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A Comparative study for Diagnostic Evaluation of Polycystic Ovarian Syndrome (PCOS) by Estimation of High Sensitivity C - reactive protein (hsCRP) and Insulin Resistance

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Abstract
Now-a-days infertility is an emerging problem involving the women with reproductive age group. Polycystic ovarian syndrome (PCOS) is one of the leading cause that affects etiology of infertility to a great extent. Hyperandrogenism, Amenorrhea and Polycystic ovaries characterize the disease. Long term complication include DM Type II, Obesity, Heart disease and Endometrial cancer. Our study was aimed to compare the level of Insulin Resistance (IR) and serum hsCRP (a marker of low grade inflammation) among PCOS patients and controls and specifically to determine whether there was any correlation between Insulin resistance and hs-CRP level among PCOS patients. In search of suitable markers for diagnosis as well as management of such patients, this case control study was conducted among 44 PCOS cases and 44 non-PCOS age matched controls. Serum Fasting Blood Sugar (FBS) by GOD-POD method in auto analyzer, hs-CRP and Fasting Insulin by enzyme linked immunosorbent assay were measured. Insulin resistance was calculated by HOMA-IR. Unpaired 't' test was used to compare different means between two groups .Statistical correlation was tested between hsCRP and Insulin resistance to find any association. Statistically significant differences of means were observed in the values of Fasting Insulin, hs-CRP and IR between cases and controls as the 'p' values were <0.05. No such statistically significant difference was observed between means of FBS and correlation between Insulin Resistance and hs-CRP among PCOS cases. So the study concluded that PCOS patients have high level of mean Fasting Insulin, hs-CRP and Insulin Resistance than controls.
Keywords: *PCOS, FBS, Fasting insulin, hs-CRP, HOMA-IR, insulin resistance.*

Introduction

PCOS is the most common endocrine disorder among women of reproductive age group (18-44 yrs). It affects approximately 5-10% of this age group worldwide[1]. It is, also called Hyperandrogenic anovulation (HA)[2] or Stein–Leventhal syndrome[3], a set of signs and symptoms due to hormonal imbalance in women. PCOS

etiology includes a combination of genetic (autosomal dominant and x-linked) and environmental factors[4,5]. Risk factors include obesity, inadequate physical exercise and positive family history. Diagnosis is based on finding two of the following three criteria (Rotterdam criteria)[6]: Hyperandrogenemia (clinical or laboratory evidence), oligo-ovulation or anovulation (manifested as oligomenorrhoea or amenorrhoea) and Polycystic ovaries. Insulin resistance can be observed in both normal weight and overweight PCOS patients, although it is more common in the later (and in those matching the stricter NIH criteria for diagnosis); 50–80% of patients with PCOS may have insulin resistance at some level[7]. Elevated insulin level has been helpful to predict response to medication and may indicate women needing higher dosages of metformin or the use of a second medication to significantly lower insulin levels. But elevated blood sugar and insulin values do not always predict who responds to an insulin-lowering medication, low-glycemic diet and exercise. Moreover many women with normal levels may benefit from combination therapy. A hypoglycemic response in which the two-hour postprandial insulin level is higher and the blood sugar lower than fasting is consistent with insulin resistance. A mathematical derivation known as HOMA-IR (Homeostasis Model Assessment of Insulin Resistance),[8] calculated from the fasting values of glucose and insulin concentrations, allows a direct and moderately accurate measure of insulin resistance [Fasting glucose level (mg/dl) x Fasting insulin-level (mu/L)/405]. High sensitivity C-reactive protein (hs-CRP) is nothing but CRP detected in blood when present in very low amount (<0.3mg/L).[9] This is a biomarker of low grade inflammation & also a predictive risk marker for cardiovascular diseases and metabolic syndrome.[10]

Limited data are available on high-sensitivity C-reactive protein (hs-CRP) levels and its relationship with Insulin resistance in PCOS patients especially in Indian women. So, the objective of this study was set to determine serum hs-CRP concentration and insulin resistance in PCOS patients and to assess possible correlation in between them. The outcome of this study was expected to help in the early diagnosis and management of PCOS patients which would save them from serious complications especially the late complication like metabolic syndrome, cardiovascular diseases, diabetes mellitus and endometrial carcinoma.

Materials and Methods

Study Area: Department of Biochemistry, in collaboration with, Department of Gynaecology and Obstetrics, in our Medical College & Hospital, Kolkata

Study population: PCOS patients attending Gynaecology OPD for management. PCOS was diagnosed by Rotterdam criteria.

Sample Size: Total forty four (44) samples were taken from review of previous research papers. 44 age and sex frequency matched samples were taken as controls.

Sample Design: For selection of study subjects from the population, systematic random sampling done meeting inclusion and exclusion criteria.

Inclusion Criteria

a. Females between 18 to 36 years age group with PCOS (Rotterdam criteria, 2003).

b. Healthy females between 18 to 36 years age group without PCOS.

