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Study of Insulin Resistance in Polycystic Ovarian Syndrome

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Authors' contributions

This work was carried out in collaboration between all authors. Author SMRU designed the study, wrote the protocol and supervised the work. Author MN carried out all laboratories work and performed the statistical analysis. Authors MN, SMRU and BMR managed the analyses of the study. Author MN wrote the first draft of the manuscript. Authors MN and PVL managed the literature searches. Author SMRU edited the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To evaluate Insulin resistance in patients with Polycystic ovarian syndrome (PCOS). To determine if HOMA & G:I can be used as a simple marker to identify PCOS patients at risk for IGT & Type II DM.
Study Design: This was a hospital based, cross sectional study.
Place and Duration of Study: The present study was carried out in the department of Biochemistry in collaboration with the Gynaecology & Obstetrics department, Rajarajeswari Medical College and Hospital, Bengaluru. It was done over a period of 6 months from February 2015 to July 2015.
Methodology: The study was conducted with 85 women, 45 PCOS cases (USG diagnosed) and 40 controls (with regular menstrual cycle) in the age group of 20-40 years. Insulin resistance indices namely, Fasting Insulin, Glucose insulin ratio (G:I) and Homeostatic model assessment (HOMA) were calculated from the values of Fasting blood sugar and fasting Insulin estimated by

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Chemiluminiscence immunoassay. Cases and control were further subdivided based on age as Group I (20-30 yrs) and Group II (31-40 yrs).
Results: Fasting blood sugar, Fasting insulin, G:I and HOMA were significantly higher (P = 0.0137, 0.0018, 0.0475 and 0.0047 respectively) in cases than in controls. There was no significant difference between cases and control with respect to age, BMI and waist circumference (P = 0.7342, 0.3538 and 0.4841 respectively). When the cases were subdivided, BMI was significantly high (P 0.0001) in Group II as compared to Group I. IR markers like Fasting insulin and HOMA were higher in Group I compared to Group II but not statistically significant.
Conclusion: Fasting Insulin, HOMA & G:I can be used as simple, practical and effective marker to identify PCOS patients who are at risk of Type II DM. The data suggests that patients having Fasting Insulin > 20 IU, HOMA >2.5 & G:I < 4.5 should be closely monitored & considered as high risk for Type II DM.

Keywords: PCOS; insulin resistance; HOMA; G:I; fasting insulin.

1. INTRODUCTION

Polycystic ovary syndrome (PCOS) is a complex, multifactorial endocrine disorder affecting 5%-10% of all women of reproductive age [1,2]. It was first described by Stein and Leventhal [3] as association of bilateral polycystic ovaries with signs of amenorrhea, oligomenorrhea, hirsutism and obesity and it was referred to as polycystic ovary disease, later on to be known as PCOS to reflect the heterogeneity of this disorder.

The etiology of PCOS is complex and incompletely understood [4,1]. However, it is generally accepted that insulin resistance plays a key role in its pathogenesis [1]. Burghen et al. [5] first noted the association between PCOS and hyperinsulinemia. Subsequently studies confirmed IR as the cause of hyperinsulinemia in PCOS and a close association was found between disturbance of insulin metabolism and IR in obese and non-obese PCOS women [6]. The association of IR and anovulatory hyperandrogenism is commonly found throughout the world and among different ethnic groups [7].

PCOS can be defined based on 3 different criteria; the National Institute of Health (NIH) [8], the European Society of Human Reproduction and Embryology / American Society for Reproductive Medicine (ESHRE/ASRM, Rotterdam) [9] and the Androgen Excess Society (AES) [10].

All of these include anovulation, hyperandrogenism (clinical &/or biochemical) & polycystic ovaries with the exclusion of other androgenic, pituitary or adrenal cause.

Insulin resistance, a subnormal target tissue response to a given amount of insulin, is a common feature, but not a diagnostic criterion of PCOS. It has a prevalence as high as 70% and is a risk factor for developing type II diabetes mellitus [11] passing through a stage of impaired glucose tolerance (IGT). IR leads to compensatory hyperinsulinemia with diverse effects on adipose tissue and increased androgen production. Androgens may lead back to IR by increasing levels of free fatty acids and increasing muscle tissue mass, perpetuating this IR-hyperinsulinemia-hyperandrogenemia cycle [12].

