ORIGINAL ARTICLE

PLASMA DOPAMINE LEVELS IN POLYCYSTIC OVARY SYNDROME WOMEN

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Background: Raised levels of testosterone, dehydroepiandrosterone-sulfate and problems in ovulation are the hallmarks of polycystic ovary syndrome (PCOS). The objective of this study was to compare the plasma dopamine (DA) and blood parameters (CBC) in polycystic ovary syndrome (PCOS) and normal women. **Methodology:** This cross-sectional comparative study was conducted at the University of Health Sciences (UHS), Lahore between Jan and Dec 2017 in reproductive women of age 18−40 years. Patients of PCOS (40) were recruited from the tertiary care hospitals of Lahore and their anthropometric measurements, physical examination, complete blood count and plasma DA levels were assessed. Age and weight matched samples (40), were also collected from normal women. CBC was performed using Medonic (MERCK) automated analyzer. Dopamine was measured according to competitive ELISA method. Data were collected on predesigned questionnaires and was analyzed using SPSS-20. **Results:** Plasma white blood cell (WBC) count (p=0.02) and platelet count (p=0.05) were significantly higher in PCOS than normal women. Median (IQR) plasma dopamine levels of PCOS women [5324 (3810–5900) pg/ml] were significantly higher (p<0.001) than the normal women [3908 (3470–4716) pg/ml]. **Conclusion:** PCOS women had higher WBC and platelet counts indicating inflammatory and hypercoagulable states in their body while raised plasma DA levels of PCOS women points towards the role of catecholamines in pathogenesis of PCOS.

Keywords: Polycystic ovary syndrome, PCOS, Dopamine, Blood pressure, Complete blood count, CBC
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INTRODUCTION

PCOS is the most common endocrinopathy in the women of childbearing age. Raised levels of testosterone, dehydroepiandrosterone-sulfate, and problems in ovulation are the hallmarks of this disease after the exclusion of other related hormonal disorders such as hyperprolactinemias, thyroid diseases and adrenal hyperplasia.[1]

Dopamine (DA) was first discovered by Arvid Carlsson as an independent neurotransmitter in the nervous system and not just a precursor of norepinephrine.[2] Granulosa cells in ovaries possess dopamine D1 and D5 receptors on their surface that perform their actions via cyclic AMP second messenger system. D2 and D4 receptors are also found inside these cells that act via the phospholipase C second messenger system.[3] The plasma DA levels can serve as an important tool for assessing the degree of sympathetic activity in humans.[4]

Exaggerated sympathetic response and altered function of innermost lining of blood vessels have been discovered in obese females with polycystic ovaries, irrespective of their excess adiposity. Adrenergic hyperactivity and defective endothelial function may pose increased chances of damage to heart in PCOS females.[5] In healthy individuals, the autonomic nervous system regulates heart rate and resistance in vessels to ensure proper functioning of cardiovascular system. PCOS women have higher heart rate, high blood pressure and increased sympathetic activity than healthy women. Higher cholesterol and triglycerides are also associated with increased sympathetic response in PCOS women. Cardiovascular diseases are highly prevalent in them because of autonomic imbalance and altered lipid profile.[6] Higher concentrations of DA and norepinephrine have been found in women with Stein Leventhal syndrome.[7] Some evidences exist on lower levels of DA in women suffering from PCOS. DA lowers GnRH levels and thus low DA levels lead to increased LH levels in PCOS.[8,9] There exists a controversy on DA levels in PCOS women.

High blood pressure was found in PCOS women as compared to women without PCOS. Hypertension occurs in PCOS women independent of effects of BMI and age.[10] Increased androgen levels are also associated with high blood pressure in PCOS women. Androgens may stimulate the proximal tubule rennin-angiotensin system and increase the absorption of sodium and water, thus increasing extracellular volume and blood pressure.[11]

The results of current study will help us in understanding complex mechanisms involved in the development of PCOS. This may help in exploration of new treatment options and decreased metabolic complications in PCOS patients and improve their quality of life. This study will also help in adding some knowledge regarding role of plasma DA levels in pathophysiology of PCOS women because previous studies have been equivocal and confusing regarding this parameter. This study will report CBC parameters and

plasma DA levels in Pakistani PCOS women and point to the importance of DA levels.

METHODOLOGY

This cross-sectional comparative study was conducted at the Physiology and Cell Biology Department of UHS, Lahore from January to December 2017 after approval from the Ethical Review Committee. Eighty (80) participants took part in this research. PCOS women (40), aged 18−40 years diagnosed by Rotterdam criteria, were recruited for the study from the tertiary care hospitals of Lahore. Weight and age matched normal (40) women, who were not relatives of PCOS patients, were taken from the local community. Convenient sampling technique was employed for sampling purposes.

Every individual was assessed by taking history and performing general physical examination. BMI cutoff values for South Asians were taken as standard for comparison. Gravid, or breast feeding women with a history of diabetes, psychotic disorders, irregularities in circadian rhythm or any other hormonal disease were excluded from the study. Those who had history of glucocorticoids use and anti-obesity drugs in last 6 months were not included.

