Research article

Association of prolactin and insulin with obesity in women with polycystic ovarian syndrome

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ABSTRACT

Introduction and Aim: Polycystic Ovarian Syndrome (PCOS) manifests with menstrual irregularities, infertility, galactorrhoea and hirsutism. Hormonal irregularities are found to be the pathogenesis behind PCOS. The study was aimed to evaluate whether obesity is associated with serum prolactin and insulin levels in PCOS women.

Materials and Methods: This case control study was conducted in a tertiary care hospital in Chennai. Control group included 30 apparently healthy women and cases included 30 PCOS patients, diagnosis based on Rotterdam criteria. The individuals were of 20-40 years of age in both the groups. The serum prolactin and insulin were analysed by ELISA. The obtained data were checked for normality of distribution and subjected to statistical analysis. Receiver Operating Characteristics (ROC) curve was done. Statistical analyses were performed using SPSS version 16.0. P less than 0.05 was considered statistically significant.

Results: The median and interquartile range (IQR) of serum insulin were 11.5 (1.8-70) and 5 (1.8-44) mIU/L in cases and controls respectively. The cut-off level of insulin was 7.0 mIU/L. The median and IQR of serum prolactin were 12.5 (4.5-53.0) and 12 (3.5-20) ng/mL in cases and controls respectively. BMI was positively correlated with age of the participants and prolactin.

Conclusion: PCOS is prevalent among obese reproductive individuals. Insulin was markedly elevated in PCOS individuals. Insulin resistance alters levels of luteinizing hormone and sex hormone binging globulins. Hyperprolactinemia is associated with anovulation or oligoovulation, galactorrhoea and hirsutism. In obese PCOS women, prolactin and insulin could be diagnostic markers of PCOS.

Keywords: PCOS; hyperprolactinemia; hyperinsulinemia; insulin resistance; phenotypes.

INTRODUCTION

Polycystic ovarian syndrome (PCOS) is a metabolic disorder affecting women, with the prevalence ranging from 4% to 18%. Prevalence varies widely based on the criteria used for diagnosis of PCOS (1). In 1996, the prevalence was found to be 6–8% (2). The prevalence has been increasing worldwide due increase in individuals with obesity and diabetes mellitus (3). Based on Rotterdam criteria, the prevalence in China and Sri Lanka were 7.5% and 6% respectively. Depending on the National Institute of Health (NIH) criteria, the prevalence was 5-8% among Caucasian middle-aged women. Wide variations in the prevalence across the globe is due to the heterogenous presentation of the disorder, different ethnic and age groups and variations in the diagnostic methods (4). Presence of type 2 diabetes mellitus (T2DM), hypertension, obesity and dyslipidemia lead to metabolic syndrome (MetS) in PCOS individuals. Thus around 40% individuals are at risk for atherosclerosis and myocardial infarction (2). Women with PCOS vary in the clinical presentations; there can be mild irregularity in menstruation, menorrhagia, amenorrhea, infertility, features of hyperandrogenism and IR. Hyperandrogenism manifests as acne and hirsutism. The clinical manifestations vary depending on the genetic makeup of the individual, race, ethnicity, and lifestyle factors (5). This study was undertaken to evaluate whether obesity is associated with serum prolactin and insulin levels in PCOS women.

MATERIALS AND METHODS

Study participants

The single centre prospective case control study included 60 women in the age group of 20 to 40 years. The PCOS individuals (n=30) were recruited from the Department of Obstetrics and Gynecology of Sri Ramachandra Medical College and Research Institute. The PCOS cases were diagnosed by Rotterdam criteria.

The control individuals included 30 apparently healthy women without features of PCOS and hyperprolactinemia, pregnant women in the luteal phase of the menstrual cycle. Individuals with diabetes mellitus, hypertension, dyslipidemia, endocrine, liver, and renal disorders, patients on anabolic or androgenic drugs, oral contraceptive pills, clomiphene citrate, smokers and alcoholics were excluded from the study.

Analysis of the variables

Serum prolactin was assayed by sandwich Enzyme Linked Immunosorbent Assay (ELISA) from Bio-Detect. (Ref no. KT 804). The methodology had the sensitivity of 0.002 ng/mL and measurement linearity was 10,000 ng/mL. The Human Insulin was assayed by sandwich ELISA from Epitope Diagnostics, Inc. (Ref KT-886) with the sensitivity of 0.1817 mIU/L and measurement linearity was 138 mIU/L.

Ethics statement

Ethics approval was obtained from the Institutional Ethics Committee (Ethics No.: CSP-MED/18/AUG/45/109, dated 24-09-2018). The participants gave written informed consent for participating in the study.

