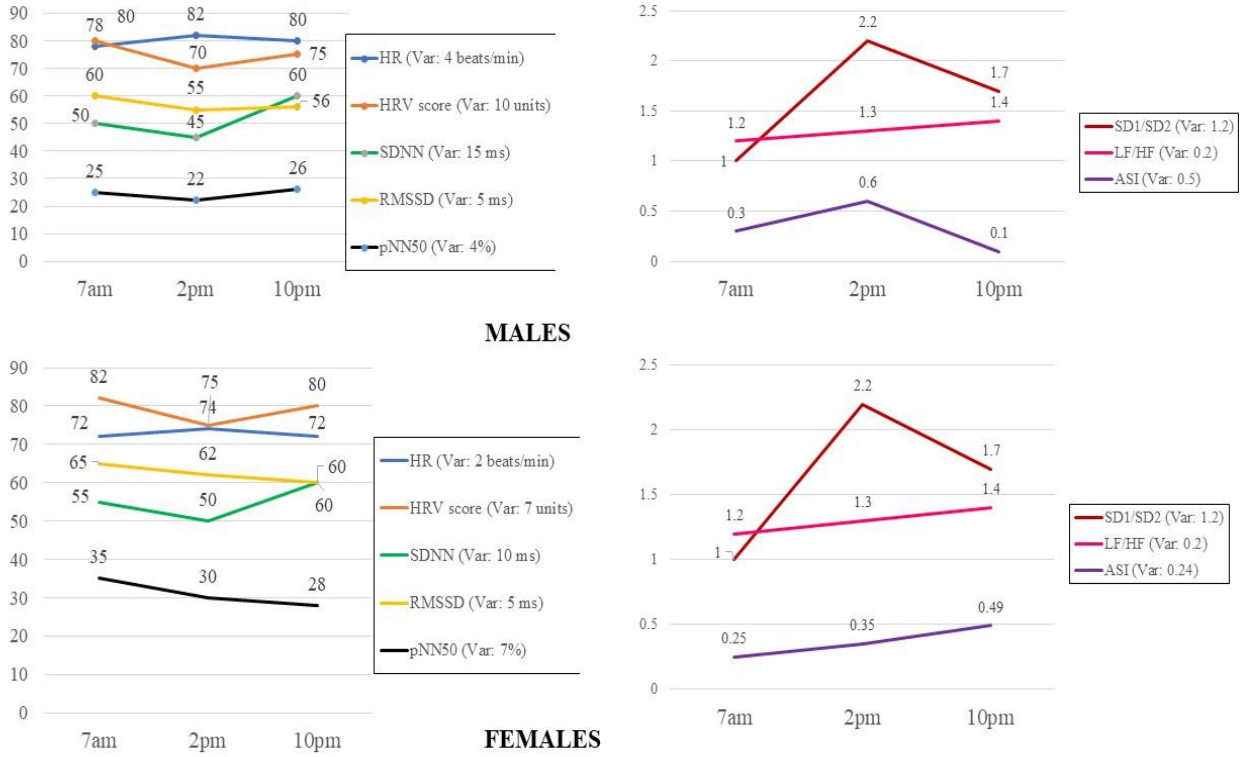


Figure 3: showcases the line plots for the hypersensitivity analysis

Table 2: The mean-deviation of CVb and ASI as the assessor of Lyfas hypersensitivity/robustness

FACTORS	MALE			FEMALE		
	Mean	Range	Variance	Mean	Range	Variance
HR	80	4	2 (7am) 2 (2pm) 0 (10pm)	72.67	2	0.67 (7am) 1.33 (2pm) 0.67 (10pm)
HRV score	75	10	5 (7am) 5 (2pm) 0 (10pm)	79	7	3 (7am) 4 (2pm) 1 (10pm)
SDNN	51.67	15	1.67 (7am) 6.67 (2pm) 8.33(10pm)	55	10	0 (7am) 5 (2pm) 5 (10pm)
RMSSD	57	5	3 (7am) 2 (2pm) 1 (10pm)	62.33	5	2.67 (7am) 0.33 (2pm) 2.33 (10pm)
pNN50	24.33	4	0.67 (7am) 2.33 (2pm) 1.67 (10pm)	31	7	4 (7am) 1 (2pm) 3 (10pm)
SD1/SD2	1.63	1.2	0.63 (7am) 0.57 (2pm) 0.07 (10pm)	1.63	1.2	0.63 (7am) 0.57 (2pm) 0.07 (10pm)
LF/HF	1.3	0.2	0.1 (7am) 0 (2pm) 0.1 (10pm)	1.3	0.2	0.1 (7am) 0 (2pm) 0.1 (10pm)
ASI	0.33	0.5	0.03 (7am) 0.27 (2pm) 0.23(10pm)	0.36	0.24	0.11 (7am) 0.01 (2pm) 0.13 (10pm)