The sample size was calculated by the following formula keeping the power of study as 95% and level of significance equal to 5%:[12]

$$n = \frac{(Z_{1-\beta}+Z_{1-\alpha/2})^2\,(\sigma_1^2+\sigma_2^2)}{(\mu 1-\mu 2)^2}$$

Patients and normal subjects provided written informed consent for participation in the study. Blood pressure (BP) was measured with a mercury sphygmomanometer. The subjects were allowed to rest for 10 minutes and then BP was taken in a sitting position. Three readings were taken and average was recorded as final BP.

After 10 hours of overnight fasting, blood samples were drawn from the antecubital veins of subjects after cleaning the area with a spirit swab. Blood was immediately shifted to a vacutainer containing ethylenediaminetetraacetic acid (EDTA). CBC was performed immediately in the laboratory using Medonic™ (MERCK) automated analyzer. Blood sample was then centrifuged for 15 minutes at 3,000 rpm at 4 °C in a centrifuge. The serum was extracted and stored at -80 °C for further analyses. DA was measured by competitive ELISA method using the kit by E-Lab Sciences (E-EL-0046; USA).

Analysis of data was done using SPSS-20. Data were presented as Mean±SD for continuous variables. The normality of data variables was checked by the Shapiro-Wilk test. Statistical significance was recognized with $p \leq 0.05$. Independent sample *t*-test was applied for normally distributed variables to detect differences between means in two independent groups. Mann-Whitney U test was used for comparison of medians of non-normally distributed data.

RESULTS

Forty patients with the clinical features or ultrasonography findings of PCOS and forty normal women were included in the study. The observed results are tabulated in Table-1 and Table-2 below.

Table-1: Anthropometric and biochemical parameters of women with and without PCOS

Parameters	PCOS (n=40)		Normal (n=40)		*p*
	Mean±SD	Median±IQR	Mean±SD	Median±IQR	
Age (Years)	-	21.0 (19.00–25.75)	-	20.0 (19.0–27.0)	0.99[b]
Height (metres)	1.56±0.06	-	1.57±0.52	-	0.35[a]
Weight (Kilograms)	69.9±12.25	-	68.40±9.96	-	0.53[a]
BMI (Kg/m^2)	28.49±4.91	-	27.46±3.47	-	0.28[a]
Age at menarche (Years)	-	12.0 (12.0–13.0)	-	13.0 (12.0–13.0)	0.20[b]
Systolic BP (mmHg)	-	110.0 (102.5–120.0)	-	110.0 (105.0–115.0)	0.13[b]
Diastolic BP (mmHg)	-	70.0 (70.0–80.0)	-	70.0 (65.0–70.0)	0.002[b*]
Dopamine (pg/ml)	-	5324 (3810−5900)	-	3908 (3470–4716)	0.001 [b*]

BP= Blood pressure, [a]*p*-value generated by Independent sample *t*-test, [b]*p*-value generated by Mann Whitney U test, *$p \leq 0.05$ is considered statistically significant

Table-2: Complete blood count parameters of women with and without PCOS

Parameter	PCOS (n=40)		Normal (n=40)		*p*
	Mean±SD	Median±IQR	Mean±SD	Median±IQR	
WBC Count (10^9 /L)	-	7.90 (6.72–11.42)	-	7.50 (6.22–8.30)	0.02[b*]
Lymphocytes (10^9 /L)	2.67±0.90	-	2.28±0.67	-	0.03[a*]
Granulocytes (10^9 /L)	-	5.20 (4.00–7.10)	-	4.35 (3.72–5.60)	0.03[b*]
Haemoglobin (Hb) (g/dL)	11.69±1.41	11.85 (10.95–12.37)	11.67±1.37	11.75 (11.30–12.57)	1.00
RBC Count (10^{12}/L)	-	4.48 (4.27–4.77)	-	4.48 (4.20–4.64)	0.32[b]
Platelet Count (10^9/L)	-	311.0 (268.5–366.7)	-	287.5 (242.7–346.5)	0.05[b*]
MPV (fL)	8.57±0.66	-	8.80±0.75	-	0.14[a]
PDW (fL)	12.43±0.90	-	12.67±1.01	-	0.26[a]

MPV= Mean platelet volume, PDW= Platelet distribution width, [a]*p* value generated by Independent sample *t*-test, [b]*p* value generated by Mann Whitney U test, *$p \leq 0.05$ is considered statistically significant

DISCUSSION

Increased leukocyte count itself contributes to a great extent in causing coronary artery blockage, heart attacks and deaths due to heart problems. Persons with leukocytosis are more prone to develop heart diseases even if they do not have other risk factors for heart disease.[13] In the current study, median WBC count was found to be 7.90 (6.72−11.42)×10^9/L in PCOS women. This median WBC count was in normal range in PCOS women but significantly higher when compared to normal women (p=0.02). WBC count was also found to be raised in Chinese obese PCOS women compared to healthy women.[14] A study conducted in 2015 also reported higher WBC count in White PCOS women that was positively correlated with BMI, androgen and triglyceride levels. This increased WBC count creates an inflammatory state in PCOS women that increases the risk of plaque formation in coronary arteries in these women.[15]

