Validating A Smartphone-based Biomarker Instrument Lyfas CovidAnxiety Detector (LAD) in the Indian Population

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Abstract: **Mind-heart-body is tightly intertwined. Anxiety is one of the most prevalent mental health issues during the COVID pandemic. Lyfas is a smartphone-based biomarker instrument that has been used as a CovidAnxiety Detector (LAD) where SD2/SD1 has been considered the key optical biomarker of anxiety and its other adverse health consequences. The objective of this study is to validate the efficacy of LAD with that of two gold-standard anxiety rating instruments – (i) 7-item Generalized Anxiety Disorder (GAD-7) and (ii) 14-item Hamilton Anxiety Rating Scale (HAM-A). A total of 1454 Indian adults (Male 955, Female 499) within the age group of 18-87 years (median and average age of 39.56 and 37 years respectively), divided into three age groups: 18-30 years, 31-50 years, and >50 years who were RTPCR positive took (i) GAD-7, (ii) HAM-A, and (iii) Lyfas tests daily till they became RTPCR negative (average span of 14 days). Internal consistency (α), Spearman's rank correlation (r), Classification metric - sensitivity, specificity, precision, accuracy, fscore, and Youden's index of LAD has been computed and finally, the inter-rater reliability between (a) LAD and GAD-7 and (b) LAD and HAM-A is measured with Bland-Altman Reliability Assessment. LAD shows high inter-rater reliability (i.e., the minimum proportional bias) around the mean difference with the Bland-Altman test, good-to-excellent internal consistency (>0.8 and >0.9, respectively), and high correlations (r>0.6) of LAD with GAD-7 and HAM-A in the 18-30 years age group. LAD shows high average accuracy (0.9953 and 0.9764 in males and females respectively), precision (>0.9), and Youden's index (0.9976 and 0.8633), for males and females. Hence, LAD is a reliable and novel smartphone-based non-invasive and ubiquitous biomarker clinical instrument to screen and monitor GAD and its adverse consequences such as sudden cardiac death and suicide in adults, especially in the ongoing pandemic situations.**

Keywords: **COVID-19, Lyfas CovidAnxiety Detector, Generalized Anxiety Disorder, Hamilton Anxiety rating scale, Sudden cardiac death, Suicide.**

I. INTRODUCTION

GAD comes under neurotic disorder along with phobia and is the most common mental health issue globally with a prevalence rate of 5.8% in the Indian population (1). Here, irrespective of any untoward incidence, patients feel extreme threat and apprehension of catastrophe, which is uncontrollable and thus abruptly brings down the quality of life. Researchers have found that GAD has certain types of personality traits (endophenotype of pathological worries, neuroticism, and frightfulness due to uncertainties) and hence genetic linkage, such as 5-HTT, 5-HT1A, BDNF, MAOA known as 'the anxious gene' (2) along with the neurochemicals has given it multilevel pathophysiological cascading

effects in the neurocognitive process of the brain (3), where the connections between the prefrontal cortex and amygdala are distorted leading to amygdala-driven fight or flight reactive behavior to even a trivial stimulus and very less cognitive involvement in rationalizing (4). There are silent traits as well, as the authors envisage and such traits are unveiled when any environmental challenge is faced, which has happened during the COVID pandemic (5). It is pertinent to state that T-type Ca^{++} channels (low-voltage-activated Ca^{++} channels are highly expressed in the stress-responsive regions of the brain and thus modulate the generalized anxiety states (6). Such deep-rooted pathophysiology proves why anxiety disorders are not only the commonest neurotic disorder but also are obstinate types, clinically.

As the mind-heart-body is tightly interlinked, GAD has several adverse health outcomes in the form of cardiometabolic (7) (8) and mental (9). During the pandemic time, such risks are quite high.

To address the challenges in catering to healthcare facilities during a pandemic-induced lockdown of nearly 12 weeks, Govt. of India legalized telemedicine with certain protocols, rolled-out for its safe practice, with a special note on protecting the privacy of the patients' data (10). Mobile health (mHealth), such as Lyfas took a few steps ahead to detect the mind-body homeostasis of the body with its AI-ML-based analytics and visualization, abreast protecting patients' privacy (11). Just by pressing the index finger on the rear main camera of the smartphone the help-seekers, using the arterial photoplethysmography (APPG) (12) and photochromatography (PCG) methods, can capture several optical biomarkers that surrogate for the cardiovascular autonomic modulation, which occurs due to 'errors' in the physiological processes that maintain the mind-body homeostasis. Lyfas application has been pre-installed in patients' smartphones and with a multilingual voice-assisted prompt, the test takers can easily complete the test and generate the analytics report with meaningful graphical representations of their mind-body state, which can be evaluated by the trained doctors to provide medical management support during the pandemic period. It is worth mentioning that recently Lyfas has been applied to screen sub-clinical depression with its further effects in generating negative thoughts and insomnia (13). It is, therefore hypothesized that Lyfas poses to be a useful instrument to evaluate the mental state of the test-takers by assessing their sympathovagal balance by examining the optical biomarker scores (SD1/SD2), which have been then corroborated with the history of the test-takers and during the series of average ten counseling sessions per GAD patient.

The *objective* of this work is to validate the reliability of Lyfas as a GAD detecting instrument to two well-acclaimed gold standard tools, such as (i) Generalized Anxiety Disorder (GAD-7) (14) and (ii) Hamilton Anxiety Rating Scales (HAM-A) (15).

II.   MATERIALS AND METHODS

Ethical measures

a. The study protocol was approved by the *Vagas Institutional Ethics Committee* review board (No. ECR/1181/Inst/KA 2019, dated 30-01-2020).

b. *Signed informed consents* of all participants' have been taken according to the declaration of Helsinki by the research team prior test.

Online test platform

An IoT-enabled online platform has been created for visitors who want to take anxiety tests (16). The platform provides health information related to anxiety disorders in line with the Diagnostic and Statistical Manual (DSM)-V version (17).

Population selection

A total of 1454 Indian adult participants (Male 955, Female 499) within the age group of 18-87 years (median age 37 and average age 39.56 years), divided into three groups: 18-30 (Male: 342, Female: 176); 31-50 (Male: 406, Female: 195), and >50 years (Male: 207, Female 128) who were RTPCR positive and home-quarantined. All are drug-naïve, i.e., not under any anxiolytics or selective serotonin reuptake inhibitor (SSRI), or other mood elevators. Lyfas tests were taken thrice daily till they became RTPCR negative with an average monitoring period of 14 days. None of them have been hospitalized or developed any acute symptoms.

