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An Evaluative Study of Cardiometabolic Risk Factors in PCOS.

J.Gnana Priya[1], Anuva Mishra[2]
[1] *(Post Graduate, Department of Biochemistry, SCB Medical College, Cuttack.)*
[2] *(Assistant Professor, Department of Biochemistry, SCB Medical College, Cuttack.)*

Abstract: *Polycystic Ovarian Syndrome(PCOS) affects about ~5-10 % of women of reproductive age. PCOS is a complex disorder involving genetic and environmental factors. Metabolic complications associated with Insulin resistance are increased in PCOS including adverse cardiometabolic syndrome. This study aims at evaluating the correlation of cardiometabolic risk factors with Serum Insulin.*

In the present study,70 numbers of diagnosed cases of Polycystic ovarian syndrome (PCOS) and 70 age matched healthy females participated as cases and controls respectively. Measurements were taken for assessment of Body mass index(BMI) and blood samples were collected for estimation of fasting plasma glucose, serum lipid profile and serum Insulin. Both the BMI and Blood pressures were found statistically significant in the study group compared to controls. Fasting plasma glucose(98 ± 15 Vs 85.7 ± 9 mg%), Insulin(12.1 ± 8.7 Vs 6.6 ± 2.5 µU/ml), HOMA-IR(7.7 ± 5.4 Vs 1.5 ± 0.9), Total cholesterol(189 ± 25.3 Vs 164 ± 9.1 mg%) and Triglycerides (130 ± 15 Vs 115 ± 24 mg%) were significantly higher in the study group compared to controls. BMI also showed positive correlation with Glucose, T.Cholesterol and Insulin levels. There was a significant positive correlation of Insulin values with those of Glucose, T.Cholesterol and LDL-C. Thus the increase in insulin values in PCOS can be considered as an important cause of future manifestations of Diabetes mellitus type II and Cardiometabolic complications.

Keyword: *Dyslipidemia, Metabolic Syndrome, Polycystic Ovary, Insulin Resistance.*

I. Introduction

According to NCEP-APT III Guidelines, Metabolic syndrome originally known as syndrome X [1] is defined as a disorder with constellation of interrelated risk factors of metabolic origin that appear to directly promote the development of CVD.[2] This syndrome is linked closely to a generalized metabolic disorder called insulin resistance, in which normal actions of insulin are impaired. The most widely recognised of the CHD risk factors associated with metabolic syndrome are atherogenic dyslipidemia, elevated blood pressure and elevated plasma glucose. Individuals with these characteristics commonly manifest a prothrombotic and pro-inflammatory state. Atherogenic dyslipidemia consists of an aggregation of lipoprotein abnormalities, including elevated plasma triglyceride and apoB concentrations, increased small LDL particles and reduced HDL cholesterol levels. The predominant underlying risk factors for the metabolic syndrome appear to be abdominal obesity and insulin resistance.

In general the diagnosis of Metabolic Syndrome requires 3 of the following 5 clinical characteristics.[3]

- Increased Waist Circumference (population specific >88cm in the US).
- Increased Blood Pressure(Systolic ≥130mmHg ; Diastolic ≥85mmHg).
- Increased Triglyceride (≥150mg/dl).
- Decreased HDL-cholesterol (<50mg/dl).
- Increased Fasting Blood Glucose (≥100mg/dl) or previously established Diabetes Mellitus.

In PCOS, women with chronic anovulation commonly exhibit insulin resistance and other risk factors for the development of type 2 diabetes and cardiovascular disease. These observations have focused a great deal of attention on the importance of incorporating risk reduction strategies into the clinical management of women with PCOS. Upto 10% of women with PCOS develop diabetes by the age of 40[4]. Obesity adds to the risk by aggravating the underlying insulin resistance. Overall the risk for developing IGT or type 2 diabetes is increased 3 to 7 fold in women with PCOS, compared to women of comparable age without PCOS.[4,5] Consequently many with PCOS have some degree of dyslipidemia, such as decreased HDL and total and LDL-cholesterol and triglycerides.[6]