In the current study, mean platelet count was 323±88×10^9/L in the PCOS women compared to 288±64×10^9/L in normal women. The mean platelet count was in normal range in PCOS women but significantly higher as compared to normal women (p=0.05). Thrombocytosis is linked with raised androgen levels and hyperinsulinemia in PCOS women.[16] Platelet function is directly regulated by insulin via a functional insulin receptor (IR) present on human platelets. Normal insulin levels prevent platelet interaction with collagen and weaken the gathering of platelets in healthy persons. Insulin resistance and hyperinsulinemia cause defective insulin signalling, thus weakening the above-mentioned effect and hastening coagulability.[17] Testosterone controls the expression of thromboxane A2 (TXA2) receptors present on the platelets in humans. TXA2 constricts the vessels and increases the aggregation of platelets, thus increasing the chances of heart diseases.[18] Raised platelet count causes increased blood clotting and atherosclerosis in coronary arteries in PCOS women, thus increasing the risk of heart attacks in these women.[16] The platelet count was reported to be 311×10^9/L in obese Turkish PCOS women as compared to 298×10^9/L in healthy counterparts.[19] Raised platelet count were also found in white obese PCOS women as compared to women without PCOS.[20]

Noradrenaline and DA are produced in considerable amounts in human ovary and these chemicals are linked with other hormones release in the ovary. This suggests a very vital role of catecholamines in the overall functioning of human ovary in normal and pathological states.[21] In the current study, median dopamine levels in PCOS were 5324 (3810−5900) pg/ml and 3908 (3470−4716) pg/ml in normal women respectively, showing a highly significant increase in PCOS women (p=0.001).

Our results are similar to a study done by Saller *et al*[12] in which raised DA levels in the follicular fluid of PCOS women were found along with increased levels of enzymes involved in DA metabolism. Increased DA metabolism by enzymes (monoamine oxidase-B and dopamine active transporter) causes the production of free radicals, thus leading to apoptosis of the granulosa cells (GCs) in these patients.[12] Follicular fluid is an amalgam consisting of water and solutes derived from plasma and metabolites of follicular cells.[22] Gap junctions are present inside the follicle that link GCs with oocytes and allow the transfer of substances between each other. This connection between GCs and oocytes plays a vital role in the meiosis and proliferation of ovum. Granulosa cells are a source of nutrition for the developing ovum and also have a role in maintaining the acid base balance of ovum.[23] Oxidative damage affects many aspects necessary for fertility like satisfactory condition of egg, degree of zygote formation and the chances of a live birth. The follicle just before the release of egg is more vulnerable to destruction by reactive oxygen species.[24] In another recent study conducted in Turkey, greater amounts of DA (4010.15 pg/ml) were present in ovarian cells taken from PCOS women as compared to controls (3093.06 pg/ml). This also supports our findings.[7]

Our results are different from a study conducted in 2018 that showed decreased DA levels along with reduced D2 (Dopamine 2) receptors in brain tissues of PCOS rats. DA decreases GnRH release and thus low DA levels lead to increased LH levels in PCOS.[8] Our results are also in contrast with a study done in 2011 which revealed lower DA levels and lesser D2R expressions in Spanish PCOS women. Decreased DA production and increased metabolism of D2 receptors were associated with increased vascularization of ovaries in PCOS patients.[9] Further studies are needed on the role of DA and other catecholamines in the pathophysiology of PCOS.

Median systolic and diastolic BP of PCOS females was 110.0 (102.5–120.0) mmHg and 70.0 (70.0–80.0) mmHg respectively. There was a significant difference between PCOS and normal women regarding their diastolic blood pressure (p=0.002). Increased blood lipids and insulin resistance may contribute to raise diastolic BP in PCOS patients.[25]

Increased androgen levels have been associated with increased hepatic lipase (HL) activity. HL breaks down phospholipids on the surface of HDL causing the conversion of high density lipoproteins (HDL-2) to the smaller denser HDL-3. HDL-3 being a better substrate for the liver increases the clearance of HDL. Androgens, through interaction with the androgen receptor, also decrease the catabolic removal of low density lipoproteins. Androgen receptor directly interacts with oestrogen receptor and blocks its activity, thus

decreasing oestrogen receptor mediated induction of LDL receptor activity.[26] Increased insulin levels and insulin resistance are linked with higher sympathetic activity, increased angiogenesis, constriction of vessels, and increased sodium reabsorption that in turn causes raised blood pressure.[27]

CONCLUSION

Significantly higher WBC and platelet counts indicate inflammatory and hypercoagulable states in the PCOS women. Higher sympathetic activity in PCOS women is linked with high blood pressure.

LIMITATIONS

Limitations of the current study were lack of assessment of insulin, androgen and LH levels due to financial constraints. The results can't be validated in entire population because it was a cross-sectional study.

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