Exclusion Criteria

- Patients/ Guardian unwilling to sign consent
- Pregnancy and Lactation
- Any psychiatric illness on antipsychotic medicines
- Infections and inflammatory conditions
- Endocrine disorders like Adrenal Hyperplasia, Hypothyroidism or Hyperpr-olactinemia
- Chronic liver disease
- Malignancy
- Terminally ill patient
- Known Hypertensive
- Known Pancreatic disease

Each participant was provided with a written informed sheet and blood samples were collected after taking a written informed consent.

5. Study design: The study was an observational, analytical, case-control study.

6. Parameters studied:

❖ Estimation of plasma glucose in autoanalyzer by GOD-POD (Glucose oxidase – peroxidase) method

❖ Estimation of serum insulin and hsCRP in enzyme linked immunosorbent assay.

HOMA-IR[11] was calculated using formula - Fasting insulin (mu/l) x Fasting glucose (mg/dl) / 405 or fasting insulin (mu/l) x Fasting glucose (mmol/l) / 22.5. Results were interpreted as follows –

Table 1: Insulin resistance category according to HOMA Score[11]:

Category	HOMA Score
Normal insulin resistance	< 3
Moderate insulin resistance	Between 3 and 5
Severe insulin resistance	>5

7.. Study techniques:

The patients were selected first from the Medicine indoor of our Medical College and Hospital, Kolkata according to the inclusion & exclusion criteria after obtaining consent of the patient in proper consent form. 44 cases were selected randomly from those patients. Detailed history was gathered. After that 10-12 hours fasting blood for biochemical investigations was collected[11]. The collected data was then analyzed.

8. Plan for analysis of data:

All recorded data was analyzed using standard statistical methods including standard diagrams and graphs. Statistical softwares like IBM SPSS[™]2020 and Microsoft excel 2013[™] were used for this purpose.

9. Funding: Funding was borne by the investigators.

10. Conflict of interest: No**.**

Result and Discussion

PCOS, its name indicates it is still considered a syndrome because of lack of understanding of so many aspects of the aetiopathogenesis of the disease. It is still full of mysteries and complexities. Insulin resistance and hsCRP are now most aptly considered to be the focus of a lot of PCOS researchers. Information on these issues are likely to help us diagnose the disease in a better perspective and will enable us to formulate an appropriate management protocol .This is why we undertook this research work to study the levels of hsCRP and insulin resistance in PCOS patients and healthy controls. The statistical analysis of the data clearly indicated that PCOS patients had significantly higher levels of serum hsCRP and Insulin resistance (p<0.05) (table 2&3). This findings also support the observation of some recent researchers like Ganie MA et al[12], in 2014, Tarkun I et al[7]. However, findings of Nitin Agrawal et al[13] in an recent north Indian study did not support our finding regarding FBS which showed no significant mean difference in FBS values between two groups (p=0.559). This was rather expected because these PCOS patients were chosen from 18-36 yrs age group, and patients with pancreatic diseases were excluded as per our predetermined study criteria. In healthy people age-related decline in pancreatic function occurs after the age of 35 yrs. So expected that a PCOS subject, although had insulin resistance, her pancreas was still able to produce more insulin which was maintaining the normal FBS status. As a result of this although the patient was still maintaining a euglycemic status her insulin level became high. This was reflected in the statistically significant 'p' value <0.05 in the mean difference of Fasting insulin levels of the cases (table 4).

Table 2: Table showing statistical results of PCOS patients

Cases	Age (yrs.)	Wt (kgs)	FBS (mg/dl)	F.Insulin (mu/l)	hsCRP (mg/l)	HOMA_IR
Mean	24.4	62.25	85.31	19.96 15.51	7.9830 3.35	4.204 3.324
Median	23.9	63.14	86.8			
Std. Deviation	5.5	11.4	16.42	15.04	8.63	2.95
Minimum	18	41	58	6.00	.03	2.00
Maximum	36	87	120	68.13	29.30	17.00

Table 3: Table showing statistical results of controls

Controls	Age (yrs.)	Wt. (kgs.)	FBS (mg/dl)	F.Insulin (mu/l)	hs_CRP (mg/l)	HOMA_IR
Mean	26.5	53.11	80.05	13.50 11.33	5.81	2.66
Median	27.45	54	78		3.0	2.18
Std. Deviation	5.92	4.06	11.32	4.812	4.63	1.81
Minimum	18	40	56	3.70	.25	.39
Maximum	36	65	112	18.93	12.0	3.36