The above Table 2 and Fig. 3 show that there is no variation in the diurnal Lyfas scores in both sexes. It indicates that Lyfas is a robust instrument, i.e., not hypersensitive.

3.3 LR and ANOVA study:

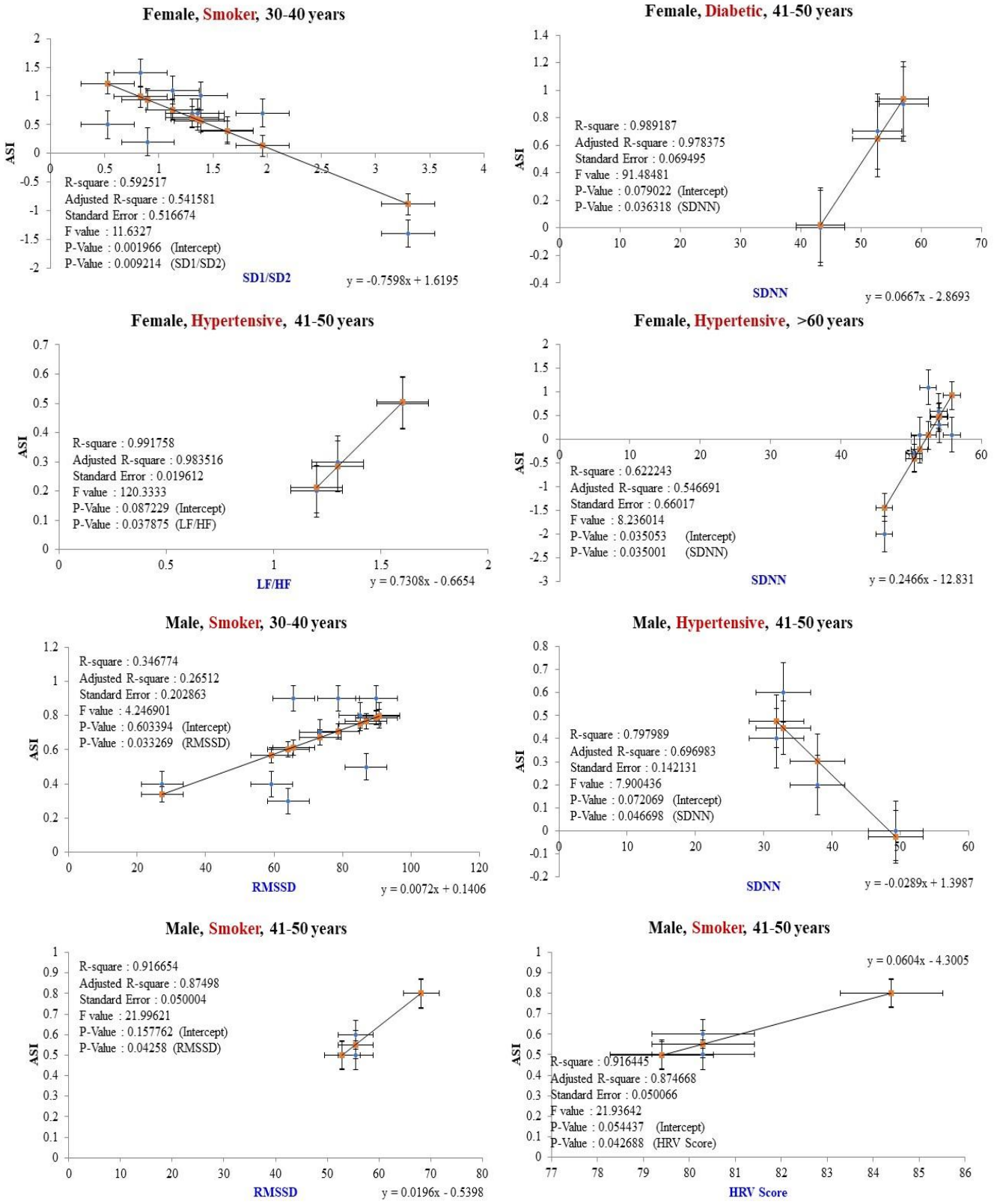


Figure 4: Significant CVb through LR and ANOVA predicting ASI in females and males