Many of the anthropometric and metabolic abnormalities of PCOS overlap with components of the metabolic syndrome, a clustering of both lipid and non-lipid risk factors that identify individuals at increased risk for coronary heart disease and type 2 diabetes mellitus.[7] Although direct evidence for an increased incidence of cardiovascular disease in women with PCOS is lacking, the prevalence of known risk factors is substantially increased.[8]

Essential hypertension is frequently associated with the several metabolic abnormalities, of which obesity, glucose intolerance, and dyslipidemia are the most common in PCOS.[9] Studies suggest that both hyperglycemia and hyperinsulinemia activate the Renin angiotensin system (RAS) by increasing the expression of angiotensinogen, Angiotensin II, and AT1 receptor, which, in concert, may contribute to the development of hypertension in patients with insulin resistance. [10] There is also evidence that insulin resistance and hyperinsulinemia lead to SNS[sympathetic nervous system] activation and as a result, the kidneys increase sodium reabsorption, the heart increases cardiac output, and arteries respond with vasoconstriction resulting in hypertension. [11] 78% of those with a systolic blood pressure greater than or equal to 130 mm Hg or diastolic blood pressure greater than or equal to 85 mm Hg met criteria for the metabolic syndrome.

Insulin resistance is defined as a decreased biological response to normal concentration of circulating insulin. The overall prevalence of insulin resistance among women with PCOS is between 50% and 75%, greater on obese than in lean women with PCOS. They are undoubtedly an important part of the pathophysiology of PCOS. However it is important to emphasize that 25-50% of women with PCOS have no demonstrable insulin resistance. Among all women with insulin resistance, the prevalence of PCOS is relatively low app.15%.[12] Therefore insulin resistance and hyperinsulinemia are not the primary cause or pathogenic factor in all women with PCOS. Elevated androgen levels predipose to increased LDL and aggravate underlying insulin resistance.

Obesity, a key determinant of insulin concentrations, appeared to have an independent effect on risk for the metabolic syndrome. IR in PCOS primarily refers to the impaired action of insulin on glucose transport and anti-lipolysis in adipocytes in the presence of normal insulin binding.[13] Hyperinsulinemia has been found to be associated with a higher risk of cardiovascular disease.[14] The increased concentrations of inflammatory markers cluster with cardiovascular risk factors such as dyslipidemia, glucose intolerance, type 2 diabetes, hypertension, and obesity.

II. Aim and Objective

Polycystic Ovarian Syndrome (PCOS) affects about ~5-10 % of women of reproductive age. PCOS is a complex disorder involving genetic and environmental factors. Metabolic complications associated with Insulin resistance are increased in PCOS including adverse cardiovascular risk profile. This study aims at evaluating the correlation of cardiometabolic risk factors with serum Insulin in women with PCOS.

III. Materials and Methods.

The present study was conducted in the Department of Biochemistry, S.C.B. Medical College & Hospital, Cuttack from between January 2014 - March 2015. 70 premenopausal women of age group between 18-40 years diagnosed to have Polycystic Ovarian Syndrome (PCOS) attending OPD and indoor in the Department of Obstetrics and Gyneacology, S.C.B. Medical College and Hospital, Cuttack were included in the study. Patients were selected based on their history, physical examination, biochemical investigation, ultrasound ovaries and according to Rotterdam criteria(2003) for diagnosis of PCOS. The control group consisted of 70 age and healthy female volunteers with regular menstrual cycles and with no clinical or biochemical features of hyperandrogenism, thereby excluding the diagnosis of PCOS in this group. The height and weight of all the subjects were recorded without shoes using standard apparatus. Body mass index (BMI) was calculated by dividing weight (kg) by height (m^2). Normal weight was defined as BMI <25, Overweight as BMI between 25.0-29.9 and Obesity as BMI >30.

All subjects answered a questionnaire which contained details of Age, Menstrual history, Medical history and Family history of Type 2 Diabetes Mellitus or Polycystic Ovarian Syndrome. All the case records were collected in a specific proforma. Written and informed consent was obtained from all subjects.

Sample Collection and Storage:

5 ml of venous blood samples were collected from healthy controls and women with PCOS after an overnight fast. 1 ml of sample was taken in a tube containing sodium fluoride as anticoagulant for Fasting plasma glucose and 4 ml of sample was taken in a plain tube for Lipid Profile and Insulin. Fasting plasma glucose & Lipid profile were estimated by Toshiba 120 FR Autoanalyser using commercially available kits. Serum Insulin was estimated by Chemiluminiscence assay.