PCOS has wide metabolic and cardiovascular implications, including not only infertility and obesity but also an increased lifetime risk of Type II Diabetes Mellitus, hypertension, oxidative stress, dyslipidemia and cardiovascular diseases [13,14-18]. Studies have shown that hyperinsulinemia is an independent risk factor for cardiovascular disease and it is a well-known fact that it precedes Type II DM [19].

Therefore, considering the severe consequences PCOS exerts on the health & lifestyle of the affected women and IR adding upon those risks, it is of utmost importance to unravel the intricate pathophysiologic cross link between PCOS and IR. The purpose of our study was to evaluate the relationship between IR & PCOS.

2. MATERIALS AND METHODS

The study was conducted by the Department of Biochemistry, Rajarajeswari Medical College & Hospitals. Women who attended the outpatient department of Obstetrics and Gynecology were selected as the study group over a period of 6 months. 45 women diagnosed with PCOS - as per Rotterdam ESHRE/ASRM criteria, in the age group of 20-40 years were taken as cases. According to ESHRE and ASRM presence of any

two of the following three criteria can be used for diagnosis (a) polycystic ovaries on ultrasound scan; (b) oligo and /or anovulation; and (c) clinical or biochemical evidence of hyperandrogenism, provided other etiologies (congenital adrenal hyperplasia, androgen secreting tumors, Cushings syndrome) have been excluded. Also the presence of 12 or more follicles in each ovary, measuring 2-9 mm in diameter, and or increased ovarian volume (>10 ml) is considered as morphological diagnostic criteria based on ultrasonography [20,21]. The basis of cases selection in our study was also the same.

Cases have been selected according to Rotterdam criteria. Ultrasonographically 9-11 cysts when identified and also considering ovarian volume, women are classified as having polycystic ovary. That does not define them as PCOS. But other than USG we have categorized them according to clinical sign and symptoms, amongst which irregular menstrual cycle is leading followed by hirsutism and infertility. So the basis of selecting control was age matched apparently healthy women with regular menses. Patients with other etiologies of hyperandrogenism (clinically diagnosed) like Congenital adrenal hyperplasia, Androgen secreting tumors or Cushings syndrome were excluded from the study. Pregnancy, hypertension, diabetes and other critical illnesses were also excluded. Patients satisfying the inclusion criteria were enrolled in the study after obtaining written informed consent from all individual participants. Ethical clearance was obtained from the Ethical review committee of the institution.

Polycystic ovarian syndrome is characterized by hyperandrogenism, anovulatory infertility and profound insulin resistance.

A thorough medical history and detailed physical examination was performed for each individual.10 ml fasting venous blood (8-10 hrs of overnight fasting) was collected in the clot activator & fluoride EDTA vacuum evacuated tubes from both cases and control (under full aseptic precautions). Serum samples were stored for not more than 30 days (at 2-8 degree C it is stable for 12 hrs & at -20 degree can be stored for 30 days) and stored samples were thoroughly mixed prior to use. Fasting insulin was assayed by chemluminescence immune assay (CLIA) analyzer MAGLUMI 1000 by Sandwich immunoluminometric assay. An anti-Insulin monoclonal antibody is used to label ABEI (N-4-aminobutyl-N-ethyl isoluminol) and another monoclonal antibody is used to label FITC (Fluorescein isothiocynate). Sample, calibrator or control with ABEI label, FITC label and nano magnetic microbeads coated with sheep anti FITC are mixed thoroughly and incubated at 37°, forming a sandwich; after sediment in a magnetic field, decant the supernatant, then cycle washing it for 1 time. The starter reagents are added and a flash chemiluminescent reaction is initiated. The light signal is measured by a photomultiplier as RLU (Relative light unit) within 3 seconds and is proportional to the concentration of insulin present in the sample.