Anxiety instrument selection

The study hypothesizes that 'anxiety' is a common state of human emotion (18), with an estimated global prevalence rate of 7.3% in the population (19). People having its trait develop the 'disorder' with time and for no obvious or any specific

reason, they suffer from a high grade of anxiety, which has been evident during the COVID period, especially during the roll-out of the vaccination process (20). This population is over-apprehensive about anything and always thinks of some forms of imminent disaster that may occur in their lives at any time (21). On the other hand, people who do not have such a trait are not found worried at all and adapted to it (19). Thus, to capture the population with that trait (as COVID might have unmasked it) and to get a more holistic picture of growing generalized anxiety in the population, COVID-specific anxiety scales or instruments are not considered in the study as these might restrict the context. In other words, the authors have used COVID as a measure to unveil the generalized anxiety disorder trait in the vulnerable population.

A systematic search has been conducted to learn about several available anxiety rating scales in Psychiatry.org, Google Scholar, Medline, Medscape, Pubmed, and PsychInfo using the keywords such as anxiety rating scale, anxiety tool, anxiety instrument, and gold standard anxiety rating tools. Among 25 scales and 529 items, two gold standard and well-acclaimed instruments, such as a (i) 7-item Generalized Anxiety Disorder (GAD-7) (14) and the (ii) 14-item Hamilton Anxiety Rating Scale (HAM-A) (15) have been chosen, based on the advice of three senior psychiatrists who has hands-on experience in using these rating scales since years of their practicing life.

### GAD-7 and HAM-A: Two well-acclaimed instruments (14) (15)

GAD-7 is a well-acclaimed popular self-administered instrument to screen and grade GAD in adults. It explores the anxiety state over the past two weeks by asking 7 questions, each having a 4-point rating. Based on the threshold score of 10, GAD-7 has 89% sensitivity and 82% specificity. Apart from GAD, it can screen for three other most common mental illnesses, such as panic disorder (74% sensitivity and 81% specificity), social anxiety disorder (72% sensitivity and 80% specificity), and post-traumatic stress disorder (66% sensitivity and 81% specificity) in adults (22).

HAM-A is an anxiety rating scale for children, adolescents, adults, and elderlies and is one of the earliest scales available for clinical practice and trials (research). It consists of 14-items to capture the rate (0-4 points) of psychic i.e., mental agitation and psychological distress, and somatic i.e., the physical effects of those psychic conditions. The advantage of HAM-A is that it is a well-acclaimed gold standard GAD rating instrument with an administration time of 10-15 minutes with a high degree of inter-rater reliability. The disadvantage of this rating instrument is that it is poor in differentiating the somatic anxiety vs somatic side effects of antidepressive and anxiolytic drugs and does not possess any standardized probe question.

Lyfas CovidAnxiety Detector (LAD): A novel smartphone-based biomarker instrument

Lyfas is a commercially available smartphone-based biomarker instrument that utilizes the principle of arterial photoplethysmography (APPG) (23). From the index finger capillary when the finger is gently kept on the rear main camera of the smartphone with the LED light 'ON', Lyfas, using its proprietary algorithm, captures the pulse rate variability (PRV) that surrogates for the heart rate variability (HRV) and its correlated optical biomarkers, which in turn refers to the state of cardiovascular autonomic modulation in maintaining the mind-body homeostasis (23). Lyfas measures the short HRV (120 seconds) and has been validated against Polar H10 gold standard biomedical instrument with 79% and 81% of Youden's indices in males and females, respectively (24), and 93.53% inter-rater reliability on Bland-Altman's test (25). It has also been stringently tested for its circadian hypersensitivity and found to be not hypersensitive and therefore can be called robust (26). The SD2/SD1 HRV optical biomarker score refers to the level of anxiety in a population that Lyfas captures alongside other PRV correlates. Chronic anxiety results in sympathetic hyperactivity through Multiunit Efferent Muscle Sympathetic Nerve Activity (MSNA) (27), which is echoed in the PRV, and the SD2/SD1 scores are the crucial optical biomarkers that quantify the extent of sympathovagal balance, where a higher value of the ratio argues for the sympathetic dominance (23). SD1 measures the diastolic blood pressure, the difference between the maximum and minimum heart rate, and other optical biomarkers such as RMSSD, pNN50, SDNN, the power in the LF and HF bands, and total power during a short-term recording of 120 seconds as mentioned before (28). On the other hand, SD2 measures short and long-term HRV in milliseconds and correlates it with the LF power and Baroreflex sensitivity (28). Baroreflex stimulates shared responses of the autonomic nervous system. When the afferent Baroreflex traffic increases blood pressure is raised, the efferent sympathetic traffic decreases, and as a result, the efferent parasympathetic traffic increases (29). The SD2/SD1 ratio, therefore, measures the mutability of the R-R time series and hence the sympathovagal balance during the stage of sympathetic activation (30), which happens during anxiety (see Fig. 1). Thus, in this work, SD2/SD1 score-based LAD) is a novel approach, which has been used during the lockdown period when the mobility of the population has been restricted as per the rate of occurrence of the infection. It is also worth

