EVALUATE RELATIONSHIP BETWEEN INSULIN RESISTANCE AND SERUM LIPOPROTEIN RATIO IN POLYCYSTIC OVARIAN SYNDROME

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Abstract- Polycystic ovarian syndrome is most common endocrine disease and metabolic disorder in adolescence and reproductive women. In PCOS women insulin resistance thought to be the uniting pathogenic factor in the development of glucose intolerance, obesity, lipid abnormalities, HTN and coronary artery disease .This study is under taken to measure insulin resistance in PCOS and to see the relationship of insulin resistance with serum lipoprotein ratio. Case control study was done taking 60 women PCOS and 60 age matched healthy   women as controls.In all the subjects, concentrations of fasting plasma glucose, serum TG, serum TC and HDL were estimated using enzymatic methods in semiautoanalyser. Fasting serum insulin   and was measured by CLIA using Lumax-CLIA microplate reader. HOMA IR, serum LDL concentration, serum TC:HDL ratio, serum TG:HDL ratio and serum LDL:HDL ratio were calculated from estimated parameters.The mean concentrations of all the parameters were significantly increased in women with polycystic ovarian syndrome when compared with healthy women except serum HDL concentration, which was significantly decreased.Insulin resistance was significantly positively correlated with serum lipid profile and serum lipoprotein ratio except with serum HDL, which was significantly negatively correlated.
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Index Terms- Lipo protein ratio, Polycystic ovarian syndrome, insulin resistance.

I.   INTRODUCTION

Polycystic ovary syndrome (PCOS) is the most frequent endocrine disorder seen in pre-menopausal women, affecting 5–10% of this population. It is characterized by menstrual irregularities and biochemical and/or clinical hyperandrogenism such as hirsutism, seborrhoea and acne. Regardless of the presence of obesity, many are also insulin resistant .[1]

A number of findings suggest that hyperinsulinaemia may play a central role in the development of hyperandrogenism ,Dyslipidemia, Type II diabetes mellitus (DM) and an increase in the risk of cardiovascular disease (CVD) [1]

According to Rotterdam (2003) polycystic ovarian syndrome is defined as having any two of following:
1) Oligo/anovulation
2) Clinical/biochemical signs of hyperandrogenism
3) Polycystic ovaries by scan with exclusion of other related
disorder.[2]

Insulin resistance defined as inability of known quantity of exogenous or endogenous insulin to increase glucose uptake and utilization in individual, as it does in normal individual. [3] Insulin resistance is pathogenic factor for association between HTN, Glucose intolerance, obesity, lipid abnormality, coronary artery disease which together constitute metabolic syndrome [SYNDROME X].[4]

In polycystic ovarian syndrome patients there is relative inefficiency of insulin receptors binding to insulin which leads to improper transfer of glucose to intracellular compartment and this result in relative hyperglycemia, in spite of increased insulin producing beta cells.so polycystic ovarian syndrome patients are at increased risk of developing insulin resistance.[5]
Hyperandrogenism leads to increased hepatic lipase.[3].In polycystic ovarian syndrome there is increased secretion of VLDL particle by liver resulting in elevated TG concentration.[6]

Insulin resistance and associated dyslipidemia are independent of obesity markers such as BMI, waist to hip ratio.[7]. Overall prevalence of abnormal OGTT in polycystic ovarian syndrome reproductive age women is 20%. It is probably beneficial to screen IR in this specific group in order to provide optimum management.[8]
This study is undertaken to find out prevalence of insulin resistance in polycystic ovarian syndrome patients and to evaluate relationship of TG/ HDL-C, TC/HDL-C, LDL-C/HDL-C with insulin resistance in polycystic ovarian syndrome patients.

II.   MATERIALS AND METHOD

A study was carried out for a period of one year. The patients were selected from Chigateri General Hospital and Bapuji Hospital, Davangere (both hospitals are attached to the teaching institute JJM Medical College, Davangere).and private hospital in and around JJMMC Davangere.

Study was carried out in clinically   60 diagnosed cases of polycystic ovarian syndrome and 60 age matched controls were selected based on inclusion and exclusion criteria.