Inclusion Criteria:

Diagnosis based on Rotterdam Criteria [2003]:
(Any 2 of the following criteria confirms the presence of PCOS)[15]

1. Oligomenorrhoea/Amenorrhea.
2. Clinical / Biochemical signs of Hyperandrogenism.
    a. Hirsutism.

b. Acne.
c. Alopecia.
d. Elevated androgen levels (Testosterone).

3. Presence of Polycystic ovaries on ultrasound scans.

Exclusion Criteria:

- Diabetes Mellitus
- Hypertension
- Thyroid Disorders
- Cardiovascular Diseases
- Cushing's Syndrome
- Pregnant or lactating women
- Oral Contraceptives
- Hypoglycemic agent /Lipid lowering drug/Hormonal Medications within previous 6 weeks

Institutional Ethical Commitee approval was obtained. The participation of the respondents was voluntary and informed consent was signed by each participant. Plasma was separated and analysed by using standard methods. Data Analysis was performed using SPSS 20 Software. All data were expressed as mean ± standard deviation. Student't' test paired two sample for means was used to compare the values. Differences with a 'p'value of less than 0.05 were considered to be statistically significant.

IV. Observation and Results.

TABLE-1: Physical Parameters in PCOS Cases & Controls.

PARAMETERS	CONTROLS [n =70] Mean ± S.D	CASES [n =70] Mean ± S.D	p Value
Age [Years]	29.4 ± 4.3	27.3 ± 5.3	0.715
BMI [Kg/m²]	22.1 ± 1.8	28.1 ± 2.4	<0.001*
SBP [mmHg]	108.4 ± 1.0	121.2 ± 2.3	< 0.0001**
DBP [mmHg]	72.5 ± 1.4	79.8 ± 2.7	< 0.0001**

TABLE-2: Biochemical Parameters in PCOS Cases & Controls.

PARAMETERS	CONTROLS [n = 70] Mean ± S.D	CASES [n = 70] Mean ± S.D	p- Value
Glucose (mg/dl)	85.7 ± 9	98 ± 15	<0.001*
T.Cholesterol (mg/dl)	164 ± 9.1	189 ± 25.3	<0.0001**
TG (mg/dl)	115 ± 24	130 ± 15	<0.001*
HDL (mg/dl)	46 ± 4.9	41 ± 5.5	<0.001*
LDL (mg/dl)	80.3 ± 11.8	118 ± 20.9	<0.0001**
VLDL (mg/dl)	22.8 ± 4	25.5 ± 3	<0.001*
Insulin (μU/ml)	6.6 ± 2.5	12.1 ± 8.7	<0.01*
HOMA-IR	1.5 ± 0.9	2.7 ± 1.3	<0.01*

TABLE-3: Correlation of Biochemical Parameters in PCOS.

PARAMETERS	Glucose		T.Cholesterol		LDL-C	
	r - value	p - value	r - value	p - value	r - value	p - value
BMI	0.181	0.033*	0.263	0.002**	0.306	<0.0001**
T.Cholesterol	0.240	0.004*	-	-	0.813	<0.0001**
Insulin	0.205	0.015*	0.249	0.003*	0.365	<0.01*