noting that LAD is a robust instrument and hence is not hypersensitive to diurnal physiological changes. Table 1 presents the comparative study of LAD, GAD-7, and HAM-A.
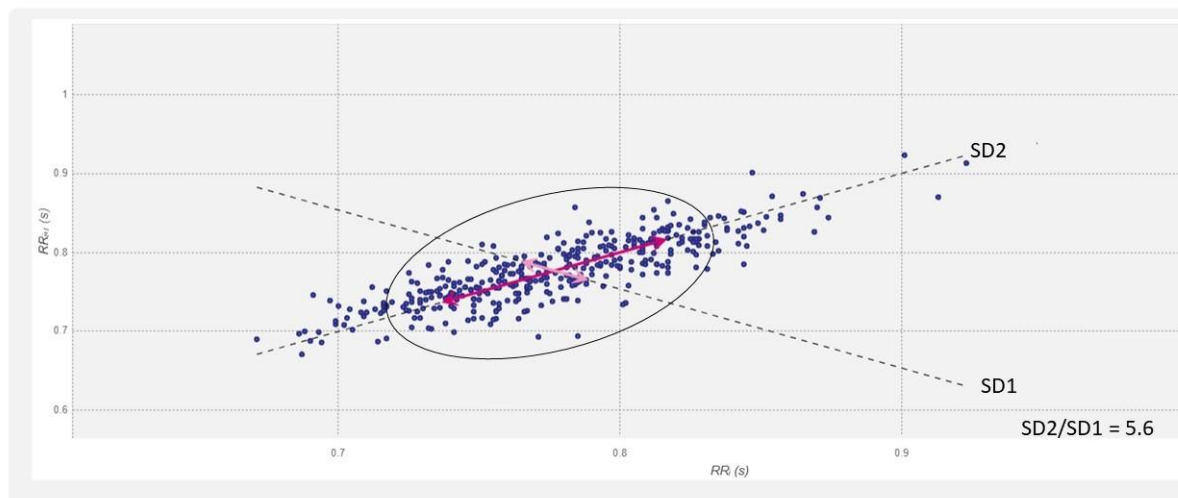
(a)
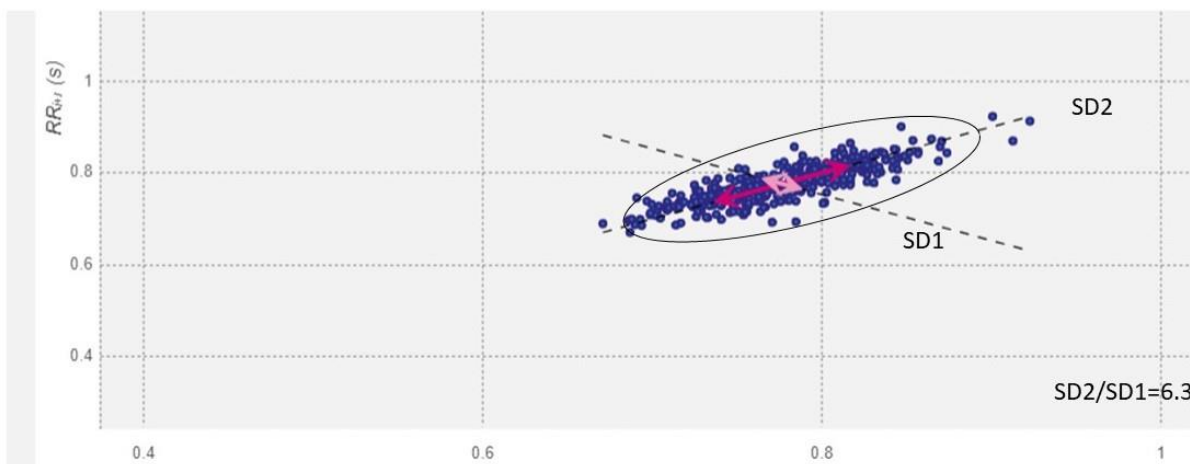
(b)

Fig. 1. Poincaré plot spectral analysis in (a) male and (b) female GAD patients.

The plots in this figure are the Poincaré plots by fitting an ellipse to the scatter plotted points. The Poincaré plots help in the visual identification of the presence of non-linear HRV components. The non-linear measurements such as SD1, SD2, and S can be derived after fitting the ellipse. The standard deviation (SD) of the distance of each point from the y axis = x-axis denotes the width of the ellipse called SD1. SD1 is measured in milliseconds and correlates with the baroreflex sensitivity (28) not only in the arteries but also on the sinoatrial node or SAN (the pacemaker of the heart) (31). On the other hand, SD2 of each point from the y = x + average of the R-R interval denotes the length of the ellipse (28). SD1 denotes parasympathetic activity (increases the beat-to-beat or R-R interval), while SD2 denotes the sympathetic drive (decreases the beat-to-beat time or R-R interval). The ratio of SD2/SD1 predicts the R-R time series which in turn denotes the sympathovagal balance and correlates with low and high-frequency (LF and HF), respectively, and its ratios (HF/LF) (28). A high SD2/SD1 ratio indicates sympathetic overdrive or parasympathetic dampening or dampening of both with relatively more dampening of the parasympathetic drive. It correlates with LF/HF and has a potential influencing effect on the SAN and the electrical conducting systems of the heart. The normal SD2/SD1 value must be below 2.5 (32).

Table 1 showcases the important parameters of LAD, GAD-7, and HAM-A.

Table 1. Notable parameters of LAD, GAD-7, and HAM-A

Instrument	Item-size	Instrument type	Scale	Test time (min)	Interpretation
HAM-A	14 Qs	Questionnaire-based self-scoring	5-point (0-4)	10	Score <17: mild; 18-24: moderate; 25-30: severe (15)
GAD-7	7 Qs	Questionnaire-based self-scoring	4-point (0-3)	5	Score >5: mild; >10: moderate; >15: severe (14)
LAD	1 optical biomarker	Smartphone-based optical biomarker type, based on short HRV variables	2-point (0-1)	5	SD2/SD1 HRV score 2.5: mild; 2.6-5: Moderate; 5-10: High; >10: severe (The classification is hypothetical which has been attempted to validate in this study)

Pilot study

A five months' (1st July 2021 to 17th November 2021) pilot study has been conducted at Acculi Labs Pvt. Ltd. Bangalore, Karnataka India after appropriate ethical measures as mentioned above. The team size consists of eight persons – (i) the creator of the Lyfas biomarker tool (LAD) who is an electronics engineer, (ii) an experienced senior data scientist who is also a practicing clinician, (iii) three senior practicing psychiatrists who chose the GAD-7 and HAM-A instruments to test the *inter-rater reliability* of the LAD, interpreted the results, and managed the patients on-line who have moderate to severe GAD, (iv) two interns for pre-test and post-test general counseling and GAD-7 and HAM-A questionnaire-based data (patients' answers) entry, and (v) one mid-career data analysts for LAD report generation for comparing to the GAD-7 and HAM-A gold standard instruments. SD2/SD1 scores (LAD), GAD-7 scores, and HAM-A scores are then graded accordingly and saved as excel files for analysis. During the study, a constant touch has been maintained by the interns with all participants as per the advice of the psychiatrists.