3.4 PIC 'r' values:

Table 3. Pearson's correlation among the 'significant' factors

No.	Gender	Age group	Attribute	Factor-1	Factor-2	'r'	Interpretation
1.	F	30-40	Smoker	SD1/SD2	ASI	-0.77	High vascular risk
2.	M	30-40	Smoker	RMSSD	ASI	+0.59	Low vascular risk
3.	F	41-50	Hypertensive	SDNN	ASI	-0.98	High vascular risk
4.	F	41-50	Hypertensive	LF/HF	ASI	+0.99	High vascular risk
5.	M	41-50	Hypertensive	SDNN	ASI	-0.89	High vascular risk
6.	M	41-50	Smoker	RMSSD	ASI	+0.96	High vascular risk
7.	M	41-50	Smoker	HRV score	ASI	+0.96	High vascular risk
8.	F	>60	Hypertensive	SDNN	ASI	+0.79	High vascular risk

Fig. 4 and Table 3 show the statistical correlation between the significant CVb and ASI obtained by Lyfas, gender and age group-wise. In females, SDNN, SD1/SD2, LF/HF, and in males, RMSSD, HRV score and SDNN have proved to be relevant biomarkers for screening vascular risks.It is evident that except for male smokers within the age group of 30-40 [24], all show high vascular risks.

Testing hypersensitivity is an important measure to assess the robustness of any novel application/tool/instrument [12]. In this study, the authors measured the hypersensitivity of Lyfas by noting any existing diurnal variations in the CVb and ASI scores as too much sensitivity is not a very good quality of a novel measuring instrument. The authors found that, throughout the day for all the subjects, the scores of CVb and ASI remain within the normal range and there is no major deviation from the mean scores. For the three tests, HR with mean 80 (M) and 72.67 (F) showed a variation of fewer than 2 beats/min; HRV Score with mean 75 (M) and 79 (F) showed a variation of less than equal to 5 units; SDNN with mean 51.66 (M) and 55 (F) showed a variation of around and less than 8 ms; RMSSD with mean 57 (M) and 62.33 (F) showed a variation of less than equal to 3 ms; pNN50 with mean 24.33 (M) and 31(F) showed a variation of less than equal to 1% units in males and less than equal to 4% units in females; SD1/SD2 with mean 1.63 (M) and 1.63 (F) showed a variation of less than 1; LF/HF with mean 1.3 (M) and 1.3 (F) showed a maximum variation of 0.1; ASI with mean 0.33 (M) and 0.36 (F) showed a variation of less than or around 0.2 units.

