Endocrine Correlates of Polycystic Ovary Syndrome in Pakistani Women

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ABSTRACT

Objective: To systematically examine the hormonal variations and their interactions in a local cohort of PCOS patients.
Study Design: A cross-sectional, analytical study.
Place and Duration of Study: King Edward Medical University and associated Mayo Hospital, Lahore, Pakistan, from June 2009 to May 2010.
Methodology: The study comprised of 50 controls, 65 PCOS patients and 25 First Degree Relatives (FDRs) of patients, aged 18 - 45 years. Clinical examination, findings, detailed menstrual and family history was recorded through a comprehensive questionnaire followed by ovarian ultrasound examination. The blood samples were drawn for hormonal analysis by radioimmunoassay and immunoradiometric assay.
Results: Body mass index, Luteinizing Hormone (LH), LH:FSH ratio, insulin, testosterone, androstenedione and prolactin were significantly elevated in PCOS patients compared to control group. The FDRs of patients did not indicate significant variations when compared to control. LH and androstenedione were found to be significantly correlated.
Conclusion: The gonadotrophins, LH to FSH ratio and androgens are dependable diagnostic parameters of PCOS. LH appears to be the most important in this regard, leading to hyperandrogenism.

Key Words: Polycystic ovary syndrome. Endocrine changes. First degree relatives.

INTRODUCTION

PCOS is the most common female endocrine disorder in the developed world, Pakistan being no exception to it. It is a multifactorial and most debatable reproductive endocrinal disorder in the young females with highly controversial pathophysiology.[1]

The National Institutes of Health (NIH) criteria 1990 included hyperandrogenism, oligo-ovulation, and exclusion of other disorders mimicking PCOS, as the diagnostic criteria. However, 20 - 25% of regularly ovulating women have PCOS on ultrasound examination. The abnormal ovarian morphology is consistent with PCOS but not essential for diagnosis. Moreover, recent reports indicate that ovarian morphology is no longer an indispensable diagnostic criterion of PCOS.[2]

The endocrine society, however, recommended that PCOS can be diagnosed if the adult women presents with two of the following features i.e., excess production of androgens, anovulation and pearl-sized cysts found in the ovaries.[3]

Despite these criteria, PCOS may show differences in clinical features, based on the degree of severity of androgen levels, gonadotrophins, and insulin resistance. It has also been suggested that ethnicity as well as religious and cultural background of PCOS patients is an important contributory factor towards heterogeneity of PCOS.[4,5]

PCOS is being strongly associated with future development of type-2 Diabetes mellitus and hyperinsulinemia.[6] Thus, recognition of PCOS may be uncertain on account of discrepancies in diagnostic criteria. This is especially true about serum levels of gonadotrophins. Elevated LH concentration has been found to be the most significant link between PCOS-associated disorders of ovulation and raised Anti-Mullerian-Hormone (AMH) in the syndrome.[7] It was suggested that LH excess may be revealed by GnRH stimulation test. Moreover, raised LH:FSH ratio after GnRH stimulation might be highly beneficial as an additional diagnostic tool in the recognition of PCOS.[8]

The prevalence of PCOS in South Asian women, especially in Pakistani women is much higher (52%) as compared to White population (20 - 25% in UK). Local research that could give an insight into the indigenous factors causing the disease is scanty. The increasingly high incidence is attributed to genetic, environmental factors and intermarriages. Moreover, the relatives of women having PCOS have a high incidence of oligo-menorrhea.[9]

The current study was, therefore, planned to systematically examine the specific endocrinal changes and hormonal interactions in Pakistani cohorts of PCOS patients.

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METHODOLOGY

The study was approved by the Ethical Review Committee (ERC) of King Edward Medical University (KEMU) and Mayo Hospital, Lahore and conducted during June 2009 to May 2010. Initially 204 subjects were recruited for the study out of whom 140 agreed to participate potentially, fulfilled the criteria and selected as the subjects of the study. They were grouped into three categories as (i) controls, (ii) patients of PCOS and (iii) first degree relatives (FDR) of the patients. The participation rate was 36% in controls (n=50), 46% in PCO patients (n=65) and 18% (n=25) in FDR of the patients. Control subjects were randomly selected as a reference population for comparative purpose. They were healthy, non-pregnant, non-lactating and non-smoking for the previous 3 years and had regular menstrual cycles. The PCOS patients were selected from the infertility clinics at Lady Wallingdon Hospital, Lady Aitchison Hospital, Abdullah Diagnostic Clinic and Private Community based Family Planning Clinics in the city of Lahore. The FDR cohort was not presenting with clinical features of PCOS, approached through PCOS patients. They were mainly the sisters or daughters of PCOS women. FDRs of only seven PCOS patients agreed to participate in the study.

