Original Research Article

Variations in sympathovagal balance, psychogalvanic reflex and isometric grip force in females with polycystic ovarian syndrome- an exploratory study

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ABSTRACT

Background: Poly-cystic ovarian syndrome (PCOS) is an endocrine ailment and recently emerging cause of infertility amongst reproductive females. The disease is associated with multifactorial etiologies. Autonomic dysfunction is cause or result of this disorder is yet a paradox. Various autonomic function tests can be done to reveal this dilemma. Heart rate variability is pivotal amongst these and assesses trivial variations between two heart beats and stands as a marker of cardiac risk. Sympathetic skin response checks integrity of sympathetic reflex tract. Isometric grip force relates contracting force and electromyography with a person's heart rate during exercise. As there is meagre published evidence on autonomic function testing in central Indian women with PCOS, this study was undertaken on the PCOS patients and their data was compared with regularly menstruating healthy controls.
Methods: A cross sectional study was performed on 35 healthy controls and 35 PCOS diagnosed patients of age range 18 to 42 years short-term HRV was recorded using ECG and RR interval using power lab system. Thereafter SSR was obtained using bio amplifier by giving standard acoustic stimuli. The patients were then asked to perform isometric grip force exercise using grip force transducer to obtain various related parameters.
Results: The mean age group of controls was 22.62±2.15 years and that of among cases was 23.29±4.26 years. Compared to healthy controls PCOS patients had higher waist circumference, lower HF Norm whereas higher VLF norm and LF/HF ratio, delayed SSR latency while hand grip strength was significantly higher in the control group as compared to the PCOS patients. LF/HF ratio, mean HR, waist circumference and SSR latency were positively correlated to each other and negatively correlated to mean hand grip strength.
Conclusions: PCOS females have altered adrenergic status as compared to normal women. Various autonomic function tests like HRV, SSR and hand grip strength exercise can be useful in evaluating these patients as the disease exposes them to various co morbid risk factors.

Keywords: Hand grip strength, Heart rate variability, Polycystic ovarian syndrome, Sympathetic skin response

INTRODUCTION

Polycystic ovarian syndrome (PCOS) is a common endocrinopathy and frequent cause of distress, pregnancy complication and infertility among the reproductive women of early middle age group. PCOS also known as Stein Leventhal syndrome, named after the scientists who first associated the disease with presence of ovarian cysts and anovulation.[1] The disease is diagnosed using classic hallmark triad consisting of anovulation, obesity and hirsutism. It indeed is a different metabolic syndrome leading to risks of cardiovascular disease too.[2,3] It has high estimated prevalence of 25% among the Indian female population.[4] Microneurography suggests increased

sympathetic activity in patients of PCOS. This is further confirmed by clinical symptoms of the disease.[5-7]

Heart rate variability (HRV) measures beat to beat variations between successive heartbeats. These measurements can be traced to body's autonomic nervous system. It has the potential to provide additional valuable insight into various pathological conditions and to enhance risk stratification. Frequency domain spectral component of HRV analysis is of major concern in our study, as it measures high frequency (HF), low frequency (LF) and very low frequency (VLF) components. HF component of HRV indicates vagal tone of the individuals while LF component of HRV indicates sympathetic activity. Sympathovagal balance (LF-HF ratio) one of the spectral components of HRV analysis is extensively used to assess cardiac autonomic function.[8,9]

Isometric grip force exercise is a sophisticated technique eliciting reflex cardiovascular responses. The grip force exercise evokes sympathetic nervous system resulting in increased in heart rate, systolic and diastolic BP due to increase in peripheral resistance and cardiac output.[10-12] The blood pressure rises by vasoconstriction mediated via alpha-adrenergic receptors of ANS. Therefore, any damage to neurological pathways involved could lead to a diminished or absent cardiovascular response to sustained hand grip.

