ASSOCIATION BETWEEN BRAIN DERIVED NEUROTROPHIC FACTOR AND HEART RATE VARIABILITY IN POLYCYSTIC OVARY SYNDROME

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Abstract:

Background and objectives: Polycystic ovary syndrome (PCOS) is the most common endocrine disorder seen in women of reproductive age and affecting 8-13% of them. In PCOS reduced HRV is due to weight gain, as obesity may deteriorate cardiac autonomic functions. Altered level of BDNF, a member of neurotrophin family, were observed in plasma and follicular fluid in women with PCOS. The aim of present study was to study the association of BDNF and HRV in PCOS.

Methods: The present study involved180 women aged between 17 to 35 years with and without PCOS. Out of 180 women: 90 women with PCOS were enrolled as cases and 90 age-matched women without PCOS were enrolled as controls. Further, on the basis of BMI, study group was divided into two subgroups: Obese (OB) PCOS Group (BMI ≥25kg/m^2 (n=50) and Non Obese (OB) PCOS Group (BMI ≤ 24.99) kg/m^2 (n=40).

Data was statistically analyzed using students unpaired t test and correlation between parameters was analyzed by Pearson's rank correlation and p value <0.05 is considered significant.

Results: HRV was significantly altered in women with PCOS in the form of increased sympathetic and decreased parasympathetic activity, especially in OB PCOS group. Additionally increased level of BDNF was observed but results were not statistically significant different.

Conclusion: HRV changes in women with PCOS reflect an alteredAutonomic balance and potential cardiovascular implications. Moreover, HRV alterations in PCOS were unaffected by plasma BDNF level.

Key words: polycystic ovary syndrome (PCOS), Heart rate variability (HRV), brain derived neurotrophic factor (BDNF)

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Introduction

Polycystic ovary syndrome (PCOS) is currently the leading cause of menstrual complications in women. PCOS is a complex metabolic, endocrine and reproductive disorder affecting approximately 8-13% of the premenopausal women. (1)

According to the Rotterdam consensus workshop, in 2003, women can be diagnosed with PCOS if they have two of the following three symptoms: (1) hyperandrogenism (elevated levels of total or unbound hormone blood levels of testosterone), or clinical signs of hirsutism (excessive male patterned hair growth), (2) oligo/anovulation, and (3) polycystic ovaries visualized by ultrasound.(2)

PCOS is closely related to several cardiometabolic risk factors such as central obesity, dyslipidemia, insulin resistance (IR), impaired glucose tolerance and hypertension. Recent studies reveal that these cardiovascular risk factors are also associated with cardiovascular autonomic dysfunction with compromised modulation of heart rate and blood pressure. Autonomic dysfunction in the form of decreased heart rate variability (HRV) has been reported to be associated with adverse cardiovascular events.(3,4)

The measurement of HRV has been widely used to evaluate the modulation of the autonomic nervous system (ANS), using cardiovascular function. The sympathetic and parasympathetic modulation could be assessed by power spectral analysis of HRV. Diminished HRV, which indicates increased low-frequency power(LF) and decreased high-frequency power (HF), is known to be related to increased sympathetic modulation and decreased parasympathetic activity.(5)

Changes in the HRV were reported in the PCOS subjects with increased sympathetic activity (LF), decreased parasympathetic activity (HF), increased LF/HF ratio and increased LF norm than controls. (6,7)

Recently it was demonstrated that the neurotrophins such as brain-derived neurotrophic factor (BDNF) and neurotrophin-3/4/5 (NT-3/4/5), nerve growth factor (NGF) are expressed in the human ovary. BDNF and the TrkB receptor expression is also seen in follicular fluid and uterus, in particular the endometrium suggest their contribution in its peripheral production.(8,9)

Plasma BDNF changes with higher levels in the luteal than in the follicular phase during the fertile cycle. Also altered expression of BDNF in plasma and in follicular fluid was observed in PCOS than in healthy controls. (10) Additionally BDNF plays an important role in HR regulation by enhancing parasympathetic activity and decreases resting HR.It was found that reduced BDNF secretion leads to sympathetic dominance in humans with BDNF polymorphism and reduced HR responses to stress.(11)

However there are limited studies of BDNF and HRV in PCOS. So, we have taken up this study with the aim to correlate the BDNF and HRV in PCOS.

Material and methods

This study was carried out in the Department of Physiology and Department of Obstetrics and Gynecology, Mahatma Gandhi Medical College and Hospital, Jaipur. After acquiring approval from the Human Ethics Committee of Institute the study was started.

For the study, 180 women of age group 17-36 years were selected as Study group and Control group, who fulfilled the inclusion criteria. Out of 180 women, 90 women were clinically diagnosed with PCOS, according to Rotterdam criteria, enrolled as Study group (PCOS Group) and90 women having regular ovulatory cycles without PCOS, as Control group with BMI between 18.5 kg/m^2 and 24.99 kg/m^2**.**

Rotterdam criteria were recruited for the research. The subject who fulfilled two out of three criteria:

1. Oligo/ammnhorrehea
2. Hyperandrogenism - Excessively high levels of male hormones.
   - Manifesting as visible signs such as increased facial/body hair or male-pattern baldness.
   - Alternatively, evident through laboratory tests like elevated FAI or free testosterone levels.
3. Polycystic ovaries displaying multiple cysts (12 or more follicles) and a volume exceeding 10ml on ultrasound.