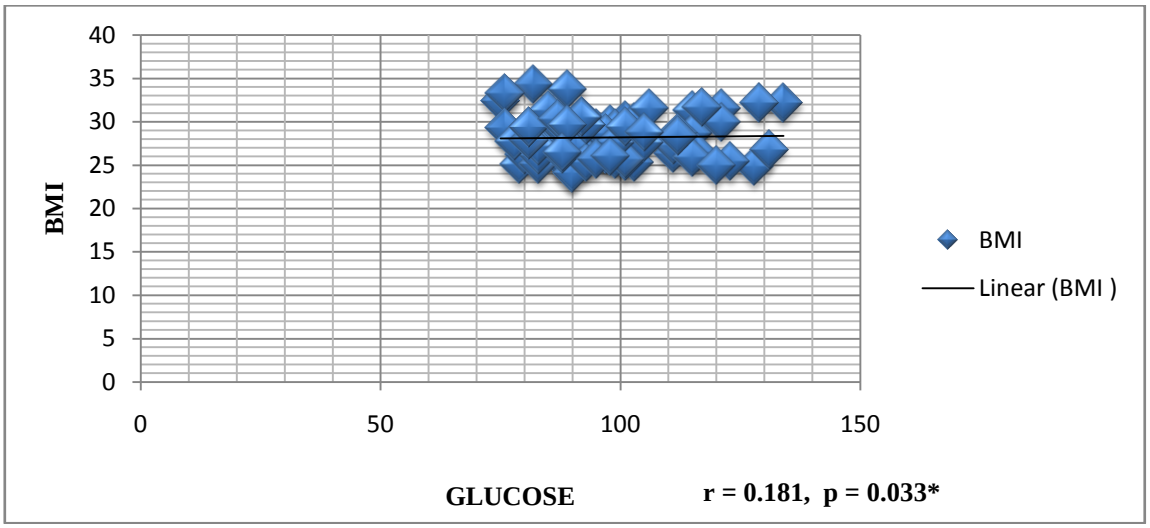

Figure 1: Correlation of BMI with Glucose in PCOS cases.

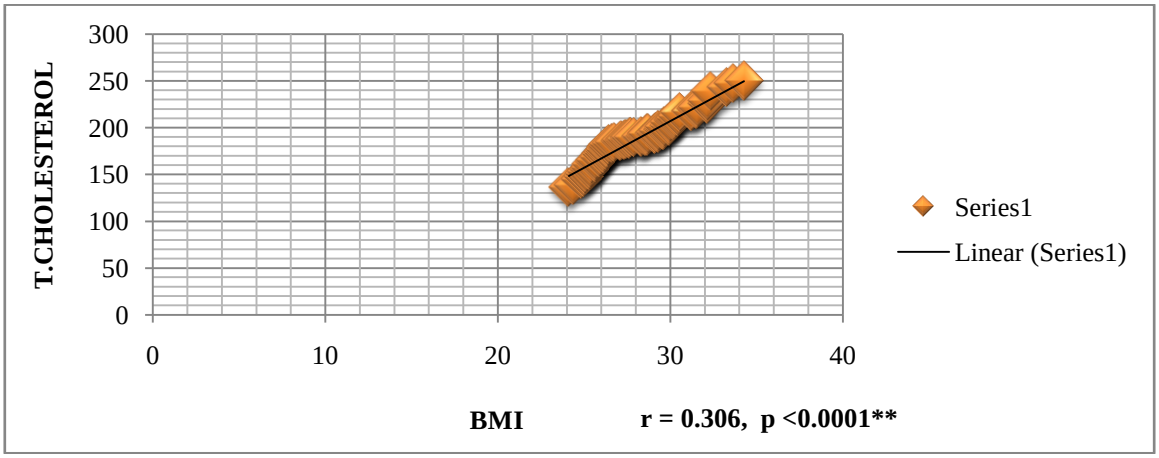

Figure 2: Correlation of BMI with T.Cholesterol in PCOS cases.