Psychogalvanic reflex popularly known as sympathetic skin response (SSR) is used to measure changes in skin potential in response to stimuli provoking sympathetic activity. It basically demonstrates sympathetic sudomotor nerve fiber activity due to postsynaptic reflex arch activation, as a result of which eccrine sweat glands gets activated. These glands get cholinergic supply by postganglionic non-myelinated type C fibers.[13]

Various autonomic function tests have been performed and used to know autonomic neuropathies. Being characterized by metabolic syndrome, PCOS is being investigated extensively these days for these parameters.

Various studies in the past investigating autonomic control in PCOS women had focused mainly on HRV.[14-20] On the contrary data about isometric hand grip strength (HGS) is sparse. Sympathetic skin response (SSR) is a recent entity and not many studies have been done on this parameter.

Hashim et al studied autonomic dysfunction in women having higher BMI with PCOS and they found that the results in both the groups were similar in terms of reduced palmar SSR latency and higher amplitude, altered HRV, higher plasma epinephrine level and faster pulse rate.[21]

In another study by Dag et al, it was found that the mean latency of SSR in PCOS patients was significantly lengthened compared with the controls. The mean SSR amplitude was significantly lower in comparison with the controls, hence sympathetic dysfunction was noted in

these patients.[22] In a cohort study on HGS by Kogure et al, women with PCOS had greater muscle strength irrespective of body composition in a bench press exercise.[23]

So, it was observed that there is dearth of data pertaining to the autonomic parameters namely SSR and HGS in PCOS patients hence this study was undertaken to determine the sympathovagal balance, psychogalvanic reflex and isometric grip force in females with polycystic ovarian syndrome and in age matched controls.

METHODS

This cross-sectional study was carried out in the department of physiology in collaboration with the department of obstetrics and gynecology of Rural Medical College which is in central India. The study population comprised 35 diagnosed patients of PCOS (non-pregnant, ideal and lean weight) referred from the department of obstetrics and gynecology in the age group of 18-42 years and 35 age matched controls. Minimum required sample size was calculated using OpenEpi, Version 3, open source calculator according to the prevalence of disease.[4] A sample size of total 70 was derived providing 80% power using a type 1 error rate of 0.05, the confidence limits as % of 100 (absolute +/- %) (d) as 5% with a design effect of 1.

Inclusion and exclusion criteria

Cases included in the study were women diagnosed of PCOS, using Rotterdam triad consisting of anovulation, obesity and hirsutism. Normally menstruating females in follicular phase of reproductive cycle comprised the control group. They were not under any medications affecting lipid and carbohydrate metabolism at least for past 2 months. The study subjects in both the groups were recruited only after they had given written informed consent.

Women below 18 and above 42 years, pregnant and lactating women, those who had undergone hysterectomy, had attained menopause, taking anti-lipidemic drugs for the past 2 months, oral contraceptives and sex steroids for the past 2 months were not included in the study. Subjects that were screened as high risk for cardiovascular disease, with any history of cardiac, respiratory or psychiatric disease, any significant co-morbidity and had who consumed any drug (like beta-blockers) affecting HRV were also excluded.

Ethics consideration

A signed written informed consent from all study participants prior to the beginning of the study was obtained. Prior approval from institutional ethics committee was obtained before the commencement of the study.

Equipments

For measuring sympathovagal balance (LF-HF ratio)

ECG was recorded using Power lab data recording system, AD instruments and analysis was done by lab chart software. The lab chart recordings of ECG were analysed with help of heart rate variability (HRV) module (Windows). After obtaining baseline characteristics the subjects were asked to wear the Equivital EQ-02-B3 ECG sensor belt and along with a Polar transmitter SPO180 (having chest strap) which is a wireless heart rate KitPTK25.
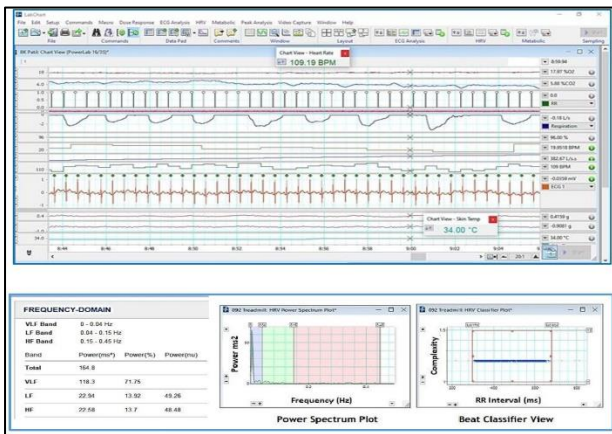