Statistical analysis

The obtained data were subjected to normality of distribution using Kolmogorov- Smirnov test. Based on the type of distribution, mean and standard deviation or median and interquartile range (IQR) were used. Comparison of the data was done by Spearman correlation. Receiver Operating Characteristics (ROC) curve was done to arrive at the cut-off values of prolactin and insulin. Statistical analyses were performed with the SPSS version 16.0. *P* value less than 0.05 was considered statistically significant.

RESULTS

The study included 30 apparently healthy individuals as controls and 30 PCOS patients diagnosed based on Rotterdam Criteria. The demographic and biochemical characteristics are shown in Table 1. The participants were 60 in number, distributed equally among the PCOS patients (cases) and controls. The participants were 20 to 40 years in both cases and controls. The median age was 26.5 years and 24.5 years in cases and controls respectively; no statistically significant difference was obtained (*P*=0.61). Most of the individuals were between 20 and 30 years of age in cases (n=25) and controls (n=21); whereas in the age groups of 31 to 40 years, there were 5 participants in cases and 9 participants in controls. There was no statistically significant difference between the age groups (*P*=0.77). Among PCOS patients, around 28% of individuals have a higher body mass index (BMI) than normal. The mean BMI of the individuals were 25.72 and 24.39 Kg/m^2 in cases and controls respectively. (Table 1)

Table 1: Demographic and biochemical characteristics of the study participants

Variables	Case (n=30)	Control (n=30)	P value
Age (years)			
All #	26.5(20-40)	24.5(20-40)	0.61
20-30	24.5±3.62	24.4±2.79	0.77
31-40	35.8±3.63	35.2±3.73	
BMI(Kg/m^2)			
All	25.72±5.26	24.39±3.51	0.23
<22.9	7±11.7	9±15.0	0.27
23-24.9	8±13.3	9±15.0	
>25	15±25.0	12±20.0	
Prolactin (ng/mL)#	12.5(4.5-53.0)	12(3.5-20)	0.11
Insulin (mIU/L)#	11.5(1.8-70)	5(1.8-44)	**0.006****

#: expressed in median and interquartile range (IQR); rest of the variables as mean ±SD; BMI: Body Mass Index; **P value: Highly statistically significant

The median and interquartile range of serum prolactin were 12.5(4.5-53.0) and 12(3.5-20) ng/mL in cases and controls respectively, and there was no statistical significance between the groups (*P*=0.11). The median and interquartile range of serum insulin were 11.5 (1.8-70) and 5 (1.8-44) mIU/L in cases and controls respectively, and there was statistical significance between the groups (*P*=0.006). (Table 1)

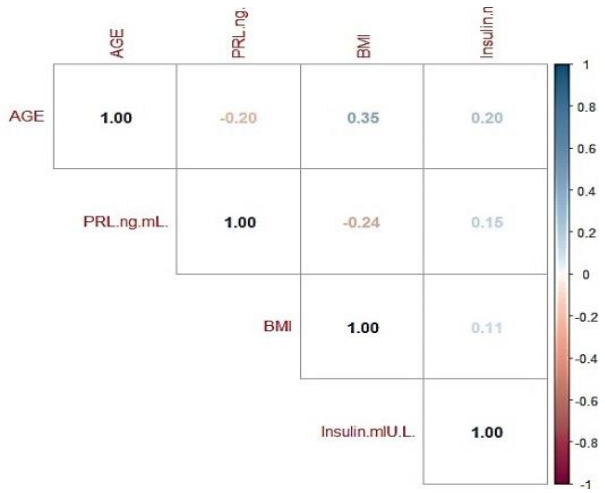

Fig. 1: Correlation among the demographic and biochemical variables (PRL: prolactin)

BMI showed positive correlation with age (r=0.35) which was statistically highly significant (P=0.001) and positive correlation with prolactin (r=0.24) which was significant (P=0.01). BMI did not show correlation with serum insulin (r=0.11). Insulin and prolactin showed positive correlation with age but were not statistically significant. Insulin did not show correlation with prolactin. (Fig. 1; Table 2)

Table 2: Correlation among the demographic and biochemical variables

Variables	Age (r value)	Prolactin (r value)	BMI (r value)	Insulin (r value)
Age		0.2	0.35 **	0.2
Prolactin	0.2		0.24*	0.15
BMI	0.35**	0.24*		0.11
Insulin	0.2	0.15	0.11	