Inclusion Criteria: CASES; patients between age 17-36yrs diagnosed as polycystic ovarian syndrome having clinical features oligomenorrea (35days) , amenorrea (6months) , hyperandrogenisum features like acne, hirsuitism, and diagnosed polycystic ovaries by ultrasound CONTROLS; 17- 36 years females having normal menstrual cycle.Exclusion criteria:Patients having history of diabetes mellitus, impaired glucose tolerance, pregnancy, breast feeding ,non fasting patients with untreated hypothyroidism, those on drug treatment like antihypertensive, antiplatelet, lipid lowering agents, drug affecting glucose &lipid metabolism, congenital adrenal hyperplasia, cushing syndrome, ovarian/adrenal androgen secreting tumors.

After taking informed consent, under all aseptic precautions about 5 ml of venous blood was collected in a sterile bulb after overnight fasting of 12 hours. 2ml was collected in EDTA vial (for plasma), 3ml in plain vial for (for serum) , it was subjected for centrifugation serum and plasma will be separated. Insulin, Serum lipoproteins estimated from serum and fasting glucose from plasma.

Fasting plasma glucose and Serum triglycerides measured by GOD POD method by Erba mannheim chem5 plus Semi Auto – analyzer”.Normal Fasting plasma glucose 70-110mg/dl. Normal Serum TG levels :- 40-150 mg/dl .Serum Total cholesterol AND serum HDL measured by CHOD PAP METHOD .Erba Mannheim Chem5 plus Semi Auto – analyzer”Normal Serum TC levels :- 150 -200 mg/dl, ”Normal Serum HDL levels :- 35-75 mg/dl. serum LDL cholesterol calculated by [9]:Friedwalds formula

$$LDLcholesterol(mg/dl) = Total\ cholesterol - HDL\ cholesterol - \frac{[triglycerides]}{5}$$

Normal serum LDL cholesterol concentration range: < 130 mg/dl

serum fasting insulin estimated by chemiluminescence immunoassay. Normal value of fasting insulin: 5-25µIU/ml.

Calculation of insulin resistance by using HOMA model (HOMA -IR). [10]

$$HOMA\ \text{-}IR = \frac{(fasting\ plasma\ glucose\ in\ mg/dl \times fasting\ serum\ insulin\ in\ \mu iu/ml)}{405}$$

The subject is considered to have insulin resistance if HOMA-IR value is more than 2.7.

Statistical analysis was done using SPSS software, version 17.0. Descriptive data were presented as mean $\pm$ SD and range values. Unpaired student t test used to compare between cases and controls. Relationship between insulin resistance and serum lipoproteins, was assessed by Pearson's correlation coefficient. For all the tests, the probability value (p-value) of less than 0.05 was considered statistically significant.

III.RESULTS

In the present study, a total number of 120 subjects were included. They were divided into 2 Groups:**Controls –** It consisted of 60 healthy women **Cases**-It consist of 60 PCOS cases

The present study shows that the mean levels of fasting insulin ,fasting plasma glucose and HOMA IR ,serum lipo protein ,serum lipoprotein ratio are significantly increased in subjects with PCOS when compared to healthy controls except HDL cholesterol which is significantally lowered in PCOS when compared to healthy controls.(Table 1, figure 1 &2)

There is significan positive correlation between HOMA IR levels and all serum lipo protein ratio.(Table 2, figure 3)

Table1: Comparision of Fasting plasma glucose, Fasting serum Insulin,insulin resistance ,Lipid profile and serum lipoprotein ratio in study groups. Results expressed as Mean ± S.D

Parameter	PCOS CASES	CONTROLS	P VALUE
Fasting serum Insulin (μIU/ml)	24.50±10.03	9.33 ±3.08	<0.05
Fasting plasma glucose mg/dl	114.20±30.38	94.38±10.36	<0.05
HOMA-IR	7.29±4.08	2.16±0.67	<0.05
S.Triglycerides (mg/dl)	202.44±30.15	124.97±13.87	<0.05
S. Total Cholesterol (mg/dl	210.05±36.96	152.43±25.78	<0.05
S. HDL Cholesterol (mg/dl)	33.71±6.74	53.84±5.18	<0.05
S. LDL Cholesterol (mg/dl)	135.85±38.60	73.59±27.45	<0.05
S. TC : HDL ratio	6.60± 2.21	2.87 ± 0.63	<0.05
S. TG : HDL ratio	6.34 ± 1.97	2.34± 0.37	<0.05
S.LDL:HDL ratio	4.33±1.90	1.40±0.61	<0.05