The steps taken are as follows:

Step-1: Online advertisement and call for the study (refer to Fig 2)

Step-2: Selection of the subject with the help of the psychiatrists

Step-3: Assigning the registration number to the selected subjects

Step-4: Selection of a day of tests

Step-5: Lyfas application installation in their mobile phones and requisite training given to the subjects before the test

Step-6: Lyfas test is taken three times – before breakfast, lunch, and dinner followed by GAD-7 and HAM-A self-rating of GAD on the same day each time Lyfas tests are taken, i.e., each test is taken three times a day for each day till RTPCR is negative (average 12 days for which tests are taken)

Step-7: Lyfas analytics is performed to capture the SD2/SD1 values (consists of the LAD) and GAD-7 and HAM-A scores are also captured and evaluated simultaneously

Step-8: Data entry of LAD, GAD-7, and HAM-A is completed for further analysis, where the average values of three tests for 12 days are considered

Step-9: Validating LAD as a smartphone-based novel GAD assessing instrument using appropriate statistical methods

Step-10: Research dissemination.

Fig. 2. Subject registration form of the anxiety study.

Fig. 2 shows the consent form of the participants (anonymously presented) on the company letterhead.

Analysis (age-group and gender-wise)

All experimental computations are performed using *Python 3.9* on the IDLE editor (33) on Windows 10 Pro PC. Following methodologies are applied to analyze the data,

- *Descriptive statistics* (refer to Table 2) to see the basic structure of the data (34) (35)

- Normality test to check data distribution whether normal or not-normal with *Kolmogorov-Smirnov normality test* (KST, see Table 3), where high ks-statistics (D) and low *p-values* refute normal distribution of the data (36) (37)

- Internal consistency (*Cronbach's α*) to examine the data fidelity (38) (refer to Table 4)

- *Spearman's rank correlation (r statistics) analysis* to test if there is any significant statistical dependence between two instruments at one time (39). In this study, the instruments are LAD vs. GAD-7 and LAD vs. HAM-A (see Table 5)

- *Classification metric:* Sensitivity, specificity, precision, accuracy, fscore (harmonic mean of sensitivity and precision), and Youden's index (24) to assess how good LAD can classify the grades of GAD when compared to GAD-7 and HAM-A (refer to Table 6), and finally

- *Bland-Altman Reliability Assessment* (BARA) has been performed here to examine the strength of agreement between three instruments i.e., LAD vs. GAD-7, and LAD vs. HAM-A (see Fig. 3) (40) (41), where LAD is the 'novel' instrument and GAD-7 and HAM-A is already two gold-standard instruments. BARA works by the following calculations:

a) The *mean difference* between the scores of any two instruments

b) The *mean* values of two instruments

c) *One-sample T-test*

d) *Mean*, *standard deviations*, and determining the *upper and lower limit* with a *95% confidence interval*, and finally

e) The *scatter graph plot*.

III. RESULTS

In this section, the results, obtained by the experiments are presented. Blue fonts refer to important findings.

Descriptive statistics

Mean, median, and standard deviations give the structure and the spread of the data. Table 2a-c presents the descriptive statistics of LAD, GAD-7, and HAM-A of male and female test-takers. Any LAD score >2.5 refers to GAD.

Table 2a: LAD score (SD2/SD1 cut-off scores 2.5)

Age group (years)	Mean		Median		Standard deviation	
	Male	Female	Male	Female	Male	Female
18-30	*2.6492*	*2.7430*	1.74	1.91	3.0739	3.1873
31-50	2.4331	2.4408	1.705	1.74	2.3225	2.5058
>50	*2.6509*	*2.9542*	1.76	1.57	3.2638	3.7958

Table 2(b): GAD-7 score (items cumulative cut-off scores 5)

Age group (years)	Mean		Median		Standard deviation	
	Male	Female	Male	Female	Male	Female
18-30	*5.1052*	4.3579	4	3	4.3781	3.5174
31-50	4.1970	3.5282	3	2	3.5347	3.7448
>50	*5.2898*	*5.0937*	4	3	4.1399	4.5687

Table 2(c): HAM-A score (items cumulative cut-off scores >0)

Age group (years)	Mean		Median		Standard deviation	
	Male	Female	Male	Female	Male	Female
18-30	*9.6315*	*14.5909*	10	12	7.8564	4.9768
31-50	*10.2980*	*14.5698*	10	12	8.5587	6.2883
>50	*13.9710*	*16.8671*	12	14.5	8.0790	6.3100

Table 2a shows that on average both the genders in the age group of 18-30 years and >50 years show *mild* GAD. Similarly, in Table 2b, it is evident that on average males in the 18-30 years age group and both the genders over 50 years age groups present with *mild* GAD when scored with the GAD-7 questionnaire. In Table 2c, *mild* GAD is visible in both genders in all three age groups on average. Hence, on average *mild* anxiety has been the most predominant feature in the test population.

Normality test (KST)

Table 3: KST results

Age group (years)	D		*p*	
	Male	Female	Male	Female
18-30	0.2606	0.2455	*0.00001*	*0.00001*
31-50	0.2093	0.2223	*0.00001*	*0.00001*
>50	0.2191	0.2501	*0.00001*	*0.00001*

The distribution of GAD scores in both genders is not normal bell-curved with average positive skewness of 4.2387 for males and 3.8709 for females, respectively.

Internal consistency (α test)

Table 4: α test results

Age group (years)	*α test* (LAD, GAD-7, HAM-A)	
	Male	Female
18-30	*0.8606, 0.8711, 0.9082*	*0.8555, 0.8911, 0.9138*
31-50	*0.8093, 0.8923, 0.8544*	*0.8223, 0.9821, 0.8890*
>50	*0.9391, 0.9128, 0.9098*	*0.9501, 0.9016, 0.0385*

The test confirms that the internal consistency of the data varies from 'good' (equals to or above 0.8) to 'excellent' (equals to or above 0.9) (42).

Now to test the statistical dependence (association strength) between LAD-GAD-7, and LAD-HAM-A, the result of Spearman's rank correlation *(r)* analysis and its respective *p-values* has been presented below (refer to Table 5).