Figure 1: Lab chart view of ECG recording for extracting and analysis of HRV.

For measuring isometric grip force (HGS)

Hand grip strength was measured using grip force transducer (MLT004/ST) from AD instruments, Australia. The raw data obtained by Power lab data recording system, was analysed by lab chart software and expressed in Newton (N). Single value of maximum voluntary contraction which represents 100% of the handgrip strength was noted. Mean value of handgrip strength was calculated from a set of values obtained during a period of 0 to 3 minutes with an interval of 20 second each. BP along with heart rate was recorded before and after the exercise.

Figure 2: Lab chart view of isometric grip force recording.

For measuring electro-myography (EMG)

Equipment required for recording EMG was AD instruments' bio amplifier BIO AMP (FE132). EMG was recorded using silver-silver chloride disposable electrodes and analysis was done by lab chart software. Root mean square (RMS) calculation from the raw EMG signal was done with a triangular smoothing window width of 0.5 second.

For measuring psychogalvanic reflex (sympathetic skin response)

Equipment required to record SSR was AD instruments' bio amplifier BIO AMP (FE132).

SSR was recorded using silver-silver chloride disposable electrodes and analysis was done by lab chart software. Amplitude and latency calculation from the raw SSR wave pattern was obtained after application of standardized auditory stimulus.

Procedure

All the patients and control subjects were subjected to full medical and gynecological history and complete physical examination. Height was measured in centimeters (cm) as the study participants stood in their upright position using the height measuring scale and the weight was measured using electronic weighing machine. Waist circumference (WC) was measured in centimeters using a measuring tape by standard measures.

Short-term HRV recording was performed as per to the standards set forth by the task force of the European Society of Cardiology and North American Society of Pacing and Electrophysiology in 1996 wherein two methods of analysis of HRV data are considered: time-domain and frequency-domain analysis.[8] Five minutes resting ECG was recorded. ECG signals were acquired at rate of 1000 samples/second using data acquisition and data was transferred to windows based laptop loaded with software for HRV analysis.

For measuring isometric grip force (HGS) and electromyograpy (EMG) right forearm of the subject was exposed and thoroughly cleaned using spirit in order to apply the electrodes. Positive (red) and negative (white) electrodes were placed over the posterior proximal aspect of forearm on the belly of muscle while ground electrode was applied on anterior distal aspect of forearm. Subject was asked to maintain a standardized arm position while seating straight without any back rest/support, that is shoulder adducted and neutrally rotated, elbow flexed at 90°, forearm in neutral position and wrist in neutral position. Two trials were given at intervals of 20 seconds to the subject to recorded maximum value of the hand grip strength. Best of two values was taken as the maximum value which corresponds to 100% of his HGS. Trials were given on the left hand to enable the subject to familiarize

with the system for maintaining 30% of the grip. Subject was asked to apply 30% of his strength on grip force transducer in his/her dominant hand for about 1-3-minute duration.[16] Maximum voluntary contraction and mean isometric grip force were noted. Root mean square EMG was also analysed.