Fasting blood glucose was measured in fully automated biochemical analyzer-ERBA 600 by colorimetric end point method--GOD/POD (glucose oxidase peroxidase method). Glucose is oxidized to gluconic acid and H_2O_2 in the presence of Glucose Oxidase. H_2O_2 further reacts with phenol & 4-aminoantipyrine by the catalytic action of peroxidase to form a red colored Quinoneimine dye complex. Intensity of the colour formed is directly proportional to the amount of glucose present in the serum sample. Absorbance is measured against the filter 505 nm.

Insulin resistance was taken as-Fasting Insulin>20 mIU/L, glucose insulin ratio (G:I) <4.5 [18] and Homeostatic model assessment (HOMA) > 2.5. The already established cut off values for HOMA, G:I and Fasting Insulin has been taken in this study [19].

HOMA was calculated by this formula

HOMA = fasting insulin (µu/ml) **X** fasting glucose (mmol/L)/22.5 [19].

BMI was calculated as-weight in kg/height in meter square.

3. RESULTS AND DISCUSSION

This was a hospital based, cross sectional study. The data collected has been tabulated and analyzed using descriptive statistics. The results are presented as mean ± SD. Students t test is used to analyze the data. P value of <0.05 is considered to be statistically significant. The software used for statistical analysis is Graph Pad Quick Calcs t test calculator.

In our study mean Fasting blood sugar, Fasting insulin, G:I and HOMA were significantly higher

(*P*= 0.01,0.001,0.04 and 0.004 respectively) in cases than controls as shown in Table 2. Table 1 shows the reference ranges taken for various parameters in the study. IR was found in 31 out of 45 (68.88%) by G:I ratio, 26 out of 45 (57.77%) cases by HOMA (<2.5) and 15 out of 45 (33.33%) by Fasting Insulin. When the cases were subdivided based on age as Group I (20-30 yrs) and Group II (31-40 yrs), BMI was significantly high (*P*= 0.0001) in Group II as compared to Group I (Table 3). IR markers like Fasting insulin, G:I and HOMA was higher but not statistically significant (*P*= 0.09, 0.25 and 0.08 respectively) in Group I as compared to Group II. Similarly when control was divided according to age as Group I (20-30 yrs.) and Group II (31-40 yrs.), BMI and FBS was significantly high (*P*= 0.0001 and 0.0001 respectively) in Group II as compared to Group I but IR markers like Fasting insulin, G:I and HOMA was not statistically significant (*P*= 0.33, 1.00 and 0.70 respectively).

3.1 Discussion

Insulin resistance in PCOS is both a reason and a result affecting 50%-70% of the PCOS patients. A cardiovascular study done in Quebec showed that hyperinsulinemia is an independent risk factor of cardiovascular diseases. It is a well-known fact that IR leads to Type II DM. Therefore IR in a PCOS patient adds on the already existing risk of developing metabolic and cardiovascular complications. Thus identifying IR in PCOS patients can reduce the doubled burden of the severe consequences. Clinical studies have shown that lowering circulating insulin levels (by weight reduction and using insulin sensitizers) resulted in reduction of serum testosterone levels and increased the frequency of ovulation and fertility in PCOS women.

Table 1. Reference ranges for various anthropometric and Biochemical parameters

Parameters	Reference ranges
Age (yrs)	20-40 yrs
Body mass index (Kg/m2)	≤25
Waist circumference (cm)	>88 cm
Fasting glucose (mg/dl)	70-110
Fasting insulin (µU/ml)	<20
HOMA	<2.5
Glucose/Insulin	>4.5

Table 2. Comparison of anthropometric and biochemical parameters between cases and controls