Table 5. 'r' statistics and p-values

Age group (years)	LAD vs. GAD-7 *(r; p)*		LAD vs. HAMA *(r; p)*	
	Female	**Male**	**Female**	**Male**
18-30	*0.7221; 2.24e-64*	*0.601; 1.09e-19*	*0.6210; 1.14e-19*	*0.6307; 1.1e-19*
31-50	0.5810; 2.33e-16	0.5811; 2.1e-16	0.5830; 2.40e-16	0.5913; 2.3e-16
>50	0.5666; 2.25e-15	0.58; 2.28e-16	0.5618; 2.75e-15	0.5892; 2.38e-16

From Table 5, it can be noted that GAD in the group of 18-30 years shows the highest correlations between LAD & GAD-7 and LAD & HAMA, followed by 31-50 years and above 50 years age groups. It is pertinent to place on record that LAD is statistically significant when compared to GAD7 and HAMA (p-values <0.05). further, Table 6 gives the classification metric of LAD when compared to GAD7 and HAMA.

Thus, until now, the anxiety scores captured by LAD (SD1/SD2, i.e., single item), GAD-7 (7-items), and HAM-A (14-items) are found to be,

- Internally consistent

- Not normally distributed (too low p-values and small values of ks-statistics)

- On average shows 'mild' GAD for both the genders in all age groups, and

- LAD has a statistically significant association with GAD-7 and HAM-A (refer to the p-values <0.05, CI 95%).

Classification metric

Table 6 presents the results, where TP, FP, TN, and FN refer to True Positive, False Positive, True Negative, and False Negative, respectively.

Table 6. Classification metric of (a) male and (b) female

MALE (N=955)	GAD-7 vs. HAM-A			
	TP	**FP**	**TN**	**FN**
	760	72	99	24
Sensitivity	0.969388			
Specificity	0.578947			
Precision	0.913462			
Accuracy	*0.795812*			
Fscore	*0.9405*			
Youden's index	*0.548335*			

FEMALE (N=499)	GAD-7 vs. HAM-A			
	TP	**FP**	**TN**	**FN**
	345	30	112	12
Sensitivity	0.966387			
Specificity	0.788732			
Precision	0.92			
Accuracy	*0.691383*			
Fscore	*0.9426*			
Youden's index	*0.755119*			

MALE (N=955)	LAD vs. GAD-7				LAD vs. HAM-A			
	TP	FP	TN	FN	TP	FP	TN	FN
	800	67	73	15	750	74	103	28
Sensitivity	0.981595				0.96401			
Specificity	0.521429				0.581921			
Precision	0.922722				0.910194			
Accuracy	*0.837696*				*0.78534*			
Fscore	*0.9367*				*0.9511*			
Youden's index	*0.503024*				*0.545931*			

FEMALE (N=499)	LAD vs. GAD-7				LAD vs. HAM-A			
	TP	FP	TN	FN	TP	FP	TN	FN
	350	23	124	2	356	12	125	6
Sensitivity	0.994318				0.983425			
Specificity	0.843537				0.912409			
Precision	0.938338				0.967391			
Accuracy	*0.701403*				*0.713427*			
Fscore	*0.9655*				*0.9753*			
Youden's index	*0.837856*				*0.895834*			

Table 6 presents the performance of three instruments as *LAD*, which is a 2-point scaled 1-item smartphone optical biomarker instrument that is aimed to be validated in this work; *GAD-7* is a 4-point scaled 7-item questionnaire-based self-rating instrument, which is already a validated instrument, and *HAM-A*, which is a 5-point scaled 14-item questionnaire-based self-rating instrument that is already well-acclaimed incorrectly classifying *GAD* as mild, moderate, and severe cases (refer to Table 1). In this study, GAD-7 has been classifying cases with 79.58% and 69.13% accuracies in males and females, respectively with Youden's indices of 0.5483 and 0.7551 in males and females. D*iagnostic accuracy* of 83.76% and 70.14% in males and females, respectively, when LAD is compared to GAD-7 and 78.53% and 71.34% accuracy in males and females, respectively when compared with HAM-A corroborates the fact that LAD is a reliable instrument in diagnosing GAD. The fscore values close to 1.0 corroborate the fact that there is a balance of precision and sensitivity. That is, there is a coherence of measures in the detection of: (i) individuals who are labeled positively for covidanxiety had anxiety in reality and vice versa (43). Moreover, LAD's *Youden's index* of 0.5030 and 0.8378 in males and females, respectively when compared to GAD-7 and 0.5459 in males and 0.8958 in females (when compared to HAM-A) corroborates that LAD is a reliable medical instrument that can be used in the clinical settings. It is important to note that a Youden's index above 0.5 qualifies a novel medical instrument/device for clinical use (44). Further to state that, LAD is statistically much closer to GAD-7, where the *closeness* of (i) accuracy is 99.53% and 97.64% in males and females, respectively, and (ii) Youden's index is 99.76% and 86.33%, respectively for males and females). Therefore, the study proposes that LAD can also be used in a clinical setup to screen and monitor GAD in adults.

BARA

Fig. 3(a) through 2(l) show the BARA plots for each gender and age group for a pair of instruments under the *agreement* test. High agreement between LAD and GAD-7 (a-f) and LAD and HAM-A (g-l) means that there is not much proportional bias in the distribution of data around the mean difference line, which can be evident in Fig.2 for both genders and all age groups.
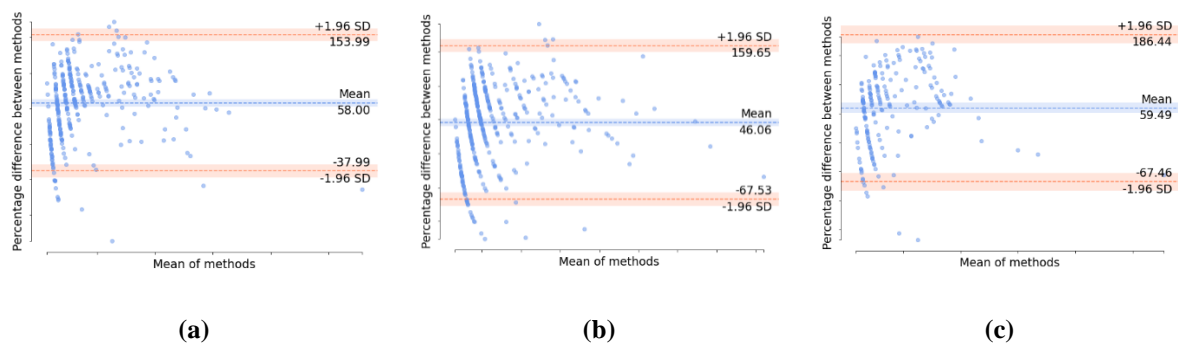
(a) **(b)** **(c)**

Fig.3a-c. LAD vs. GAD-7 in *males* (18-30 years, 31-50 years, and >50 years).