. **Table 2 :Corelation of HOMA IR with serum lipoprotein ratio**

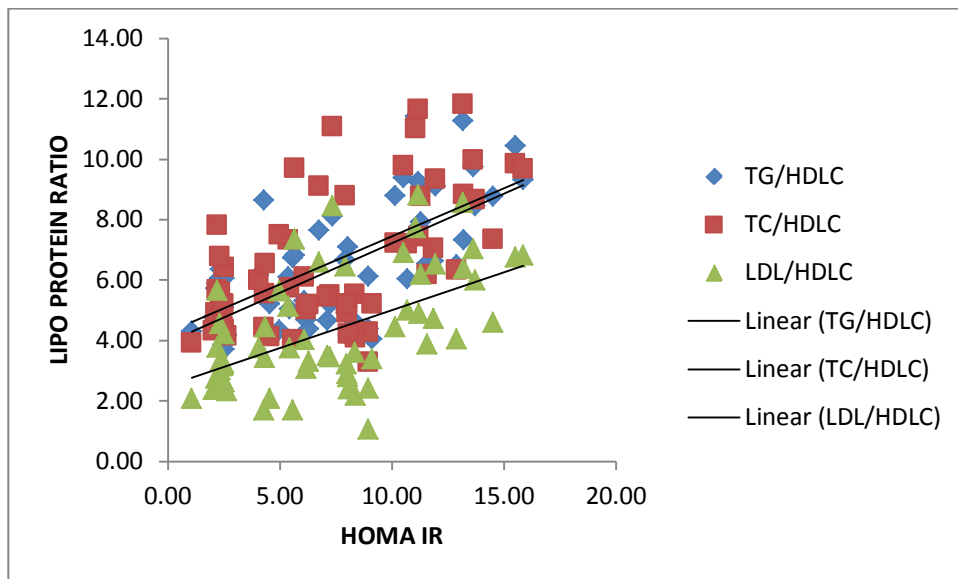| Variables	r value	p value
Serum TG:HDL ratio	0.68	0.00 **
Serum TC:HDL ratio	0.58	0.00 **
Serum LDL:HDL ratio	0.54	0.00 **

Graph 1:Fasting glucose , fasting insulin and insulin resistance HOMA IR in cases and controls.
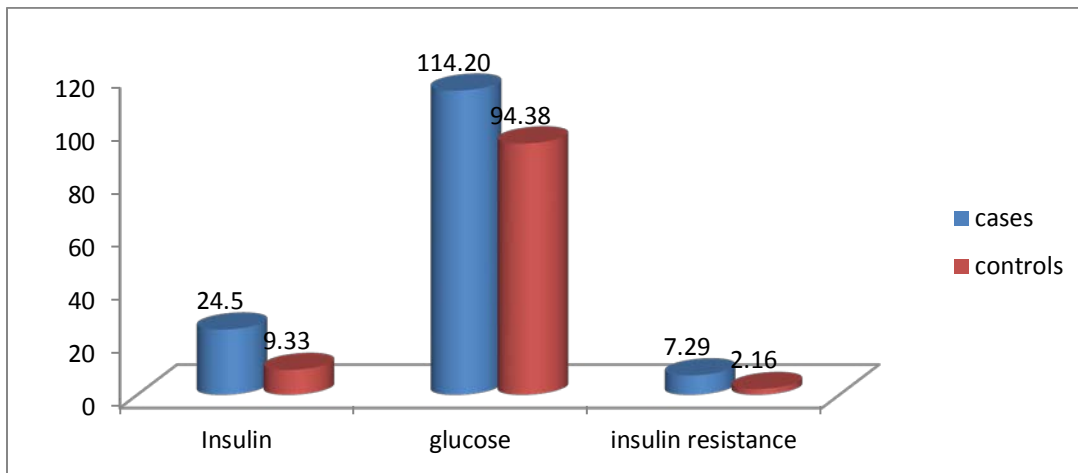
Graph 2: Lipid profile in PCOS cases and controls.
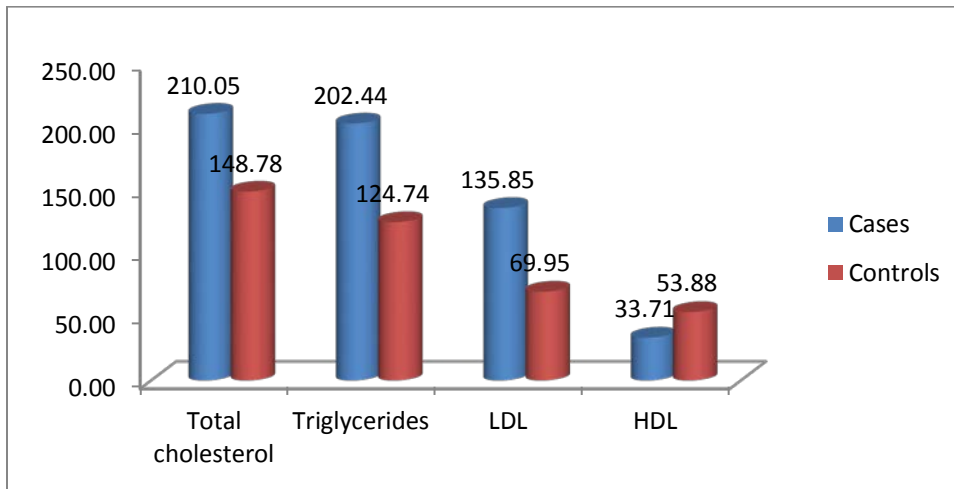
Graph 3 : Correlation of insulin resistance with serum lipo protein ratio