In female hypertensives-

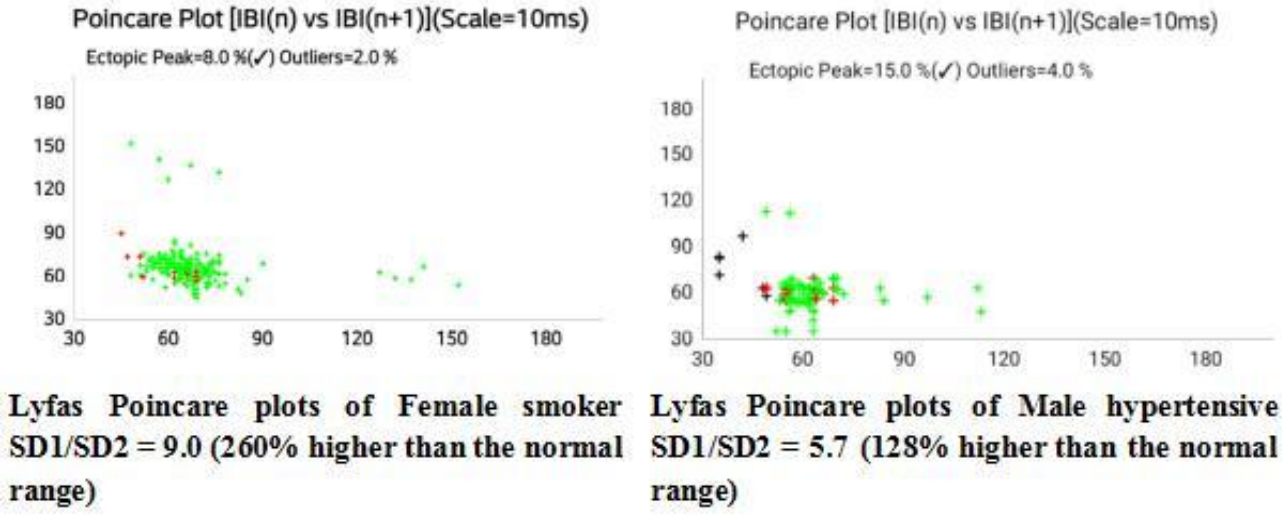
i) SDNN (mean 51.99 ms) for >60 yrs is correlated positively (r=0.79) with the ASI (mean -0.01, max value 1.1, median 0.1, and the standard deviation 0.98). SDNN is a CVb for the state of cardiac health and the mean value refers to the moderate risk as mentioned in section '2.5 a(i)'. Studies have established that menopausal or perimenopausal women have higher cardiac risk due to the natural depletion of estrogen [17]. Females also show a greater refractory effect of antihypertensive agents. Anxiety disorders, psychosis, and dementia are also predominant in this age group due to estrogen attenuation [17]. As the cognitive ability declines, BP increases, irrespective of the extent of arterial stiffness at the very initial stage of the illness [18]. Later in their life, the intimal endothelial function declines due to the lack of estrogen stimulation of nitric oxide (NO) synthesis, which dilates the vessels [17]. In this work, that is why the cardiac risk is still lying moderately high despite the Lyfas ASI mean score falling within the normal limit.

ii) SDNN (mean 49.83 ms) for 41-50 yrs is correlated negatively (r=-0.98) with ASI (mean 0.33, max value 0.5, median 0.3, and the standard deviation 0.15). In this case, as well, the mean of CVb SDNN value lies in the range of moderate risk as in the first case. Perimenopausal women start witnessing the decline in estrogen levels [17] leading to decreasing SDNN values (moving closer to the critical value of 40 ms, below which lies the risk of sympathetic dominance and hence the CVD risk). Studies have witnessed that women who are in the transitioning phase through menopause have greater arterial stiffness (attributed to another inflammation-like phenomenon) when compared to premenopausal and postmenopausal women [19]. This is also evident in the ASI value for a female being significantly more and abnormal for 41-50 years women than >60 years women in this work.

iii) LF/HF (mean 1.37) for 41-50 yrs is correlated positively (r=0.99) with the ASI (mean 0.33, max value 0.5, median 0.3, and the standard deviation 0.15). LF/HF is a CVb to gauge sympathovagal balance and the mean value being closer to 2 depicts the progression of cardiac risk as mentioned in section '2.5b(i)'. Perimenopausal women experience increased sympathetic activity (and reducing parasympathetic activity) due to endocrinological, somatic, and psychological alterations [19]. Studies have proven that estrogen protects from atherosclerosis which is the reason behind a lower number of cases of CVD in women than in men in the reproductive age groups [17]. However, with reducing levels of estrogen starting from the perimenopausal phase, the sympathetic branch starts becoming dominant as is evident from the higher value of mean LF/HF (although it remains in the range) responsible for the onset of AS and vasoconstriction as can be seen in ASI mean, max and median values lying very close to the upper limit of the normal range.

In female smokers-

i) SD1/SD2 (mean 1.43) for 30-40 yrs is correlated negatively (r=-0.77) with ASI (mean 0.53, max value 1.4, median 0.7, and the standard deviation 0.76). SD1 and SD2 ratio from the Poincare Plot denotes a snapshot of HRV (refer to Fig. 5) and hence, sympathovagal balance, and thereby depicting the vasoconstriction or vasodilation and the mean value is within the normal range as mentioned in section '2.5c(i)'.