For measuring psychogalvanic reflex (SSR) right hand of subject was sterilized using spirit on both dorsal and palmar side for application of electrodes. Positive (red) and negative (white) electrodes were placed over dorsal and palmar aspect respectively. Ground electrode was applied on the anterior distal aspect of the forearm. Subject was asked to plug earphones in ears and told to keep eyes closed. He was instructed to maintain hand position undisturbed till the procedure was completed. The resting sympathetic skin response was recorded for about 50 seconds. A sudden standardized acoustic stimulus of an air horn was given from the system for 2 seconds. Psychogalvanic response was then observed.

Components of psychogalvanic reflex[13]

Latency of SSR was measured from the stimulation artifact to the first deflection from the baseline.

SSR amplitude was measured from the peak of the first deflection to the peak of the next one (peak to peak). Peak achieved was compared with resting SSR. This difference was noted as SSR amplitude in mV.
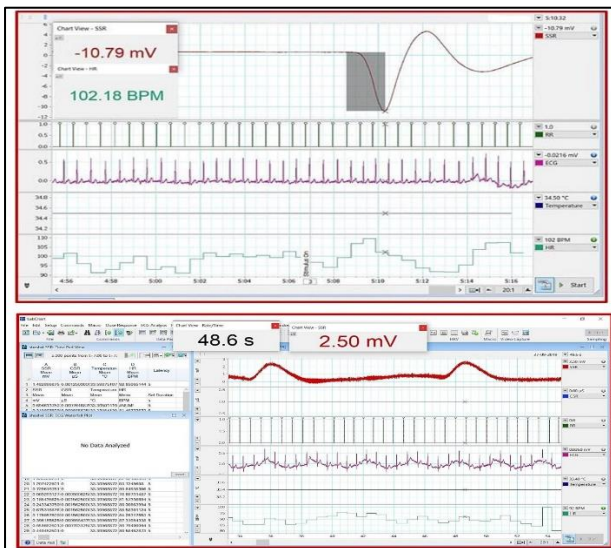

Figure 3: Lab chart view of psycho-galvanic reflex (SSR) recording (P- Type and N- Type).

Shape of the SSR response has a slow, biphasic or rarely monophasic form.

According to the polarity of the wave with the maximum amplitude, the SSR has two kinds- P-type with maximum positive deflection (a), N-type with maximum negative deflection (b).

Statistical analysis

All the investigations were done during morning hours so as to avoid any diurnal variation. The data thus obtained was entered in Microsoft Excel spreadsheet and later expressed in mean±SD. Then data was analysed by using the SPSS (statistical package for social sciences) version 20.0, SPSS Inc, Chicago, Illinois, USA. Unpaired two-tailed student's t tests were used to determine differences between groups. The parameters of HRV, SSR and HGS were correlated using Pearson correlation. The correlation coefficient value "r" either positive (direct correlation) or negative (inverse correlation) was calculated for the correlation between two quantitative variables with its t test for testing the significance of correlation. The significance level $p<0.05$ was considered as significant.

RESULTS

The present study recruited 35 females diagnosed with PCOD as per Rotterdam criteria in the age range 18-42 years and were compared with 35 healthy women free from PCOS in the same age range. The mean age of cases was 23.29±4.26 while that of controls was 22.62±2.15 years. The baseline characteristics of participants in PCOS and control groups in terms of Mean±SD are expressed in Table 1. It was evident that the difference between means of age, height, and weight was not statistically significant. The cases demonstrated significantly higher waist circumference (p=0.006, S) than controls.

Table 1: Demographic profile and clinical characteristics of participants.

Parameters	Control females (n=35) Mean±SD	PCOS females (n=35) Mean±SD	P value
Age (years)	22.62±2.15	23.29±4.26	0.4091, NS
Height (cm)	152.62±4.01	151.13±4.24	0.1356, NS
Weight (kg)	57.64±9.04	57.98±16.81	0.9165, NS
Waist circumference (cm)	77.47±9.11	85.50±14.04	0.006, S
Systolic BP (mmHg)	109.55±5.07	112.13±9.56	0.162, NS
Diastolic BP (mmHg)	71.11±8.31	74.75±9.85	0.0993, NS

(S)- Significant, (NS)- non-significant

Mean±SD of systolic BP of the PCOS was 112.13±9.56 and the controls was 109.55±5.07. Diastolic BP of the PCOS was 74.75±9.85 and the control was 71.11±8.31 which shows there was no significant difference between systolic and diastolic BP with p value 0.1629 and 0.0993 respectively.