Exclusion Criteria:

- Oral contraceptives, ovulation induction drugs, steroids, anti-diabetic agents, anti-androgen medications, or other hormonal medications.
- Any drugs affecting ANS activity.
- Pregnancy
- Hypothyroidism, any benign conditions related to the uterus or ovaries, as well as anyliver, kidney or heart disease.
- Alcoholics and smokers

Methodology:
All the subjects, were required to provide a written informed consent and demographic and medical history was procured. After clear explanation of the procedure, through physical examination of subjects was done. Following which height, weight, body mass index, waist circumference, hip circumference, waist hip ratio, vital parameters (body temperature, pulse rate, BP, respiratory rate) were measure. After that blood sample was collected and HRV recording was done.

Blood sample collection
About 10 ml of venous blood sample was drawn from antecubital vein after overnight fasting from every subject and collected in EDTA coated tubes for BDNF estimation.

Recording of HRV
After that participants were subjected to HRV recording using a RMS Polyrite D HRV recorder. The recording of HRV was performed in a peaceful setting inside a room with controlled temperature between 25-28°C and dimmed lighting. Prior to commencing the recording, all electronic devices were removed from the recording site to prevent interference with the recording. Subsequently, the subjects received instructions to lie in a supine posture, to keep their eyes open and unwind for twenty minutes prior to the commencement of the recording

Electrode placement:
Before placing the electrodes on the subjects the area was cleaned thoroughly. For this test, three limb electrodes were utilized. One electrode was placed above the medial malleolus of the left lower limb, while the other two electrodes were placed over the right and left wrists respectively. These electrodes were then joined to the ECG recorder that was linked to the RMS Polyrite D. The lead II, which is primarily used for the HRV recording analysis, was derived from the electrical signal difference "between the left lower limb and left upper limb electrodes". (96)

Method of recording of HRV:
After 20 minutes of rest and positioning of electrodes, an electrocardiogram (ECG) was obtained for a duration of continuous 5 minutes continuous recording. After recording the result of HRV was analyzed by inbuilt software. The resting ECG signal was converted from analog to digital and analyzed using Fast Fourier Transformation (FFT) analysis.

Plasma BDNF level assessment:
Thermo Scientific Human BDNF ELISA Kit (EH42RB) was used for assaying the plasma BDNF levels and procedure was done according to manufacture's guidlines. The procedure adhered to all prescribed national guidelines for biohazard safety.

For BDNF assessment blood sample was centrifuged at 2-8^{o} C for 15 min at 1000xg and again at 10000xg for 10 min at 2-8^{o} C to remove platelet completely and the separated plasma was stored at -20^{o}C in the deep freezer.

Data analysis:
Data analysis was done by SPSS version 25 statistical softwear. Unpaired t test and Pearson's rank correlation were used to analyze and compare data and a p value less than 0.05 was considered significantly notable.

Results:
This study was carried out to study the association of BDNF and HRV in PCOS. For this purpose 90 women who were clinically diagnosed with PCOS, enrolled as PCOS Group and90 women who were with normal ovulatory cycles without PCOS, enrolled as Controls**.** Further, according to BMI, women in PCOS group were divided into two subgroups: Obese (OB) PCOS Group had BMI ≥25kg/m^2 (n=50) and Non Obese (OB) PCOS Group who had BMI <25kg/m^2 (n=40). Allwomen were evaluated for HR variability and plasma BDNF levels.

Table 1: Comparison ofAge, BMI and WHR between the PCOS group and Controls, using unpaired student t test:

Variables	**PCOS (n=90)**		**Control (n=90)**		
	Mean	**SD**	**Mean**	**SD**	**p-value**
Age (years)	23.37	±5.21	24.52	±4.25	0.105**(NS)**
BMI(kg/m^2)	26.44	±5.19	20.52	±1.62	**<0.001*** **
WHR	0.89	±0.99	0.78	±0.04	**<0.001*** **
SBP (mmHg)	114.67	±8.26	111.16	±7.68	**0.004****
DBP (mmHg)	75.13	±7.11	72.62	±5.86	**0.011***

(*P) (**P) =significant (***P) = highly significant and (NS) = not significant.

The age of PCOS group and controls was found to be comparable. PCOS group had significantly higher BMI, SBP and DBP as shown in Table 1.