Lyfas Poincare plots of Female smoker SD1/SD2 = 9.0 (260% higher than the normal range)

Lyfas Poincare plots of Male hypertensive SD1/SD2 = 5.7 (128% higher than the normal range)

Figure 5: Sample Poincare plots with high SD1/SD2 from the Lyfas report

Studies have shown that smoking causes tachycardia and increases BP, due to arterial stiffness, which then acts through baroreflexes causing sympathetic inhibition [20]. Moreover, sympathetic nerve supply (SNS) levels remain stable during the menstrual phases in habitual smokers, unlike the normal days of fluctuating SNS. This non-fluctuation, if consistent, poses a greater risk of cardiac issues due to dysregulation of CVb in female smokers [21]. This is characterized by impaired baroreflex suppression of SNS and/or increased central responses due to the hemodynamic changes [20]. It is evident in the SD1/SD2 value being in range, showing the sympathetic impairment, in this work even though the ASI mean and max values depict the evidence of AS.

In male hypertensives-

i) SDNN (mean 38 ms) for 41-50 yrs is correlated negatively (r=-0.89) with ASI (mean 0.3, max value 0.5, median 0.3, and the standard deviation 0.26). Mean SDNN refers to high risk as mentioned in section 2.5a(i). Studies have found that low testosterone levels in middle-aged men, unlike women, are associated with increased AS as is witnessed in this work of Vlachopoulos et al. as well [22]. Men, unlike women, exhibit a linear stiffness and cardiovascular risk [23]. However, in hypertensive men, due to more sympathetic activation, increased AS (due to declining testosterone) interplays with sympathetic dominance (due to coexisting hypertension) [23]

evident from the SDNN mean value below the critical value of 40 ms in this work.

In male smokers - ii) RMSSD (mean 72.14 ms) for 30-40 yrs is correlated positively (r=+0.59) with ASI (mean 0.61, max value 0.9, median 0.6, and the standard deviation 0.24). RMSSD is a CVb and the mean value is significantly above the cut-off value below which sympathetic dominance becomes a risk. Studies have established that smoking significantly decreases the arterial distensibility, thereby, suppressing the mechanoreceptors, and causing AS, as an attempt to maintain the homeostasis in them [24]. However, till the baroreceptors' sensitivity is not impaired, there is sympathetic inhibition, as was seen in the case of female smokers as well [24]. This is evident by normal range values of RMSSD even though the ASI mean, max, and median values corroborate with the presence of AS. In the age group 30-40 years, though, there is a lower correlation, smoking in this age group poses lower cardiovascular risks even with a significant amount of AS caused by smoking.

iii) RMSSD (mean 58.03 ms) for 41-50 yrs is correlated positively (r=+0.96) with ASI (mean 0.6, max value 0.8, median 0.55, and the standard deviation 0.14). With the increasing age, the RMSSD mean has moved closer to the critical value of 54 ms as mentioned in section '2a(iii)', depicting the distribution moving towards sympathetic dominance. Also, the correlation is very strong between RMSSD and the ASI in this age group in male smokers. This is established by studies that, smoking causes sympathetic inhibition due to baroreceptors' reactivity initially, but with age or time or usage, as the baroreceptors' sensitivity gets impaired, the baroreflex regulation of the CVb also gets impaired leading to sympathetic dominance [24]. Hence, in this work, it is evident that with the increasing age group, AS develops as a consequence of the slow but seamless process of autonomic impairment, which is principally due to the baroreceptors impairment and hence, manifolds the cardiovascular risks.

iv) HRV (mean 81.1) score for 41-50 yrs is correlated positively (r=+0.96) with ASI (mean 0.6, max value 0.8, median 0.55, and the standard deviation 0.14). HRV score is a CVb and the mean is at the borderline of critical value, the values below which show autonomic dysfunction. Studies have found a complex interrelationship between smoking and HRV. Smoking induces AS is universally established in studies, however, its relation with HRV is found to be much complex [25]. HRV results, according to some studies backed by statistical analysis, are normally found to be lower in smokers than non-smokers, while other studies do not show this pattern. These complex dynamics have been attributed to differential mediation mechanisms of several types of nicotine acetylcholine receptors (nAChRs), which are initially activated and then desensitized by nicotine from smoking, regulating either the sympathetic or parasympathetic or both at the ganglionic level [26]. Smoking, hence, impacts the AS in this age group and it is well-evident by the ASI mean score in this research and has a strong positive correlation with HRV, but the HRV mean score and the 'r' value are indicative of the score increasing further with ASI. Hence, it is important to consider RMSSD along with HRV for this age group of male smokers to get a clearer picture of the cardiovascular risk.