Parameters	Cases n=45	Controls n=40	*P* Value	Confidence interval
Age (yrs)	29.7 ± 6.02	30.15 ± 5.79	0.73	-3.0792 to 2.1792
Body mass index (Kg/m^2)	26.50 ± 4.79	25.57 ± 4.10	0.35	-1.0547 to 2.9147
Waist circumference (cm)	91.47 ± 9.96	90 ± 8.7	0.48	-2.6929 to 5.6329
Fasting glucose (mg/dl)	86.8 ± 7.84	82.72 ± 7.0	0.01	0.8569 to 7.3031
Fasting insulin (µU/ml)	20.20 ± 19.67	9.91 ± 4.39	0.001	3.9459 to 16.6341
HOMA	4.23 ± 4.68	2.03 ± 0.99	0.004	0.6942 to 3.7058
Glucose/Insulin	8.04 ± 4.69	6.25 ± 3.10	0.04	0.0203 to 3.5597

Table 3. Comparison of BMI and IR markers among Group I and Group II, cases

Age(yrs)	N	BMI(Kg/m^2	FBS(mg/dl)	FI(µU/ml)	G:I	HOMA
Group I (20-30) (26.04)	25	24.47 ± 3.8	87.16 ± 8.77	24.52 ± 28.57	7.41 ± 5.03	5.18± 5.71
Group II (31-40) (35.94)	20	30.48 ± 3.55	87.35 ± 8.52	13.20 ± 10.17	8.99 ± 3.77	2.83± 2.15
P		0.0001	0.94	0.09	0.25	0.08

Table 4. Comparison of BMI and IR markers among Group I & Group II, control

Age(yrs)	N	BMI(Kg/m^2)	FBS(mg/dl)	FI(µU/ml)	G:I	HOMA
Group I (20-30)	21	22.73 ± 3.12	83.47 ± 7.63	10.56 ± 4.86	6.25 ± 3.28	2.08 ± 1.03
Group II (31-40)	19	28.70 ± 2.43	81.89 ± 6.32	9.19 ± 3.82	6.25 ± 2.72	1.96 ± 0.95
P		0.0001	0.0001	0.33	1.00	0.70

The present study was done with 85 subjects, 45 cases and 40 controls. The selection of cases was based on ESHRE & ASRM criteria. IR Markers used to study Insulin Resistance were; Fasting Insulin, G:I and HOMA. IR was found in 68.88% of PCOS women by G:I, in 57.7% of PCOS women by HOMA & in 33.33% of cases by Fasting insulin, all statistically significant as compared to control (*P*= 0.001, 0.004 & 0.04 respectively). This was 60%, 64% and 56% respectively by a study done by Sachan Rekha et al. [22] which was also statistically significant as compared to control (*P*=0.001). It correlated with the findings of Mine Yavuz et al. [23] where Fasting Insulin & HOMA were taken as IR Marker (*P*=0.001) and with the study done by S. Kandasamy et al. [24] where all the 3 markers of IR were taken as in our study (*P* <0.05 for all the 3).

When the cases were divided according to age as 20-30 (Group I) and 31-40 (Group II), IR was found to be higher in (Group I) as compared to (Group II), although P value was not statistically significant. This was in concordance with the study done by Legro et al. [18] 254 PCOS women were evaluated prospectively in Legro et al. [18] study and it was concluded that PCOS women are at significantly increased risk for IGT & Type II DM at all weights & at a young age and they concluded that PCOS may be a more important risk factor for IGT than ethnicity or race in young women [14].

No significant difference was found among cases and control with regard to BMI and WC. This was comparable to the study by Mine Yavuz et al. [23].

In our study we could not find a significant correlation between IR indices (FI, G:I & HOMA) & BMI and WC among cases. In contrast there was a significant correlation for the same in control. This was in concordance with the study done by Bhattacharya Sudhindra Mohan et al. [24] where the cases were divided according to BMI. This can be explained by the fact that in control it is only the body fat reflected by BMI which is influencing the insulin resistance whereas in cases there are so many other factors like androgen & free fatty acid levels effecting IR. In this study we did a comparison of BMI and IR markers between Group I of cases versus control and Group II of cases versus control. But we did not get statistically significant values.

4. CONCLUSION

In the present study we examined the association of IR in women with and without PCOS. IR was high in PCOS women as compared to control. The data suggests that patients having Fasting Insulin>20 IU, HOMA >2.5 & G:I < 4.5 should be closely monitored & considered as high risk for Type II DM.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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