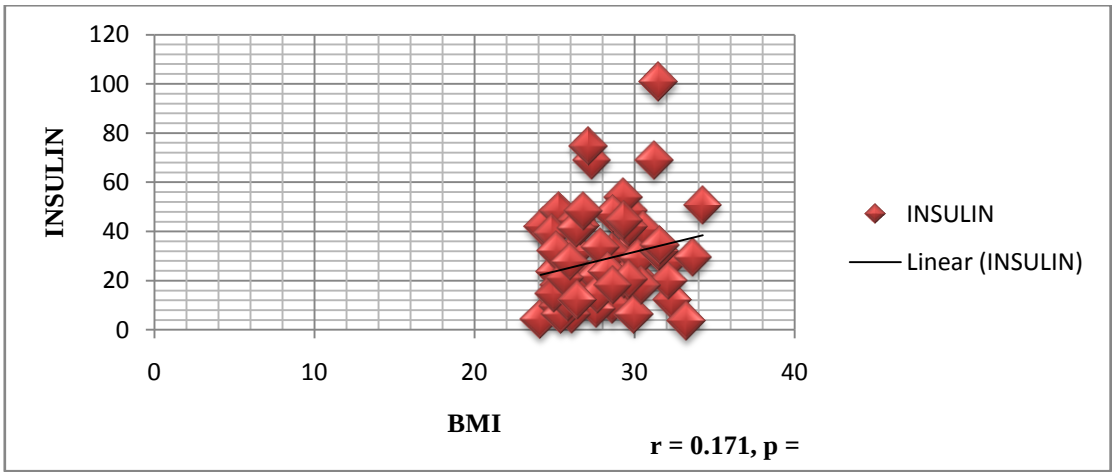

Figure 3: Correlation of BMI with Insulin in PCOS cases.

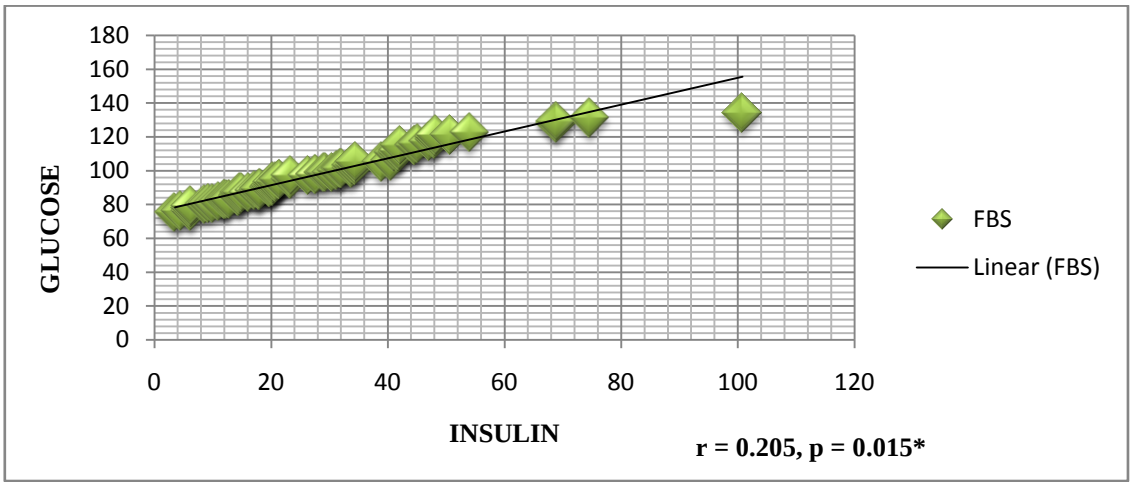

Figure 4: Correlation between Insulin & Glucose in PCOS cases.

IV. Discussion

Obesity was one of the important pathogenesis, which may contribute to determine the health state in PCOS patients. The risk for PCOS increases with obesity and the common measure of excess body weight is the body mass index (BMI). BMI, Glucose intolerance and serum Insulin levels are interrelated in women with PCOS and without PCOS, particularly in obese. Obese women with PCOS are more Insulin resistant, hyperandrogenic and hyperlipidemic compared to lean women with PCOS.

In our present study, BMI levels were significantly higher in PCOS group (28.17±2.47) compared to that of the control group (22.16±1.88) (p <0.001*). 72% of PCOS cases had BMI between (25.0-29.9) and 20.5% of cases had BMI (>30) and 7.5% had normal BMI (<25) among the 7.5% of non-obese cases, 1.5% cases had increased insulin level. Xioli chen et al.,[16] in his study on BMI in PCOS, showed significantly higher BMI in PCOS patients compared to controls (p <0.0001) with BMI of above ≥23 in 34.6% cases. In our study, BMI showed significant positive correlation with Glucose (p <0.033*), T.Cholesterol (p <0.05*) and Insulin (p <0.043*) (Fig-1,2,3).