IV.DISCUSSION

Polycystic ovarian syndrome is most common endocrine disease and metabolic disorder in adolescence and reproductive women which is first reason for female infertility [3].

PCOS is a heterogenous disorder for which several pathogenic mechanism have been proposed. The main mechanism is abnormal gonadotropin secretion, with excess circulating LH and low FSH. Hypersecretion of androgen by ovarian and adrenal gland which provide substrate for pheripheral aromatisation to estrogen also play role in development of PCOS. Estrogen in turn negatively feed back on pituitary to decrease FSH production.[11]

PCOS women have insulin resistance, which results in compensatory hyperinsulinemia. The defect in insulin action in PCOS appears to be at the post binding level which involves glucose transport. Insulin resistance may be accompanied by a defect in insulin induced inhibition of lipolysis.[11]Both insulin resistance and hyperandrogenemia contribute to atherogenic lipid profile. Testosterone decreases lipoprotein lipase activity in abdominal fat cells, and insulin resistance impaires ability of insulin to exerts its antilipolytic effects.[12]

Prevalence of insulin resistance

Prevalance in this study is 45/60 i,e 75%. This is similar to anuradhaclara et al[1] where prevalance of IR in PCOS found to be 76.9 % and is in consistant with previous studies that showed Indian PCOS women to be more insuin resistant then there white counterparts[1]

In study done by Thanyarat et al[8] showed the overall prevalence of IR in there study population was 20.0%. The prevalence of IR in there PCOS women was lower than that of many reports which revealed the prevalence up to 75%. The difference in techniques used to define IR was responsible for such large discrepancy. They used FPG AND OGTT. There finding was in line with the previous information that FPG alone is not a sensitive indicator for IR in women with PCOS. One more factor is that there study population was relatively young and thin. Previous studies showed that the prevalence of IR increased with age and BMI.[8]

QUICKI, HOMA-IR and fasting insulin to fasting glucose ratio are the most useful index in the evaluation of resistance to insulin. However, DeUgarte et al believe that the greatest predictive value is characterized by HOMA-IR.[13]

The cellular mechanism of insulin resistance in the polycystic ovary syndrome remains controversial. It may be because of reduced binding of insulin to its receptor, whereas two recent studies using peripheral adipocytes (recognized target cells for insulin action) have shown normal binding but reduced insulin-mediated glucose transport, suggesting a postreceptor defect. The mechanism underlying this phenomenon has not been fully characterized, but decreased expression of the insulin-dependent glucose-transporter protein GLUT-4 has been described. [14]

There is a postbinding defect in insulin signalling in adipocytes and skeletal muscle isolated from women with PCOS. In cultured skin fibroblast of women with PCOS, impaired insulin action on glycogen synthesis is associated with constitutively increased insulin receptor beta subunit serine phosphorylation and decreased insulin receptor tyrosine kinase activity. A serine kinase extrinsic to receptor is responsible for these abnormalities. [15]

Both insulin resistance and hyperandrogenemia contribute to dyslipidemia in PCOS.[12] Dyslipidemia may be caused by combination of overproduction of VLDL (apo B 100), decreased catabolism of apo B containing particles and increased catabolism of HDL apo A1 particles. These abnormalities may be the consequences of global metabolic effect of insulin resistance.[16]