Table 2: Comparison oftime domain, frequency domain and BDNF between PCOS group and Controls, using unpaired t test:

Variable	PCOS (N=90)		Control (N=90)		
	Mean	**SD**	**Mean**	**SD**	**p value**
Mean HR (bpm)	77.11	±4.68	75.10	±2.95	**0.001****
SDNN (ms)	60.13	±14.64	73.57	±13.19	**<0.001*****
rMSSD (ms)	50.54	±15.12	63.08	±13.98	**<0.001*****
LF (ms^2)	324.23	±116.21	361.07	±154.4	0.07 **(NS)**
HF(ms^2)	295.35	±159.13	565.75	±226.59	**<0.001*****
LFnu	53.51	±12.08	41.2	±9.19	**<0.001*****
HFnu	45.30	±10.15	59.74	±9.03	**<0.001*****
LF/HF	1.28	±0.53	0.67	±0.24	**<0.001*****
BDNF(pg/ml)	528.20	±220.49	505.31	±206.65	0.473**(NS)**

(**P) =significant, (***P) =highly significant

Table 2 shows, for time domain HRV parameters, a statistically significant difference in the Mean HR was noticed when PCOS group (77.11 ± 4.68, p=0.001) was compared to Control group (75.10 ± 2.95), highly significant difference in SDNN and RMSSD was noticed when PCOS group (60.13 ± 14.64 and 50.54 ± 15.12, respectively, p <0.001) was compared to Control group (73.42± 13.73 and 63.08± 13.98, respectively).

The frequency domain HRV parameters i.e LF, HF, LFnu, HFnu and LF/HF ratio of PCOS group. Table 2 shows, no notable difference in LF(p=0.07) when PCOS group (324.23±116.21) was compared to control group (361.07±154.4). Also, Table 2 shows, show a high statistically significant difference in the mean value of HF, LFnu, HFnu and LF/HF when PCOS group (295.35 ± 159.13, 53.51±12.08, 45.30 ±10.15, and 1.28 ±0.53 respectively, p <0.001) was compared to Control group (560.33±222.95, 41.2±9.19, 59.74±9.03 and 0.67±0.24 respectively).

The BDNF levels were comparable in both the groups as no significant difference was observed for BDNF levels when PCOS group (528.20±220.49, p=0.473) was compared to Controls (84.83± 7.26).

Table 3 Correlation analysis of BDNF and Frequency domain parameters of HRV in PCOS group, using Pearson's rank correlation

Variable	BDNF	
	r	**p value**
LF (ms^2)	-0.04	0.74**(NS)**
HF(ms^2)	**0.49**	**<0.001*****
LFnu	**-0.65**	**<0.001*****
HFnu	**0.56**	**<0.001*****
LF/HF	**-0.66**	**<0.001*****

(***p) = highly significant and NS = not significant

In order to find, any relationship between the frequency domain HRV parameters and BDNFin PCOS group, correlation analysis was performed among the HRV parameters and BDNF. There was no correlation observed between LF and BDNF (r=-0.04, p=0.74) andnegative correlation was observed among LFnu and BDNF (r=-0.65, p<0.001) andLF/HF and BDNF (r=-0.66, p<0.001). However, statistically notable correlation among HF and BDNF (r=0.49, p<0.001) and HFnu and BDNF (r=-0.56, p<0.001) was observed.

Discussion

In this study, BMI and waist hip-ratio were signifcantly higher as compared with that of controls. PCOS women also had significantly higher SBP and DBP values as compared Controls. These observations are suggestive of presence of obesity along with increased cardiometabolic risk in PCOS women. Balamurugan M *et al* (12) and Tiwari R et al(13)found that the LF (nu) and LF/HF to be remarkably higher in PCOS women as compared with that in controls. However, HF and

HF (nu) values to be significantly lower in PCOS women as compared with that in controls. Their findings suggestsympathetic dominance in PCOS women.

As it is known, more follicles are present in polycystic ovaries than in healthy ovaries during the different phases of development. BDNF and the TrkB receptor were found in "mural and cumulus granulosa cells" of human preovulatory follicles [(14)]. A higher level of BDNF was also observed in the follicles of women with PCOS and elevated level of BDNF shows coping mechanism for the underlying disease process.(15)

Kadoya M et al. established a significant connection between BDNF levels and several HRV parameters in humans, which serve as indicators of cardiac autonomic activities.

The current research indicates that both BDNF and HRV are influenced by autonomic nervous system. Disruption in the regulation of this system has been observed in PCOS implying the BDNF might play a role in the pathogenesis of PCOS.

Conclusion:

Sympathovagal imbalance was present in PCOS women, according to HRV analysis, in the form of increased sympatheticactivity and parasympathetic withdrawal in PCOS women. The continuous activation of the sympathetic nervous system over time increases their susceptibility to experiencing negative cardiovascular events at a younger age. Moreover, BDNF appears to be essential for regulation of CVS as it is required for heart rate modulation by increasing parasympathetic activity. Further, altered level of BDNF in plasma was observed in PCOS. Although there is no direct evidence linking BDNF and HRV in PCOS, it is plausible that there could be some interplay between these factors. Therefore, more research is required to fully comprehend the extent of BDNF involvement in the etiopathogenesis of PCOS.

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