(d) **(e)** **(f)**
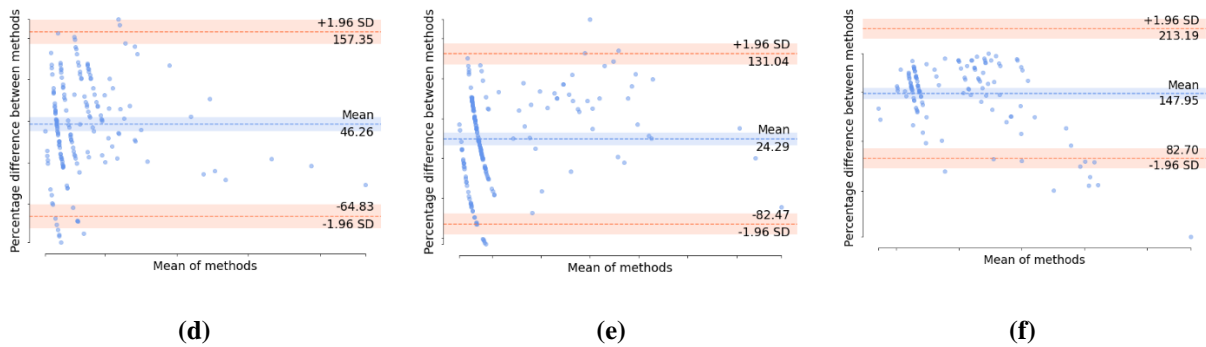
Fig.3d-f. LAD vs. GAD-7 in *females* (18-30 years, 31-50 years, and >50 years).

(g) **(h)** **(i)**
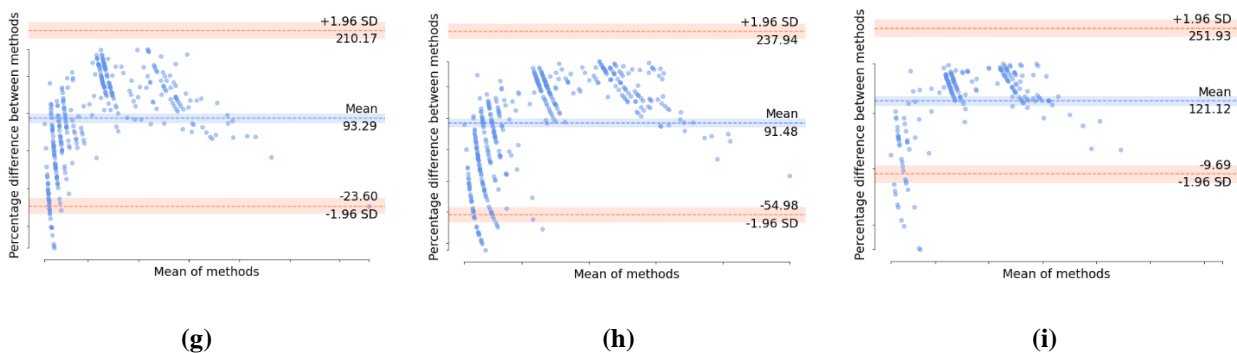
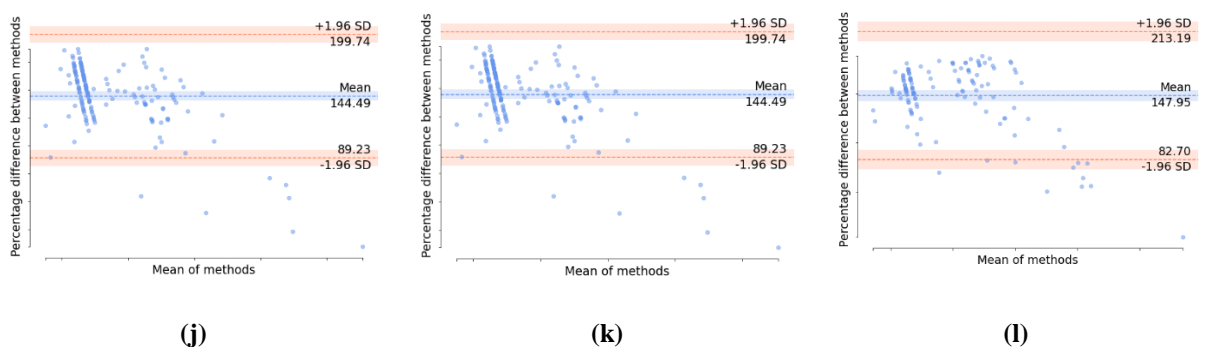**Fig.3g-i. LAD vs. HAM-A in *males* (18-30 years, 31-50 years, and >50 years).**

(j) **(k)** **(l)**

Fig.3j-l. LAD vs. HAM-A in *females* (18-30 years, 31-50 years, and >50 years).

Fig. 3 shows the pair-wise BARA plots. In each plot, the red lines are the upper and lower bound SDs and the blue line in the middle is the mean. It is expected that the percentage difference of the methods must be around the mean to predict good inter-rater reliability. Here, the plots show high inter-rater reliabilities or less proportional bias as most of the data points lie close to the mean of methods.