**: P value statistically highly significant; *: P value statistically significant

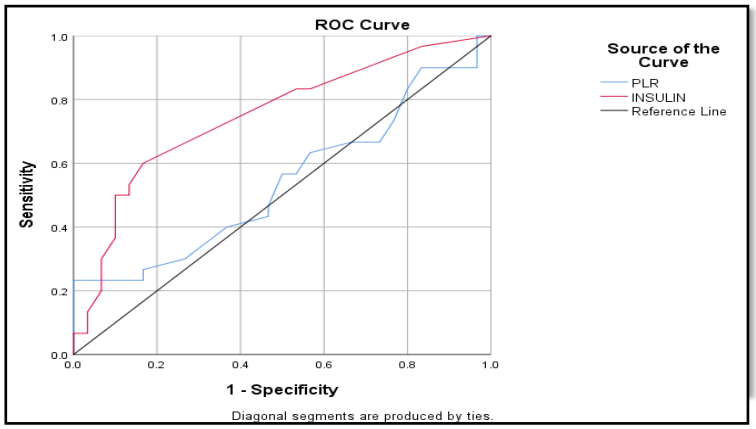

Fig. 2: Receiver Operating Characteristic (ROC) curves insulin and prolactin

Table 3: The cut-off value of serum insulin

Variable	AUC	Cut-off values	95% CI	P value	Sensitivity	Specificity
Prolactin	0.541	8.75	0.35, 0.68	0.584	60	17
Insulin	0.749	7.0	6.2, 8.7	0.001**	95	83

95% CI: 95% confidence interval; **: P value statistically highly significant

That ROC curve showed the area under the curve (AUC) for prolactin was 0.541 with cut-off value of 8.75 ng/mL (95% CI: 0.35, 0.68) with sensitivity of 60% and specificity of 17% with P value of 0.584. The AUC for insulin was 0.749 with a cut-off value of 7.0 mIU/L (95% CI: 6.2, 8.7) with sensitivity and specificity of 95% and 83% respectively (*P* =0.001). (Fig. 2; Table 3)

DISCUSSION

Polycystic ovarian syndrome (PCOS) is a common multisystem disorder in women of child bearing age (4). In addition to infertility and menstrual disorders, PCOS is associated with various metabolic disorders such as obesity, diabetes mellitus and cardiovascular disorders. Various genetic, epigenetic and environmental factors are found to play roles in the complex pathogenesis of PCOS (6). Based on the NIH criteria (1990), the prevalence of PCOS was 5 to 9%; but as per Rotterdam criteria (2003) the prevalence was as high as 19.9% (7). Various diagnostic criteria for PCOS (Table 4) have been defined at different time intervals (1).

In the present study, the predominant distribution of participants was in 20 to 30 years which could be due to the presenting clinical characteristics such as menstrual irregularities or fertility problems, in addition to other phenotypic clinical features of PCOS. (Table 1). According to Louwers *et al.*, PCOS is common in 40% of adolescent girls. The features of PCOS are present within 8 years after menarche; usually diagnosed by ultrasound of ovaries. Women with normal menstrual cycles but with high androgen levels are at risk of developing anovulation in future (9). As per Azziz *et al.*, prepuberty children present with premature puberty whereas in adolescents it presents with features of androgenization and postmenopausal women show features of atherosclerosis, diabetes mellitus and dyslipidemia. There is increased intima media thickness of carotid arteries and calcification of coronary arteries (7).

In the present study, when the study participants were classified as per the Asia-Pacific Classification on BMI, 15 individuals were obese in the case group whereas 12 individuals were in obese category in controls (Table 1). In the present study, BMI showed positive correlation with age (r=0.35, P=0.001) (Fig. 1; Table 2). According to Wang *et al.*, PCOS individuals are prone to be obese and chances of abortion are common in these individuals (10). As per

Alexandraki *et al.*, obesity is more prevalent in the USA compared to that in Europe. Obese individuals also develop nonalcoholic steatohepatitis and nonalcoholic fatty liver disease, in addition to diabetes mellitus (2). According to Joshi *et al.*, PCOS individuals are obese with high serum testosterone and free androgen index (FAI). Obese individuals have low sex hormone binding globulin (SHBG) and hyperinsulinemia (4). Obese PCOS individuals have lower serum adiponectin levels compared to nonobese PCOS women. Polymorphisms of genes involved in insulin function and steroidogenesis are seen in obese PCOC women (6).