Table 2: Analysis of HRV, HGS and SSR.

Frequency domain components of HRV			
	Control females	**PCOS females**	**P value**
	Mean±SD	**Mean±SD**	
VLF	263.58±236.25	387.10±507.48	0.1961, NS
VLF norm	15.96±11.23	25.72±13.49	0.0016, S
LF	397.33±307.9	387.62±397.49	0.9094, NS
LF norm	24.15±9.71	27.86±10.10	0.1219, NS
HF	900.75±755.43	772.92±888.05	0.5188, NS
HF norm	55.42±14.70	43.44±16.76	0.0022, S
LF/HF	0.5 ± 0.3	0.8 ± 0.49	0.0029, S
Comparison of isometric grip force indices in cases and controls			
Max. voluntary contraction (N)	201.91±46.26	194.53±51.88	0.5320, NS
Mean hand grip strength (N)	54.01±5.41	50.80±6.48	0.0277, S
Mean RMS EMG (mV)	0.25±0.15	0.20±0.10	0.1055, NS
Mean heart rate (BPM)	80.61±23.17	85.69±11.38	0.2484, NS
Comparison of psychogalvanic reflex among PCOS and controls			
SSR amplitude (mV)	2.24±3.15	2.08±0.88	0.7689, NS
SSR latency (sec)	1.99±0.23	2.43±0.61	0.0002, S

Table 3: Correlation analysis between study parameters.

LF/HF ratio versus mean HR				
	LF/HF ratio	**Mean HR**	**r value**	**P value**
Control	0.5±0.3	80.61±23.17	0.377	0.021, S
Cases	0.8±0.49	85.69±11.38	0.441	0.006, S
LF/HF ratio versus waist circumference				
	LF/HF ratio	**Mean WC**	**r value**	**P value**
Control	0.5±0.3	77.47±9.11	0.17	0.314, NS
Cases	0.8±0.49	85.50±14.04	0.29	0.0817, NS
LF/HF ratio versus SSR latency				
	LF/HF ratio	**SSR Latency**	**r value**	**P value**
Control	0.5±0.3	1.99±0.23	0.13	0.443, NS
Cases	0.8±0.49	2.43±0.61	0.18	0.286, NS
SSR latency versus mean HR				
	SSR latency	**Mean HR**	**r value**	**P value**
Control	1.99±0.23	80.61±23.17	-0.27	0.106, NS
Cases	2.43±0.61	85.69±12.63	0.30	0.071, NS
Mean isometric grip force and root mean square (RMS) of EMG signals				
	Mean HGS (N)	**Mean RMS (mV)**	**r value**	**P value**
Control	54.01±5.41	0.25±0.15	0.21	0.212, NS
Cases	50.80±6.48	0.20±0.10	0.20	0.235, NS
Mean isometric grip force versus mean HR				
Control	54.01±5.41	80.61±23.17	-0.22	0.190, NS
Cases	50.80±6.48	85.69±12.63	-0.546	0.0004, S

Spectral analysis of frequency domain HRV parameters of PCOS and control subjects are enlisted in Table 2. It clearly shows that mean LF, VLF and HF powers and low power frequency in normalised units (LF nu) of the study population were not significantly different. On the other hand, high power frequency in normalised units (HF nu), very low power frequency in normalised units (LF nu) and LF/HF ratio showed highly significant difference between the study population with p values 0.0022, 0.0016, 0.0029 respectively. Elevated LF/HF ratio, increased LF nu and decreased HF nu showed that there was increased sympathetic activity in PCOS cases.