HOMA-IR also showed a significant mean difference ($p < 0.01$) between our case and control groups.(table 4) This result was quite matching with our existing knowledge of insulin resistance in PCOS patients. 50 to 80% of PCOS patients are known to suffer from insulin resistance. This is considered to be of crucial importance. Insulin resistance causes hyperinsulinemia which results in a lot of pathological effects in PCOS patients like hyperandrogenemia, anovulation, acanthosis nigricans, glucose intolerance, dyslipidemia and many other metabolic defects. Independently and in combination with LH, insulin enhances androgen production by the ovaries directly and through hypothalamus. As per the research outcome of Andrea Dunaif[14], hyperinsulinemia causes more androgen production by stimulating the enzyme P450c17,20 lyase by phosphorylation of the serine residues of the enzyme via serine/threonine kinase pathway (PKC) of insulin receptor signal transduction . This is an example of selective inhibition and activation mechanism of action of insulin in patients with insulin resistance and hyperinsulinemia. Insulin selectively inhibits PI-3K pathway, in these patients, but stimulates PKC (Protein Kinase C) pathway causing a lot of conspicuous metabolic abnormalities.

Daskalopoulos G et al[15] in an observational study found that there was no significant difference in hsCRP levels between their PCOS case and control groups, although there was a higher trend in the case group. This did not match our study outcome as mean difference of hsCRP values between our two study groups were found to be significant (p value <0.05). (table 4). This higher values of hsCRP in patients group support the hypothesis that PCOS has got an inflammatory component in its aetiopathogenesis. In fact the late sequelae of PCOS like Diabetes Mellitus, Hypertension and Cardiovascular diseases all have a persistent chronic low grade inflammatory pathology in their background. In fact some of the researchers hypothesize that CRP is not only a marker of inflammation but also an active mediator of it. So expectedly we found our study cases to have higher values of hsCRP which corroborate their findings[16-18].

Table 4: Difference between mean of FBS, Fasting Insulin, High sensitivity CRP and HOMA IR among cases and controls (Independent Samples Test)

		F	Sig.
FBS	Equal variances assumed	0.345	0.559
	Equal variances not assumed		
F_Insulin	Equal variances assumed	6.710	0.012
	Equal variances not assumed		
hs_CRP	Equal variances assumed	5.843	0.019
	Equal variances not assumed		
HOMA_IR	Equal variances assumed	9.574	0.003
	Equal variances not assumed		

As we wanted to know, in our study, whether there was a correlation between hsCRP and insulin resistance (HOMA-IR) our result showed that although a very little positive correlation was there between these two parameters (correlation coefficient 0.167) that was statistically insignificant (p value 0.361). (Figure 1). So there might be no causal relationship between these two components of PCOS. Might be this result is indicating that although individually these two parameters are related to inflammatory pathology they do not have a proportional relationship in PCOS. This can be explained by the fact that other than inflammatory pathology insulin resistance has got other important causative factors in its aetiopathogenesis like genetic and environmental factors. On the contrary, Sahin S et al[19], in their PCOS study in 2014, showed that HOMA-IR had significant positive correlation with hsCRP. But they could not find any significant difference in hsCRP levels in PCOS case and control groups. And their study showed only 30% of PCOS patients were having insulin resistance which was actually much lower than the expected percentage. These unexpected findings could not be explained by our existing knowledge. Mark O. Goodarzi et al[20] observed in 2003, that treating insulin resistance in PCOS patients with metformin might improve their fertility, lipid profile, weight loss, reduce the incidence of diabetes and prevent atherosclerosis, myocardial infarction and stroke. They recommended an investigation to detect insulin resistance in every hirsute woman.
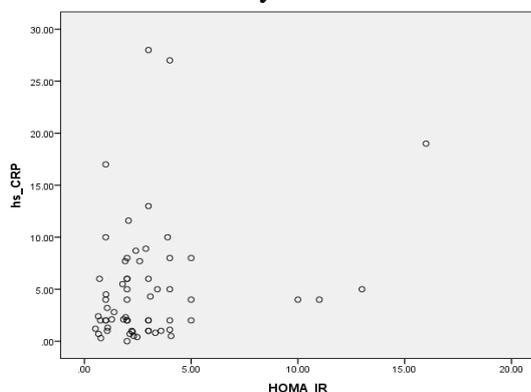
Figure 1: Scatter diagram showing correlation between Insulin Resistance (HOMA-IR) and hsCRP among PCOS patients (n=44)

From our study we can agree on this recommendation. As the onset of PCOS is at around menarche, all the suspected PCOS cases and the women with the predisposing factors for PCOS should be properly investigated at perimenarchal age. Measurement of blood androgens, especially free testosterone, and insulin resistance levels can help us catch the disease early. An early diagnosis and prompt management will save the PCOS patients from serious complications like diabetes mellitus, hypertension, cardiovascular diseases, metabolic syndrome and endometrial carcinoma.

Conclusion

After considering several recent research, from this study we can come into conclusion that hs-CRP along with insulin resistance in PCOS cases may be useful diagnostic marker other than imaging study. Although there is still lot of lacunae in this field which are required to clarify to come into definite conclusion. Future studies on these issues are necessary which ideally should be community based and multicentric, and should include greater number of related parameters so that more accurate and more informative results can be obtained.

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