Fig. 6 shows the APPG signals of each significant attribute and gender-wise with the respective ASI scores, below.

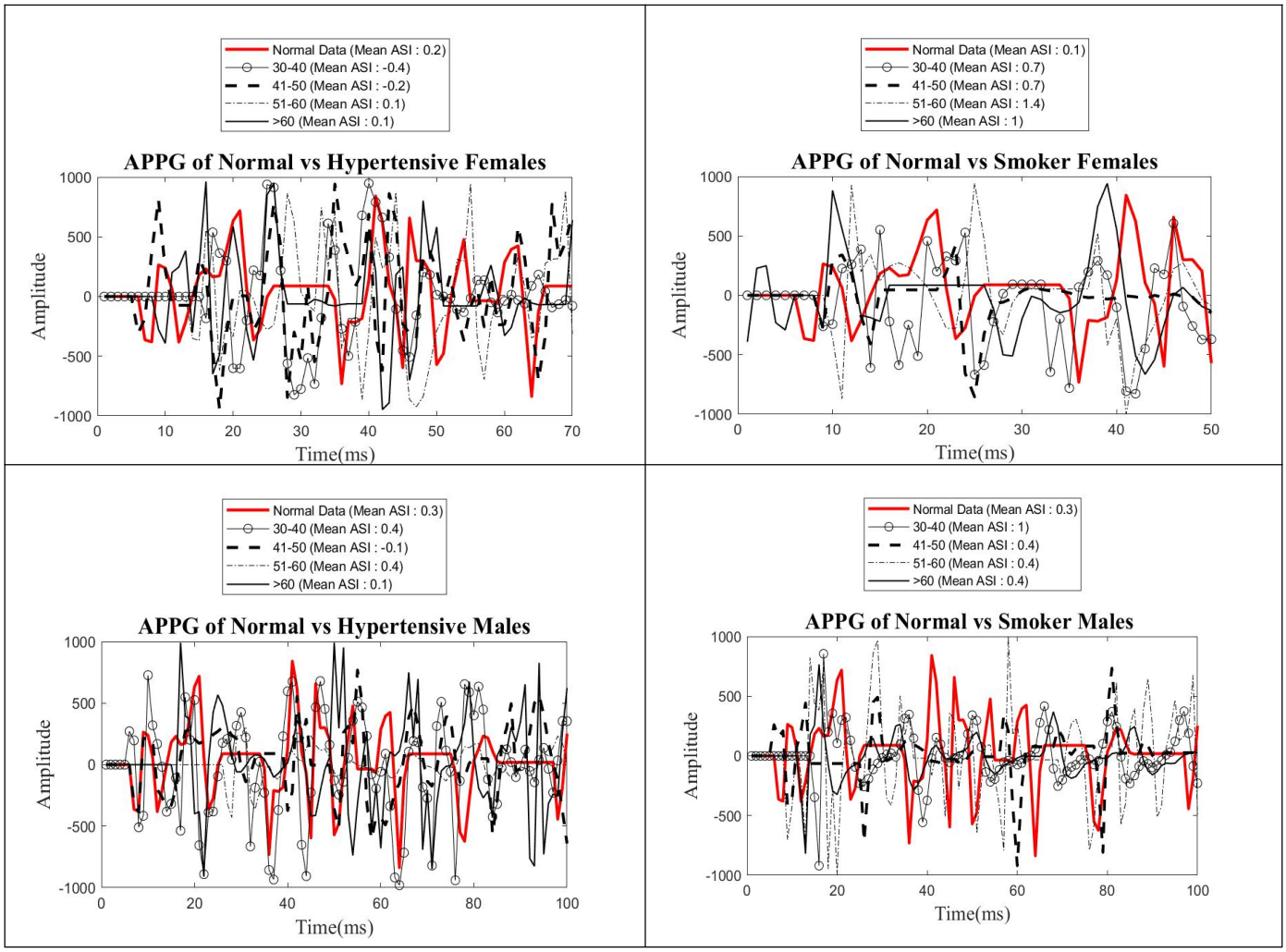


Figure 6: APPG signal plots of Normal vs. 'significant' groups (attaribute, age, and gender)

Thus, hypertension and smoking, independently, have a strong correlation with the progression of disturbed autonomic nervous system interplay, thereby, posing cardiovascular risks due to non-regulation of the cardiovascular biomarkers.