All the participants belonged to the same socio-economic status and age group (18 - 45 years). The objectives of the study were explained to each of the women and a written consent was obtained.

A detailed history of past gynaecological events of each patient was recorded on a proforma designed for the study. History of hypertension, diabetes mellitus, menstrual irregularities, weight gain, and malignancy of genito-urinary tract was recorded. Detailed menstrual history, including the date of the onset of first menstrual period, last menstrual period, duration of cycles, history of missed periods, or complete loss of cycles was also recorded. Each woman was examined for the presence of signs of acne. History of hair growth on the body and face region was recorded. Women with hepatic, renal or with other evident endocrine disorders, history of immuno-suppressive therapy, smoking or those on any form of drug treatment were excluded from the study.

The ultrasound diagnosis of PCOS was made on the basis of ESHRE/ASRM criteria i.e., 10 or more cystic follicles per ovary, varying in size from 2 - 9 mm or ovarian volume of 10 cm^3.[10]

The women were interviewed and examined for the signs and symptoms of PCOS. The clinical diagnosis was based on history, general physical examination and ultrasound (US) assessment (trans-abdominal in young, unmarried girls and vaginal US in the married women). The height (m) and weight (kg) were recorded for calculation of body mass index (kg/m^2). Body weight was recorded by digital weighing machine. During weighing, subjects were without shoes and wearing light clothings. The weight of average clothing was substracted to calculate the actual body weight of the participants. It was then interpreted and compared with reference to height (m), measured by a metric scale adjusted with the wall, through body mass index (kg/m^2).

The hormonal analysis were performed at RIA Laboratories, Centre for Nuclear Medicine (CENUM), Mayo Hospital, Lahore. Serum androstenedione and testosterone were determined by competitive radio-immunoassay while serum LH, FSH, prolactin and insulin were determined by a sandwich type immuno-radiometric assay, using commercially available kits.

All data was entered and analyzed by using Statistical Package for Social Sciences (SPSS) version-20. Mean ± standard deviation was used for quantitative variable like BMI and Median (IQR) inter-quartile range were used for LH, FSH, LH:FSH, testosterone, andro-stenedione and prolactin. Shapiro Wilk test was used to check the normality and found to be skewed except BMI. Therefore, non-parametric Kruskal Wallis test was applied for comparison and Spearman's rank correlation was applied to see any relationship between quantitative variables. One way analysis of variance (ANOVA) was applied on ranking data for Tukey's Post-Hoc multiple comparison. P-value ≤ 5% was taken as statistical significant.

RESULTS

The categorical variables of age-matched cohorts comprising of control subjects (n=50), patients of PCOS (n=65) and first degree relatives of PCOS patients (n=25) are presented in Table I, whereas, BMI and hormonal profiles are presented in Table II. Significant alterations were observed in comparable groups as indicated by one way ANOVA and Kruskal Wallis test at p ≤ 0.05 and CI of 95%. The results related to BMI, LH, FSH, LH:FSH ratio, insulin, testosterone, androstenedione and PRL are summarized in the ensuing lines.

The BMI in PCO group was significantly high indicating a 26% rise as compared with control group (p < 0.001). There was no significant difference in the control versus FDR group.

The LH elevated significantly in PCO group as compared with control group indicating a 60% rise (p < 0.001). In FDR group, LH varied insignificantly as compared to control group.

The FSH levels declined significantly in PCO and FDR groups as compared to controls. The reductions were 33% and 35% in PCOS and FDR group, respectively but there is no significant difference between FDR and PCOS.

The LH:FSH ratio in PCO versus controls was observed to be significantly higher (p < 0.001). The ratio of

Table I: Summary statistics of categorical variables in different cohorts of study.