IV. DISCUSSION

Anxiety disorder is predominant these days across the globe and at a much higher grade during the COVID-19 pandemic period due to biopsychosocial disjoints across various sectors. The authors envisage that the actual load of anxiety morbidity is much larger than what is being noticed. There is evidence that persistent anxiety manifolds not only the suicide risks and vulnerabilities to substance use including alcoholism and tobacco smoking (45), but also it raises the cardiac risk, such as sudden cardiac deaths (SCD) in the vulnerable population due to uncontrolled and abnormal tachycardia (46). The key pathophysiology lies in the cascading effect of dysregulated hypothalamus-pituitary-adrenal (HPA) axis due to an uncontrollable hyperarousal from the recurrent anxiety. Uncompensated sympathetic overdrive (known as the autonomic stress or AS) could be irregularly irregular due to the randomly increased secretion of norepinephrine from the suprarenal medulla is the lead cause of unregulated tachycardia and finally, cardiac arrest where heart muscles fail to pump oxygenated blood to the brain (refer to Fig. 4). Hence, persistent anxiety must not be overlooked.
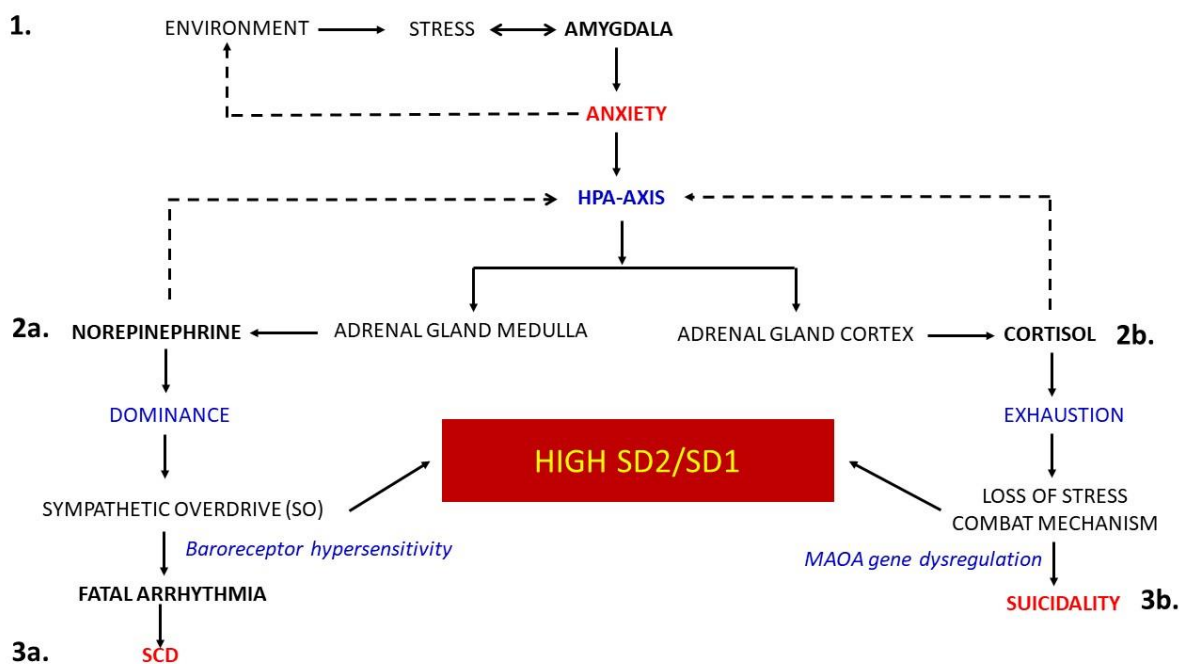

Fig. 4. The brain-HPA-AS-genetic cascade of adverse health outcomes of GAD in the population at risk.

In figure 4, it can be noted that the brain is uncontrollable and susceptible to environmental stress which is targeted at the amygdala which is responsible for the fight-flight-freeze response to the stress (step 1). Amygdala stimulates the HPA-axis where the hypothalamus, under a stress situation, secretes corticotrophin which is the precursor of the adrenal corticotrophic hormone (ACTH) that the pituitary gland secretes from its ventral part (step 2a). ACTH triggers the adrenal cortex and helps synthesize and secrete the cortisol that helps meet the additional metabolic demand of the body by increasing the blood glucose level through gluconeogenesis and glycogenolysis in the liver cells. For a faster supply of glucose to the demanding cells, norepinephrine is secreted from the adrenal medulla (step 2b). Prolonged stress leads to cortisol exhaustion which plays a significant role in depression and suicidality (step 3a). Continuation of norepinephrine shooting results in sympathetic overdrive (SO), which if continues for a long time, causes fatal tachycardia and sudden cardiac death (SCD) in the vulnerable population (step 3b). it is important to note that a persistently high SD2/SD1 could be a useful marker here to predict the risk of SCD and suicide where the monoamino oxidase A (MAOA) (47) and the tryptophan hydroxylase (TPH) gene (48) dysregulation when the stress combating mechanism is lost in the GAD, as observed in the population at risk.

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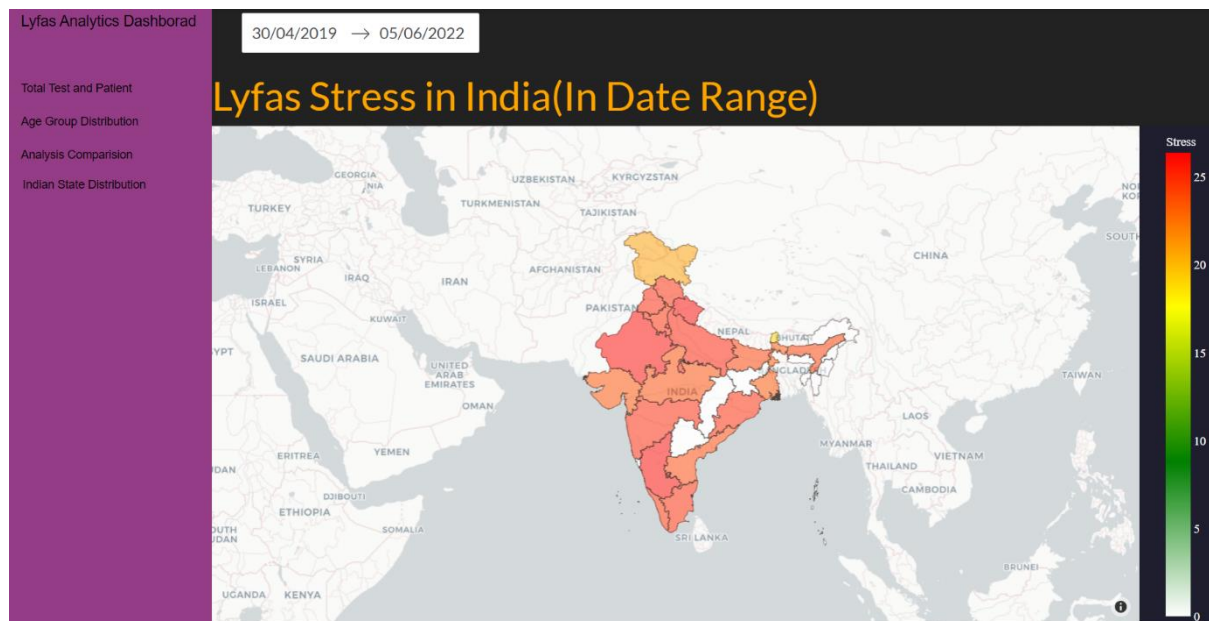The distribution of anxiety stress in India can be seen in Fig. 5 as the heatmap.