It is interesting to note that, statistically there was no significant correlation observed between the CVb values and respective ASI for both males and females in this study. For the age group of 30-40 years in males and females, mean ASI is -1.1 and 0.73 respectively, which are beyond the normal range and require clinical attention as per the Lyfas score. In 41-50 years, the mean ASI for females is 0.53, which is slightly higher than that of the upper cut-off values, while males present with the normal mean ASI scores. In the case of 51-60 years of age groups in both genders, the ASI falls within normal limits. Finally, in the age group >60 years, females present with abnormal ASI scores (mean 0.52, slightly higher than that of the upper cut-off values). Therefore, the early age group diabetics in both genders show abnormal ASI in the sample, although the CVb is not able to directly correlate with this finding statistically.

To explain the above findings, the authors propose that insulin resistance, found in diabetes, is associated with a higher basal metabolic rate (BMR) to cater to higher energy in metabolizing an increased amount of glucose in the body [27]. High BMR mandates sympathetic dominance [28], which could be evaluated by a high LF/HF score, indicating compromised cardiovascular health. However, such a dominance remains transient at the beginning of the illness due to the compensatory mechanism inducing a normal LF/HF for homeostasis. A study shows that Type 1 Diabetes has much more significantly profound cardiovascular implications than Type 2 Diabetes

[29]. Moreover, Type 1 Diabetes with AS (vasculopathy) may result from many pathways like hypertension, chronic endothelial inflammation, or nephropathy [29] and these factors cause differential pathophysiological cardiovascular risks [30]. Pittsburgh Epidemiology of Diabetes Complications (EDC) Study has found that duration of diabetes, nephropathy (impaired renal function), vasculopathy have cardiovascular implications [29] and the presence of these risk factors along with diabetes, rather than independently persisting diabetes, cause a synergistic impact on cardiovascular health. CVD is found to be associated with increased age and BMI [31]. However, few studies have quantitatively correlated the effect of age on the prevalence of CVD along with Type 2 Diabetes [32].

CVb is reflective of sympathovagal balance, as the body inherently attempts to compensate for underlying pathophysiology. It is important to conceive that these are not direct indicators.

The above observation corroborates the fact that statistical measures often fail to describe the science behind human pathophysiology, especially, a complex multiorgan metabolic disorder such as diabetes.

Recently, Lyfas has been tested against the gold-standard Polar H10 HRV electrical sensor and Kubio software in measuring HR, HRV, and CVB in healthy adult males and females, respectively with the recall of (84%, 70%); specificity (95%, 87%); accuracy (82%, 79%), precision (87%, 69%), fscore (1.4836, 1.3706), and Youden's index (79%, 81%) for HR, SDNN, RMSSD, and pNN50, respectively [33].

4. Conclusions

The paper is an attempt to correlate the CVb, captured by Lyfas with that of the 'Lyfas ASI' scores using SDPPG algorithm and FRS to evaluate the CVD risk in the smoker, hypertensive, and diabetic males and females of various vulnerable age groups. The gist of the study observes that (a) Lyfas is not a hypersensitive instrument as diurnal variations are aligned to its biomarker ranges and thus, it is a reliable instrument; (b) Male smokers and female hypertensives at all age groups are much vulnerable to AS and its related cardiovascular consequences. Although the present work has considered attributes as mutually exclusive, the study proposes that a combination of smoking and hypertension could induce an early cardiovascular risk at a greater severity due to accelerated AS [34]. Pathological ASI scores in diabetic subjects show non-correlation with the CVb scores such as LF/HF, although the vulnerability prevails, which may be attributed to autonomic compensatory mechanisms to prevent vascular damage at a very early stage of the disease [35]. Lyfas ASI correlation with CVb is a novel approach to screen the chances of early vasculopathy in smokers, diabetics, and hypertensives, and monitor its course of progression with the CVb biomarkers. Other advantages lying with Lyfas are its pervasiveness, user-friendly approach, low-cost technique, and interpretation of biological age by assessing the vascular health (e.g., ASI) using its proprietary heuristic algorithm.

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