Hypertension is frequently associated with the several metabolic abnormalities of which obesity, glucose intolerance, and dyslipidemia are the most common in PCOS. [9] Studies suggest that both hyperglycemia and hyperinsulinemia activate the Renin angiotensin system (RAS) by increasing the expression of angiotensinogen, Angiotensin II, and the AT1 receptor which, in concert may contribute to the development of hypertension in patients with insulin resistance.[10]

In our study, Systolic blood pressure (108.4 ± 1.0 Vs 121.2 ± 2.3) (p <0.0001**) and Diastolic blood pressure (72.5 ± 1.4 Vs 79.8 ± 2.7) (p < 0.0001**) were significantly higher in PCOS cases compared to controls. E.O.Talbott et al [17] in his study showed significantly increased Systolic and Diastolic blood pressure in PCOS cases compared to controls.

Fasting blood glucose level is higher in obese PCOS women secondary to increased basal hepatic glucose production, which reflects hepatic insulin resistance. [18] High glucose level can indicate insulin resistance, diabetes related condition that contributes to PCOS. Impaired glucose intolerence is a prediabetic

state of hyperglycemia that is associated with insulin resistance. And conversion from IGT to frank diabetes is also substantially enhanced in obese PCOS.[19] The inability of insulin to suppress hepatic glucose production (HGP) is a key defect found in, type 2 diabetes.

In our study, levels of Fasting plasma glucose was higher in PCOS cases (98 ± 15mg/dl) compared to controls (85.7 ± 9mg/dl) ($p < 0.001$*). A study by Azevedo MF et al., (2011) [20] reported higher fasting glucose levels in PCOS women which was statistically significant whereas, V.M. Vinodhini et al [21] showed no statistically significant differences in the mean concentrations of fasting plasma glucose between PCOS patients and healthy controls. Legro et al.,(2005) Talbott et al.,(2007) showed that there insignificantly higher conversion rates from normal glucose tolerance to IGT or DM2 for women with PCOS compared with women without PCOS.[22]

In our study, Glucose showed significant positive correlation with BMI ($p = 0.033$*),T.Cholesterol ($p < 0.0001$**), Insulin ($p < 0.015$*) (Table-3) (Fig-1,4). Jarosław Kozakowski et al[23] in their study on body composition and Glucose metabolism markers showed significant positive correlation of Glucose with Body weight, Total Cholesterol and abdominal fat. Legro et al[24] in their study showed significant correlation between Dyslipidemia and Glucose.

Obesity increases hyperandrogenism, hirsutism, infertility and pregnancy complications both independently and by exacerbating PCOS.[25] In general populations, obesity and insulin resistance further increase type 2 diabetes (DM2) and cardiovascular disease (CVD). Some evidence shows that women with PCOS have enhanced peripheral 5α- reductase activity compared with age and BMI matched control women there by generating higher tissue concentrations of more potent androgen DHT [Dihydrotestosterone]. Increased 5α -reductase activity in the adipocyte could therefore be one mechanism by which obese women with PCOS display increased androgenicity & it is clear that obesity adds to the pathophysiology of PCOS in affected or predisposed women by aggravating the degree of insulin resistance and hyperinsulinemia.[26] Likewise, in PCOS obesity worsens insulin resistance and exacerbates reproductive and metabolic features[25] Furthermore, women with PCOS have increased risk factors increased impaired glucose tolerance (IGT), DM2 and potentially increased CVD.

In our study, Lipid profile was also estimated in PCOS patients and compared with the control groups. Table-2 which shows higher levels of total cholesterol (164 ± 9.1 Vs 189 ± 25.3) ($p < 0.0001$*), triglycerides (115 ± 24 Vs 130 ± 15) ($p < 0.001$*), and LDL-C (80.3 ± 11.8 Vs 118 ± 20.9) ($p < 0.0001$*) and VLDL-C (22.8 ± 4 Vs 25.5
± 3) ($p < 0.001$*), in cases compared with controls. On the contrary, serum levels of HDL-C (46.91 ± 4.9 Vs 41.9 ± 5.5) ($p < 0.001$*) were significantly lower in women with PCOS. Our results were consistent with the study conducted by Olivier Valkenburg et al.,(2008)[27] on serum lipid profile of PCOS patients showed higher levels of Total Cholesterol, Triglycerides and LDL-C compared with controls. On the contrary, serum levels of HDL-C were significantly lower in women with PCOS. **24% cases** had significantly higher cholesterol and LDL value above the normal range. Though the TGL mean value is higher in PCOS cases compared to controls, most of the cases had values within the normal limits.