The mean±SD values of isometric grip force indices and SSR parameters in females of PCOS and control group are enumerated in Table 2. Significantly delayed latency and

non-significant lower amplitude of psychogalvanic reflex (p<0.001, p=0.031, respectively) was observed in obese PCOS when compared to control women. In order to determine if there was any relationship between the study parameters, a correlation analysis was undertaken. The results of this analysis are shown in Table 3.
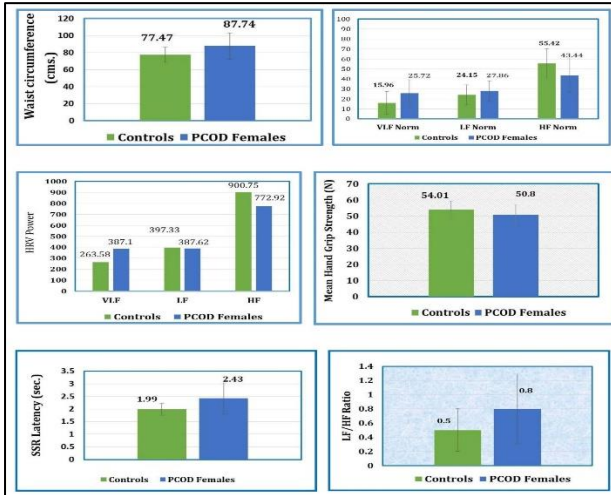

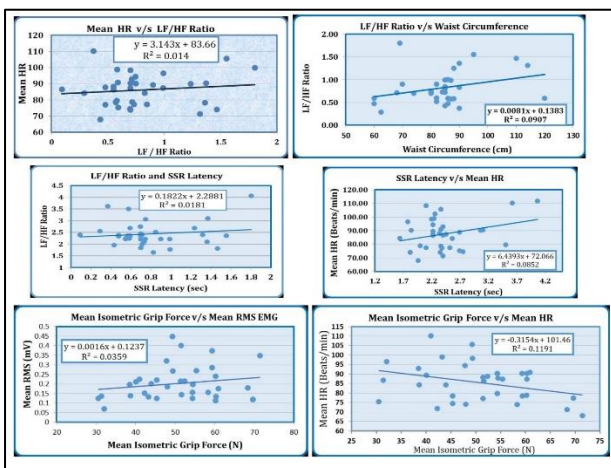**Figure 4: Comparison of study parameters PCOS and controls.**

Figure 5: Scatter plots of correlation analysis between study parameters.

DISCUSSION

Polycystic ovarian syndrome is a disorder of present day which requires keen observation and treatment studies show that among the PCOS, around 40% are generally obese. Obesity has long been known to cause alterations in autonomic functions in the form of increased sympathovagal modulation. In order to study this alteration, HRV was chosen for evaluating the same, as it is a powerful tool to evaluate autonomic activity. In the current study, PCOS cases demonstrated higher waist circumference when compared to the control group.

Upon frequency domain analysis, in PCOS patients the total power which is an index of overall HRV was reduced. Decreased HRV depicts sympathetic dominance, which is a potential CV risk. Further, the HF norm value was significantly lower in PCOS cases (p=0.0022) whereas the LF norm, HF power, LF power and VLF power were not statistically different. Nevertheless, the VLF norm was found to be statistically significant which does not hold any clinical value.

LF/HF ratio was found to be significantly higher (p=0.0029) in cases indicating enhanced sympathetic activity. These observations are in with the findings of Yildirir et al.[17]

This concurrence is in harmony with the study conducted by Balamurugan et al who have also demonstrated that the sympathetic activity was high in the PCOS people.[20]

A considerable proportion of our cases were in younger age group and impaired autonomic tone with respect to sympathetic activity was found which is also a typical finding reported by Domenico et al.[19]