Fig. 5. The heatmap of anxiety in India.

In this figure, the heatmap is denoted by the right-sided bar where colors toward dark red indicate high scores (maximum score of 30) and colors close to white refer to low anxiety stress scores (0 as the minimum). The map shows the anxiety-stress situations state-wise. High stress can be seen in northern and southern states. It is important to note that the states showing white do not imply '0' stress, actually the relevant data is not available in these cases.

To the best of the knowledge of the authors, LAD is the only smartphone-based non-invasive and pervasive clinical instrument having a *single-item* optical biomarker SD1/SD2-based scale that detects GAD in adults and there is no other similar instrument reported so far. Its inter-rater *reliability* and *agreement* are high when examined against GAD-7 and HAM-A using BARA, where it is evident that there is no proportional bias in the distribution of data around the mean difference line between the novel instrument LAD and the well-acclaimed gold-standard instruments such as GAD-7 and HAM-A. Furthermore, when compared to GAD-7 and HAM-A, the average accuracy and Youden's index of LAD are 98.6% and 93.05% in the test population, respectively although their modalities are different, which is an encouraging finding. It is worth noting, therefore, that the high SD2/SD1 is a useful clue to the doctors that the test-taker has an anxiety trait, irrespective of the overt signs and symptoms, which are captured by GAD-7 and HAM-A. Since an SD2/SD1 score gives the snapshots of cardiovascular autonomic modulations towards maintaining the mind-body homeostasis of the body that is reflected through the HRV and its correlates, which are altered in GAD. Therefore, the advantage of LAD lies over the GAD-7 and HAM-A in terms of 'early screening' of the vulnerable trait of GAD in the suspected population, which is quite prevalent during the ongoing pandemic stage.

Abreast the afore-mentioned advantages, the proprietorial heuristics-based diagnostic and the AI-ML algorithms have made LAD a robust instrument to capture the *physiological truth* at the test timestamp. In this respect, it is important to state that the questionnaire-based instruments can be manipulated by the test-takers for various interests such as social, financial, legal, professional, and so forth. Since LAD yields the physiological snapshot of the cardiovascular autonomic modulation due to the anxiety trait and the environmental threat and hence is a single biomarker-based *truth-teller*. The snapshots of cardiovascular autonomic modulations can not be manipulated or tampered with, which poses a great advantage of using LAD over GAD-7 and HAM-A or any other questionnaire-based instruments.

There could be sampling bias due to false scoring by the subjects in GAD-7 and HAM-A questionnaire-based instruments and not divulging the intake of any anxiolytic during the selection process might have influenced the study.

Moreover, the efficacy of Lyfas as an assistive healthcare instrument has been reliably validated in various clinical settings, such as (i) Duchenne Muscular Dystrophy (49), (ii) estimating the vascular aging (50), (iii) risk of heart failure in COPD patients with low FEV_1 and SpO_2, (26) (iv) silent depression in adults (13), (v) polycystic ovary syndrome (51),

(vi) the impending risk of depression in empty nest syndrome in the geriatric population (52), (vii) risk of early vascular aging in the borderline personality disorders with romantic jealousy (53), (viii) assessing the impact of hypertension-anxiety-anger triad (54), (ix) evaluating the risk of cancers with sudden high-intensity stress (55), and (x) mental health assessment during COVID-19 pandemic in the Indian adults (32). Validation of Lyfas is continuing in other healthcare fields.

V. CONCLUSIONS

Mind-heart-body is tightly interlinked. The current clinical practice is 'specialty-based' and that is the biggest disadvantage to the medical science itself and its stakeholders both financially and psychologically. The specialists are entrapped inside their specialty domain and usually overlook or remain uncared for other areas which also contribute to the morbidity. E.g., GAD is usually managed by a psychiatrist with anxiolytics and its consequential adverse health outcome in the body are managed by different domain specialists such as cardiologists, hepatologists, endocrinologists, and so forth. Alike other mental illnesses, GAD has several adverse health (mind-heart) consequences ranging from atrial fibrillation (56) and sudden cardiac death due to fatal ventricular arrhythmias (46) to suicides (9) due to the dysregulated autonomic homeostasis in the population at risk. Baroreceptor dysregulation plays a key role to affect the cardiovascular system as mentioned in Fig. 1 legend. Studies have also shown that people suffering from cardiac and associated mental health issues, such as severe degrees of depression, suffer from Generalized anxiety disorder GAD (7) which corroborates such intertwining from both ways.

The study concludes that SD2/SD1 can be regarded as the singleton cardiovascular optical biomarker to evaluate not only the anxiety in the population non-invasively and pervasively in a much more personalized manner but also the risk of SCD and suicide in the vulnerable population. A persistently high SD2/SD1 value indicates high AS in an individual. Other associated risk factors such as tobacco smoking, substance abuse, alcoholism, sleep deprivation, added sympathetic overdrive as a result of weight training, and socioeconomic stress due to rat race competitions result in high AS and manifolds the risk of SCD and suicides.

Lyfas, being a non-invasive and pervasive smartphone instrument, can be used in a large epidemiological survey of anxiety detection. Those with persistently high SD2/SD1 scores can be assistive not only to the psychologists and psychiatrists but also to the cardiologists to save lives.

Finally, the authors pay their sincere tributes to the biopsychosocial theory called 'Dirk Hamer Syndrome' coined by Dr. Ryke Geerd Hamer who mentioned that the brain is the key conspirator behind the occurrence of the dreaded diseases in the body (57). GAD-led SCD, other metabolic illnesses, and suicides validate his wisdom.

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