Variables	Control group	PCOS group	FDR group
Mean age (years)	19.67	26.71	19.86
Amenorrhea (%)	Nil	75.38	Nil
Acne (%)	18.00	32.31	16.00
Hair growth (%)	10.00	63.08	05.71
Obesity (%)	06.00	81.54	08.00
Hypertension (%)	Nil	10.77	Nil
Mean age of menarche (years)	13.05	13.85	16.00
Family H/O infertility (%)	Nil	26.15	100.0
Family H/O hypertension (%)	34.00	44.62	36.00
Family H/O diabetes (%)	32.00	32.31	32.00

FDR = First degree relatives of PCOS patients; H/O = History of.

Table II: Comparison among Controls, PCOS patients and their first degree relatives (FDR).

Parameters	Controls (n=50)	PCOS (n=65)	FDR (n=25)	p-value
BMI (kg/m²)	20.81 ± 3.01	26.23 ± 4.46	20.11 ± 2.19	< 0.001#
LH (IU/L)	3.94 (7.27)	31.21 (43.15)	4 (2.81)	< 0.001*
FSH (IU/L)	5.12 (3.21)	3.86 (3.32)	3.67 (1.72)	< 0.001*
LH:FSH	0.79 (1.08)	10.04 (12.81)	1.15 (2.04)	< 0.001*
Insulin (µIU/ml)	5.97 (5.12)	36.3 (31.9)	3.89 (3.55)	< 0.001*
Testosterone (ng/ml)	0.70 (0.37)	1.12 (0.57)	0.32 (0.29)	< 0.001*
Androstenedione (ng/ml)	2.09 (1)	5.65 (3.38)	1.29 (1.18)	< 0.001*
Prolactin (ng/mL)	3 (2.18)	8.44 (6.49)	2.61 (1.24)	< 0.001*

*p-value is calculated by One Way ANOVA and data are represented as mean ± standard deviation.
#p-value is calculated by Kruskal Wallis test and data are represented as median (inter-quartile range).

LH:FSH differed non-significantly in FDR as compared to control group (p=0.997).

The PCO group manifested a five-fold highly significant rise in insulin level (p < 0.001). The FDR group demonstrated non-significant difference when compared with control group.

The testosterone level, in PCO group, was highly significantly raised, depicting 86% elevation as compared with control group (p < 0.001). The level, however, declined significantly by 40% in FDR group in comparison with control group (p=0.008).

The androstenedione was greatly elevated in PCO group as compared to controls indicating a highly significant rise of 163% (p < 0.001), however, no significant difference was observed between controls and FDR group. Furthermore, a significant correlation (r_s=0.375) was observed between LH and androstenedione.

As compared to control group, prolactin was significantly raised in PCO patients indicating 145% elevation of prolactin in this group (p < 0.001). No significant alterations, in the level of PRL, were observed when FDR were compared with control group.

Correlations of various hormones within different groups were worked out using Spearman's rank correlation. LH and androstenedione were found to be significantly correlated (r_s=0.375, p=0.0073, CI of 95%). No significant correlations were observed for any other hormones of the study.

DISCUSSION

Over the current years, hormonal analyses are being regarded as the principal diagnostic criteria of PCOS. It has been reported that insulin appears to disrupt all components of hypo-thalamo-hypophyseal-ovarian axis; and that hyperandrogenemia is the major factor responsible for the clinical features and complications of PCOS.[11]

The present study was, therefore, carried out to determine endocrine attributes of PCOS in the back drop of local ethnic, socio-economic and environmental element in Pakistani population. The elevated LH in PCOS has also been reported earlier.[12] However, the elevation in LH level, in this study, was significantly intense than the reported values.

The LH:FSH ratio in PCOS group, in comparison to control subjects, was raised by 18-folds in this study. This highly raised ratio may be on account of very high LH and significantly lower FSH values in PCOS patients.

It has been reported that most of the women (75%) with PCOS have an elevated LH level in the early follicular phase; and 94% have an enhanced LH to FSH ratio.[13] It has, therefore, been hypothesized that gonadotrophin dysfunction plays a key role in generating anovulatory amenorrhoea characteristic of PCOS women.[14] Moreover, raised LH:FSH ratio may prove to be beneficial in revealing PCOS by Gonadotrophin Releasing Hormone (GnRH) stimulation test. This test is suggested to be an important additional investigation in the diagnosis of PCOS based on elevated LH in serum.[8]

The LH:FSH ratio in PCOS demonstrates greater accuracy in prediction of PCOS than total testosterone and average ovarian volume in these women presenting with oligomenorrhea or anovulation. Further, an LH:FSH ratio of more than one presented the best combination of sensitivity and specificity.[15] It, therefore, appears that hormonal analysis especially LH levels and LH:FSH ratio are reliable predictors of PCOS as observed in the current study.