In the present study, when the levels of serum insulin were compared between cases and controls, there was statistical significance between the groups (*P*=0.006). (Table 1) Insulin showed positive correlation with age but was not statistically significant. Insulin did not show correlation with BMI (r=0.11). (Fig. 1; Table 2) ROC showed that the AUC was 0.749 with cut-off value of 7.0mIU/L with sensitivity and specificity of 95% and 83% respectively (*P* =0.001). (Fig. 2; Table 3). Barber *et al.*, have shown that insulin resistance (IR) is a common abnormality in 50-90% of PCOS individuals (11). In women with PCOS, IR appears to be related to abnormal insulin-independent serine phosphorylation of the beta-subunit of insulin receptor and insulin receptor substrate 1 (IRS1) (2). PCOS women have decreased uptake of insulin by liver with consequent hyperinsulinemia (7). Hyperinsulinemia stimulates ovaries to produce more androgens resulting in ovulation problems (6). Insulin acts along with luteinizing hormone (LH) and activates *CYP17* which codes for an enzyme involved in the ovarian androgen synthetic pathway. Insulin also impairs preantral follicle development. (Fig. 3) In patients with PCOS, phosphatidylinositol 3-kinase (PI3K) pathway does not function normally while mitogen-activated protein kinase (MAPK) pathway functions normally resulting in glucose intolerance and steroidogenesis (11).

Table 4: Various Diagnostic criteria for PCOS (1)

Adult Diagnostic Criteria (Rotterdam) Otherwise, unexplained alternative phenotypes:	**Adolescent Diagnostic Criteria Otherwise, unexplained combination of:**
Phenotype 1 (classic PCOS) - Clinical &/or biochemical evidence of hyperandrogenism (H) - Evidence of oligo anovulation/oligomenorrhea (O) - Ultrasonographic evidence of a polycystic ovary (P)	***Abnormal uterine bleeding pattern*** - Abnormal for age or gynecologic age - Persistent symptoms for 1-2 years
Phenotype 2 (Essential NIH Criteria) - Clinical &/or biochemical evidence of hyperandrogenism (H) - Evidence of oligo anovulation/oligomenorrhea (O)	***Evidence of hyperandrogenism*** - Persistent testosterone elevation above adult norms - Moderate-severe hirsutism is clinical evidence of hyperandrogenism
Phenotype 3 (ovulatory PCOS) - Clinical &/or biochemical evidence of hyper androgenism (H) - Ultrasonographic evidence of a polycystic ovary (P)	
Phenotype 4 (non-hyperandrogenic PCOS) - Evidence of oligo anovulation/oligomenorrhea (O) - Ultrasonographic evidence of a polycystic ovary (P)	

H: hyperandrogenism; O: oligomenorrhoea; P: Polycystic ovary

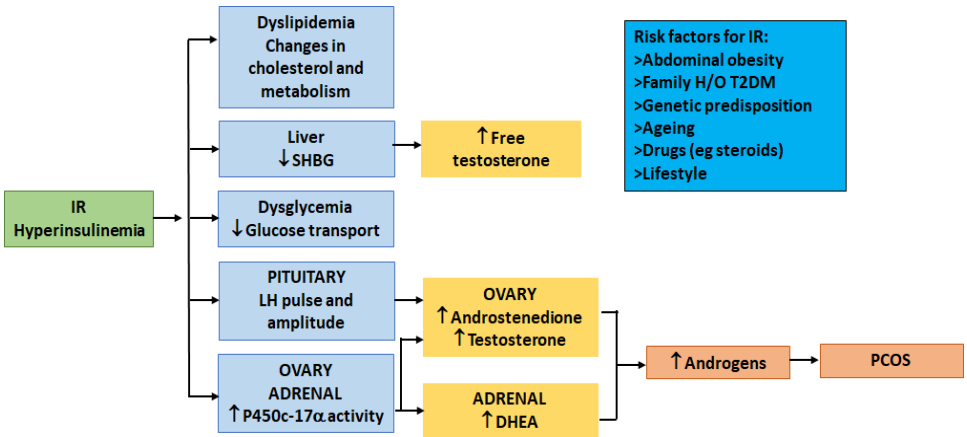

Fig.3: The overview of insulin resistance in PCOS (11)
IR: insulin resistance, LH: Luteinizing hormone, SHBG: Sex hormone binding globulin, DHEA:Dehydroepiandrosterone, PCOS: polycystic ovarian syndrome

In the study by Joshi *et al.,* PCOS women have high LH, testosterone and insulin compared to non-PCOS individuals (4). As per Rosenfield *et al*., there is hyperinsulinemia and IR in PCOS, increased lipogenesis with further adipogenesis and decreased lipolysis; these are presumed to contribute to the development of obesity. Insulin-resistant hyperinsulinemia causes low-grade inflammation which upregulates ovarian androgen production (8). Women with features of severe ovarian dysfunction also have severe IR (12). Proinflammatory markers are elevated in PCOS (13). CRP/albumin ratio is a stronger predictor of PCOS than either androgen or insulin resistance alone. (14). Pro-inflammatory cytokines suppress insulin-mediated glucose uptake in adipocytes. PCOS women have lower lipoprotein lipase activity and impaired catecholamine-induced lipolysis (6).