Pronounced sympatho-excitation in PCOS cases is well portrayed by a highly statistically significant positive correlation (r=0.441, p= 0.006) between mean HR and LF/HF ratio. This significant relationship was evident in the control group as well authenticating the superiority of LF/HF ratio in the diagnosis of sympathetic overstimulation in an individual with elevated HR. The LF/HF ratio was found to be positively correlated with WC. Although PCOS females have higher levels of androgens and hyperandrogenism enhances muscle strength as found in study of Kugure et al but we obtained contradicting results in our cases showing significantly (p=0.027) diminished grip force.[23] A possible contention in favour of this observation could be sympathetic over activity persistent in PCOS. This was further substantiated by the robust negative correlation (r=0.55, p=0.0004) noticed between mean HR and HGS. Handgrip grip strength might, thus, allow early identification of women at risk for development of CVD. Also a positive correlation between mean RMS and mean HGS values both in controls (r=0.21, p=0.212) and cases (r=0.20, p=0.235) however they couldn't achieve statistical significance.

The mean latency of SSR in PCOS patients was significantly delayed compared with the controls (p=0.0002) in the present study whereas the mean amplitude of SSR was slightly reduced in comparison with the controls indicating sympathetic dysfunction in these cases.

Monitoring the correlation of the SSR latency with LF/HF ratio, a positive correlation was revealed among both cases and controls although statistical significance could not be achieved. These observations are in concordance with

those of Dag et al while in contradiction to the results of Hashim et al.[21,22]

Therefore, the SSR could be a useful auxiliary electrophysiological test to predict autonomic dysfunction in those patients as the method of SSR recording can be considered technically simple and realizable using acoustic stimulation as has been done in the past by Péréon et al.[24] In the view of current knowledge it is possible to consider SSR measurement as a useful complementary method in neuropathy diagnostics when lesions of thin non-myelinated fibers are supposed. On correlation of SSR latency with mean HR we found a positive relationship between them and further a positive correlation between SSR latency and LF/HF ratio validated this finding.

To spell out the crux of the gamut of interrelationships among the multiple parameters assessed in this study, it gives a solid and strong evidence of altered autonomic activity manifested by pronounced sympathovagal tone, diminished psychogalvanic reflex and reduced Isometric grip force all of which could be important risk factors for additional cardiovascular comorbidity in already existent polycystic ovarian syndrome.

Like all observational studies, our study also had some limitations. Study group mainly included rural population so the results can't be generalized. A study with a higher sample size which is inclusive of different age groups and subjects from different socioeconomic status and wide geopolitical areas, is to be proposed. SSR being a very sensitive parameter, few undesirable artefacts were also faced while measuring it.

Despite above bottlenecks our study had several notable strengths like the study is unique in combining so many parameters to come to a prompt conclusion; such a thorough study on this disease has so far not been performed to the best of our knowledge.

SSR studies are generally done using electrical stimulus. Our study uses innovative standard acoustic stimuli. Very few HGS studies on PCOS females could be found as far as literature could be traced. This parameter is the apogee of our study.

This study is unique as it attempted to measure three standard parameters confirming sympathetic dominance. This has so far probably not been explored in central Indian population of Maharashtra which has high prevalence of this disease-causing infertility and distress amongst young couples of this region. These observations have implications for the mechanisms underlying the increased risk of developing hypertension, including the higher incidence of CVD later in life, in PCOS women with normal or lean BMI. The current evidence from this study would support the view that in PCOS there is decreased dynamic activity in their autonomic function, possibly by suppression in the parasympathetic component and a chronic elevation of sympathetic component. This

sympatho-vagal imbalance might expose them to cardiovascular morbidities

CONCLUSION

Autonomic dysfunction in patients with PCOS females has been documented with the assessment of sympathovagal balance, psychogalvanic reflex, isometric grip force tests. Women with PCOS exhibited pronounced sympatho-excitation. Hence assessment of sympathovagal balance (LF/HF ratio) is that autonomic function test which is superior to others in predicting sympathetic over activity in such patients.

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