In addition, we have observed in this study, highly significantly raised insulin levels in PCOS patients indicating a 5-fold rise in this group (p < 0.001) may be attributed to high levels of LH. It has further been reported that women suffering from PCOS have higher rates of obesity and hyperinsulinemia.[14]

The higher levels of insulin can be a consequence of different ethnic backgrounds.[16] In Muslim immigrant women, infertility on account of PCOS was attributed mainly to ethnicity and socio-cultural background in Austria. It has been described that Islamic women had a

very high reproductive pressure like stress of being barren and not producing males for economic bases.[5] However, Korean women having PCOS do not suffer from hyperandrogenism; and are less likely to develop metabolic dysfunction, insulin resistance and hypertension. These women have reduced possibility of having metabolic syndrome, and are mild phenotypes of PCOS without hyperandrogenism.[6]

Circulating testosterone levels in the PCOS group, in this study, were highly significantly raised. Further, androstenedione was greatly elevated in PCO group as compared to controls. There was no significant difference, however, in controls and FDR group for circulating androstenedione levels. A significant correlation was observed between LH and andro-stenedione ($p < 0.05$) in the PCOS group only. It has been reported that administration of supplements of essential amino-acids significantly decreased total testosterone, and LH to FSH ratio. This consequently improved the clinical features of hyperandrogenism in PCOS.[17] It appears that hyperandrogenism is related to and is a consequence of hyperinsulinaemia. Insulin resistance is highly prevalent in PCOS and is also closely associated with its clinical features such as hyperandrogenism, oligo- or amenorrhea.[18,19] Moreover, insulin resistance is a common metabolic abnormality seen in obese and to a lesser degree in lean women with PCOS.[20] It has been reported that treatment of PCOS patients with diet, exercise, medication or surgical wedge resection improves not only insulin resistance but also corrects hyperandrogenemia and restores normal ovarian function.[21] Moreover, it has been studied, *in vitro,* that insulin directly influences steroid biosynthesis in ovaries.[22] The ovarian follicular immaturity, specially 2 - 5 mm follicles, has been associated with hyper-insulinism. Moreover, waist circumference is strongly related with insulin resistance and follicular arrest. This may lead to excessive LH production by feedback stimulation of pituitary.[23] The highly significantly raised serum insulin, testosterone and androstenedione levels in PCOS patients, in this study, strongly support that hyperinsulinaemia could contribute towards patho-physiology of PCOS.

The Body Mass Index (BMI) in patients of PCOS, in this study, was significantly higher. The BMI was linearly related with LH, in this study. The effect of BMI on LH is reported to be mediated at the pituitary level in PCOS. However, it has been observed by some researchers that BMI has an inhibitory effect on LH pulse and LH response to a weight-based dose of GnRH.[14] It has, hence, been suggested that there might be some link between body mass index and LH; it may provide clues for deeper insight in the patho-physiology of PCOS.[15]

Most recently, raised leptin levels have been found to be associated with obesity; it has also been implicated to influence FSH, Testosterone (T), obesity and PCOS.[24] It has also been observed that PCOS when associated with obesity leads to elevation of proinsulin concen-trations, that is a known indicator of fertility outcomes in PCOS women. Moreover, treatment with metformin antagonises proinsulin and decreases the fertility outcomes in these patients of PCOS.[25]

The present study of PCOS patients is focused on gonadotrophins, androgens and insulin levels mainly. Our data supported the renewed interest in the importance of endocrine parameters in the diagnosis of PCOS. However, attempts to predict PCOS risk in asymptomatic subjects could not be successful as the FDR group was small in number (n = 25). This was on account of decreased participation (18%) of the first degree relatives of PCOS patients who avoided investigations on account of being labeled as "infertile family".

CONCLUSION

The present results reinforce that the criteria of endocrinal diagnosis of the syndrome, especially gonadotrophins and LH to FSH ratio are important aids in the description of PCOS. It, however, seems mandatory that molecular investigations like protein biomarkers in PCOS, in addition to endocrinal correlations, be studied to arrive at exact and early diagnosis of the syndrome.

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