In the present study, when the levels of serum prolactin were compared between cases and controls, there was no statistically significant difference between the groups (Table 1). Prolactin causes milk secretion from the breast and dopamine in the median eminence and inhibits the effects of gonadotropins (3). As per Saleem et al., the mature prolactin is a 23 kDa monomer; in circulation exists in several forms- monomeric prolactin, dimeric prolactin (48-56 kDa) and polymeric macroprolactin (>150 kDa). Macroprolactin comprises]23kDa prolactin and IgG autoantibodies. Both dimeric and polymeric forms have minimal biological activity (15). Hyperprolactinemia is characterized by menstrual irregularities, galactorrhoea and hirsutism. By inhibiting the gonadotropin releasing hormone (GnRH) pulsatile secretion, hyperprolactinemia causes anovulation. By treating hyperprolactinemia, the normal menstrual cycle and fertility return back to normal (16). According to Delcour et al., hyperprolactinemia in PCOS could be due to a common hypothalamic-pituitary abnormality. Women with PCOS have decreased FSH and increased LH. Normally dopamine slows down the secretion of LH. There is no convincing link between PCOS and hyperprolactinemia (16).

In the present study, prolactin showed positive correlation with BMI (r=0.24, P=0.01). Prolactin showed positive correlation with age but was not statistically significant. Prolactin did not show correlation with insulin. (Fig. 1; Table 2) The ROC showed the area under the curve for prolactin was 0.541 with cut-off value of 8.75 ng/mL (95% CI: 0.35,0.68) with sensitivity of 60% and specificity of 17% with P value of 0.584. (Fig. 2; Table 3) According to Goyal *et al.,* both PCOS and hyperprolactinemia induced hyperinsulinemia (3). Prolactin receptors are present in endometrium, prostate, pancreatic islets, and adipocytes, where it is also involved in effects on food intake, body weight gain, and IR via inhibiting adiponectin and IL-6 production in the adipose tissue leading to T2DM (17). Murdoch *et al*, stated that hyperprolactinemia in PCOS is due to increased estrogen and decreased dopamine (18). When serum prolactin is within the biological reference interval, insulin sensitivity is good with normal blood glucose and absence of MetS (19). Samuel *et al.,* stated that increased fatty acid entry into the liver results in hypertriglyceridemia and fatty liver disease (20).

Women with PCOS are at risk of atherogenicity as evidenced by subclinical atherogenesis in the carotid arteries (2). The predisposing factors include dyslipidemia, diabetes mellitus, obesity and insulin resistance. Around 40% of women are found to be hypertensive also, but the findings are inconsistent. These risk factors have the potential to reduce the concentration of SHBG. SHBG levels could predict the endothelial dysfunction and is correlated with BMI and percentage body fat (2). Increase of SHBG levels is associated with an improvement in endocrine and metabolic parameters in these women. Obesity in these individuals leads to increased production of proinflammatory cytokines by visceral adipose tissue (21). Obese women with PCOS have impaired lipolysis, production of proinflammatory cytokines, and insulin resistance in adipocytes (7).

Limitations

The same size is small which prevents us from arriving at the actual results. The study could becarried out as a cohort study to assess the prognosis of the individuals with PCOS. To study the association of insulin and prolactin with other hormonal irregularities expected in these individuals. To correlate with clinical presentation and ultrasonography of the ovaries.

CONCLUSION

Polycystic ovarian syndrome is a complex disorder with high heterogeneity in presentation either clinically or variations in biochemical markers. Usually, the diagnosis is done by disease of exclusion. It is prevalent among obese reproductive individuals. The clinical presentation in PCOS individuals is highly variable; hence diagnosis is supported by alterations in laboratory biomarkers. Insulin seems to be more promising with marked elevations in PCOS individuals. Hyperinsulinemia and insulin resistance alter luteinizing hormone and sex hormone binging globulins. This results in altered hormone production by the ovaries. Hyperprolactinemia is characterized by menstrual irregularities, galactorrhea and hirsutism. Hyperprolactinemia inhibits pulsatile secretion of GnRH resulting in anovulation. BMI was correlated with serum prolactin and age. Even though the cut- off value of insulin had better sensitivity and specificity, prolactin shows a better relationship with BMI. Hence in obese PCOS women, prolactin and insulin derive the attention as potential diagnostic markers of PCOS.

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CONFLICT OF INTEREST

The authors declare that they did not have any conflict of interest.

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