In our investigation, significant positive correlation was found between Glucose and T.Cholesterol ($p < 0.0001$**). And LDL/HDL ratios was not significantly increased ($p = 0.061$) in PCOS cases compared to controls and it was within the normal range in both cases (2.8:1) and controls (1.7:1) which is also not significant. Abnormal lipid metabolism is one of the main metabolic characteristics of PCOS patients. Dyslipidemia is a very common metabolic abnormality in women with polycystic ovary syndrome (PCOS) and obesity can alter lipoprotein lipid profiles and glucose metabolism in PCOS. Decrease in HDL-C and increase in TG levels are well known lipid profile characteristics in women with PCOS.[13] Jarosław Kozakowski et al,. Wojciech Zgliczyński et al[23] in their study on body composition and glucose metabolism markers showed significant positive correlation of glucose with Body weight, Total Cholesterol, Glucose and Abdominal fat.

Insulin resistance is an important defect in the pathogenesis of non-insulin-dependent diabetes mellitus (NIDDM).[28] 20% of the obese PCOS women had impaired glucose tolerance or frank NIDDM by National Diabetes Data Group Criteria.[29] In the presence of Peripheral insulin resistance, pancreatic β-cell insulin secretion increases in a compensatory fashion. NIDDM develops when the compensatory increase in insulin levels is no longer sufficient to maintain euglycemia.[30]

In our present study, serum Insulin level was significantly increased in PCOS cases when compared to control group (6.6 ± 2.5 Vs 12.1 ± 8.7) ($p < 0.01$*). Roy et al,[31] in their study found significant increased level of serum insulin in PCOS women compared to controls group. According to study conducted by Dunaif et al[32] based on the prevalence of glucose intolerance in women.[33] PCOS-related insulin resistance contributes to approximately 10% of cases of glucose intolerance in premenopausal women.

In PCOS, Insulin resistance were closely related to lipid abnormalities, independent of obesity. Studies have shown that serum Insulin level and Fasting plasma glucose have significant positive correlation with serum lipid parameters in patients with PCOS and could be considered as a simple reliable indicator to determine

insulin resistance.[34] In our study, Insulin showed significant positive correlation with FBS (p = 0.015*) and Cholesterol (p = 0.003*) (Fig-4). Kalra et al[35] showed significant positive correlation between Insulin and Lipid Profile. They found significantly higher mean T.Cholesterol and Triglycerides and significantly lower HDL cholesterol in the Insulin-resistant group.

HOMA-IR is a sensitive method for evaluation of IR in PCOS, and it was used to identify IR in the present study. Insulin resistance has been associated with metabolic and hemodynamic alterations and higher cardio metabolic risk. Insulin resistance (IR) is a feature of disorders such as diabetes mellitus type 2 (DM2) and is also implicated in obesity, hypertension.[36] The HOMA-IR value was given as mean±SD, and appraised the values >2.5 as an insulin resistant state and the values <2.5 as an insulin sensitive state.

In our present study, PCOS cases had higher HOMA-IR values compared to controls [7.7±5.4 vs 1.5±0.9]. Shou kul et al[37] in their study found that the HOMA-IR of PCOS women was significantly higher than the age matched healthy controls, which suggested the crucial role of insulin resistance in pathogenesis of PCOS.

V. Conclusion

In our present study we observed a significant positive correlation between BMI, Glucose, Total Cholesterol,

Triglycerides, LDL-C, VLDL-C and Insulin. These abnormalities, together with increased BMI, explain the increased prevalence of glucose intolerance in PCOS. Since PCOS is an extremely common disorder, PCOS-related insulin resistance is an important cause of NIDDM and cardio-metabolic syndrome in women. Although numerous risk factors such as obesity and age increase the risk of glucose intolerance, women with PCOS of all ages and weights appear to be at greater risk for glucose intolerance than normal controls. Future studies to identify the risk of cardiovascular events in women with PCOS will show clear, consistent, and extensive picture of reproductive and metabolic abnormalities.

In conclusion, the use of these simple and cost-effective biochemical parameters might help in early detection of these metabolic changes in women with PCOS at risk of cardio metabolic syndrome.

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