In 2013 Kim J and co-workers conducted study which aimed to study detailed profile of dyslipidemia in PCOS of Korean women. They found that in young and non-obese Korean women with PCOS have substantially increased prevalence of dyslipidemia. Dyslipidemia in women with PCOS may be consistent with those found in the insulin resistant state: decreased levels of HDL-C, ApoA-I, increased levels of TG, and increased LDL. So women with PCOS should receive a complete lipid test, and lifestyle modification is the first line therapy for all women with PCOS and is particularly important for those with dyslipidemia.[17]

Women with polycystic ovaries will have abnormal lipid profile, higher level of cholesterol, triglycerides and LDL cholesterol and lower level of HDL cholesterol.By its action on hormone sensitive lipase (HSL), insulin inhibits release of FFA from adipose tissue. When insulin resistance is increased, the uptake of FFA by adipose tissue is decreased and its release from adipocytes is increased. Thus there will be increased availability of FFA to liver. This leads to increased triglyceride (TG) synthesis and overproduction of VLDL.[18]

In the liver, FFA not only stimulates TG synthesis and VLDL production but also down regulates the expression of ATP binding cassette protein A1 (ABCA 1) by interfering with binding and activation of nuclear factor LXR alpha by oxisterols.[18]

Insulin resistance results in reduced activity of endothelial bound LPL, which contributes to impaired TG hydrolysis and uptake of chylomicrones and VLDL by muscle and adipose tissue .Insulin resistant states also result in increase level of APO C111:an inhibitor of LPL and impaired APO E mediated receptor uptake of TG rich lipoprotein and their lipolytic remanants. The net effects of changes in TG rich lipoprotein metabolism are an increase in plasma transport and prolonged residences of these lipoproteins and their potential atherogenic catabolic products.[19]

Low HDL is often considered as secondary to raised TG. In presence of increased plasma TG level the CETP (cholesterol ester transfer protein) mediates TG –CE exchange between LDL with VLDL and HDL forming TG rich HDL .These TG rich but cholesterol depleted HDL are more prone to be catabolised .They under grow hydrolysis of their TG component apo A.[20,21]

Several epidemiologic studies demonstrated that the total cholesterol TC/HDL-C and the LDL-C/HDL-C ratios are better predictors of atherosclerosis and cardiovascular disease than any other single lipid marker. Likewise, the TG/HDL-C ratio was demonstrated to be as significant a predictor of cardiovascular disease as the two other lipid ratios persons with increased TC/HDL-C ratio were shown to exhibit resistance to insulin-stimulated glucose disposal and to have higher blood pressure, increased TG concentrations, and hyperinsulinemia; each of these factors is part of the metabolic syndrome and is an independent risk factors for cardiovascular disease.[22]

Increased TG/HDL-C ratios are able to identify insulin- resistant overweight individuals with normal glucose tolerance and are markers of insulin resistance with specificities and sensitivities similar to those for fasting plasma insulin concentration. Increased TG/HDL-C ratios also indicate the presence of atherogenic small, dense LDL particles and could serve as a good predictor of myocardial infarction and the presence of coronary atherosclerotic lesions. The better ability of lipid ratios to predict cardiovascular disease compared with single lipid markers is of particular clinical relevance and can be possibly explained by association of lipid ratios with a cluster of cardiovascular risk factors that are at least in part unrelated to cholesterol metabolism.[22]

In a study Shou-Kui Xiang et al[1] it was found that TG/HDL-C, TC/HDL-C, and LDL-C/HDL-C of PCOS patients were significantly higher than those of the age-matched healthy women and had a significant positive correlation with HOMA-IR.

V.CONCLUSSION

This study shows close relationship between insulin resistance, altered atherogenic lipid profile in PCOS patients. Insulin resistance may be the first important marker of metabolic disease in PCOS women, who are at risk of development of type 2 diabetes, obesity, hypertension, dyslipidemia and coronary artery disease. Screening of dyslipidemia in PCOS is essential in order to prevent complications like development of early atherosclerosis and premature clinical presentation of cardiovascular disease.Insulin resistance is cause for dyslipidemia. Hence PCOS patients should be screened for insulin resistance and dyslipidemia and take treatment for insulin resistance in order to prevent occurrence of complications.

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