Assessment of relationship between hormones and insulin resistance in PCOS

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ABSTRACT

Introduction: Polycystic ovarian syndrome (PCOS) is the most common heterogenous disorder of reproductive age. Increased frequency of GnRH pulses from hypothalamus elevates LH levels this in turn causes increased androgen production. The chronic hyperandrogenic state have multiple long and short term complications which includes DM and CVD. Insulin resistance can be characterized as impaired action of insulin on glucose metabolism which increases risk of developing T2DM. Hyperandrogenism with hyperinsulinaemia also leads to dyslipidemia.

Aim: To estimate LH/FSH ratio, testosterone in PCOS patients and to correlate its significance with the insulin resistance.

Materials and Methods: This case-control study was conducted on clinically, diagnosed 50 PCOS patients, aged 15 to 35 years were included as cases. Age-matched 50 apparently healthy women were included as controls. Serum leutinising hormone (LH), Follicle stimulating hormone (FSH), testosterone, serum insulin were analysed by chemiluminescence immunoassay (CLIA) on Maglumi 1000. Mindray BS 300, fully automated analyser was used for estimation of Total cholesterol (TC), High density lipoprotein (HDL), Triglyceride (TG), Fasting Blood Glucose (FBG). Low density lipoprotein (LDL) was calculated using Friedewald’s formula. Insulin resistance was assessed by HOMA IR. Descriptive statistics analysis was done using unpaired student’s t-test. Chi-square/ Fisher Exact test has been used to find the significance of study parameters on categorical scale. Pearson’s correlation coefficient was applied to analyse the correlation. p value <0.05 was considered statistically significant.

Results: In our study the LH /FSH ratio, HOMA IR and Testosterone was increased in cases compared to controls with p 0.01 and for testosterone p 0.001. In contrast HDL decreased in cases compared to controls (p<0.001). Triglyceride (p<0.01) was higher in cases compared to controls in our study. However LDL and Total cholesterol were not significantly increased in cases however we can find that LDL was still increased in cases. Average BMI was within the normal range (p=0.06). On correlation study FSH showed a negligible negative correlation with HOMA IR, Follicle stimulating hormone(LH), Triglyceride(TG), Fasting Blood Glucose (FBG). Low density lipoprotein (LDL) was calculated using Friedewald’s formula. Insulin resistance was assessed by HOMA IR. Descriptive statistics analysis was done using unpaired student’s t-test. Chi-square/ Fisher Exact test has been used to find the significance of study parameters on categorical scale. Pearson’s correlation coefficient was applied to analyse the correlation. p value <0.05 was considered statistically significant.

Conclusion: In our study we propose that in PCOS cases IR seems to underlie many clinical features of PCOS. It encompasses long term health problems like CVD, DM and increased exposure to estrogen can lead to endometrial carcinoma. It is need of the time to follow up the patient to early identify and prevent the consequences of this syndrome.

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1. Introduction

PCOS is the most common heterogenous endocrinological disorder of reproductive age and also the leading cause
for infertility. Polycystic morphology is consistent with, but not essential for, the diagnosis of the syndrome. These changes, however, can be present in women who are endocrinologically normal. Thus, the ovarian morphological change must be distinguished from the endocrine syndrome of hyperandrogenism and anovulation.

Due to unknown reasons there is increased frequency of GnRH pulses from hypothalamus which elevates LH levels in turn cause increased androgen production from theca cells of ovary. The increase amplitude and frequency of LH pulses in PCOS leads to increased circulating levels of LH and its significance with FSH levels are significantly increased in PCOS women. Altered LH and FSH are reasons for anovulation.

Androgen excess is contributed by ovary and also adrenals due to multifactorial causes. There is no hormonal test to discriminate between a case of PCOS and normal cycling women. The chronic hyperandrogenic state have multiple long and short term complications which in particular includes DM and CVD along with risk of endometrial cancer. The pathogenesis of PCOS involves HPO axis, ovarian theca cell hyperplasia, hyperinsulinemia and multitude of either cytokines and adipocyte driven factors.

PCOS is associated with increased risk of metabolic dysfunction such as obesity, dyslipidemia, hypertension, IR, infertility. Increased risk for diabetes, its complication, the metabolic dysfunction features are major risk factors for CVD. PCOS imposes several consequences to female health. Insulin resistance can be characterized as impaired action of insulin in the uptake and metabolism of glucose. There is a particular emphasis on its relationship with Insulin Resistance(IR) which increases risk of developing T2DM. IR with hyperinsulinemia also stimulates excess androgen from theca cells of ovary. Insulin effects on the hypothalamo-pituitary axis and also ovarian tissue. The resultant insulin resistance leads to impaired metabolic signalling but its steroidogenic activity is retained favoring hyperandrogenism which perhaps is the major factor in clinical manifestation of PCOS.

Hyperandrogenism along with hyperinsulinemia are causes for dyslipidemia as well and when it is associated with obesity there is increased risk. Testosterone both free and total are increased with increase in serum androgens. Androgens have source from both adrenals and ovary so however the ovarian source of androgen is indicated by testosterone but DHEA-SO4 is used for adrenal production of androgen. Because of the heterogeneity of this disorder a complete understanding of the underlying pathophysiology of PCOS is still lacking, there are most likely multiple underlying pathophysiologic mechanisms. Several theories have been proposed to explain the pathogenesis of PCOS.

Thus in this study we aimed to estimate LH/FSH ratio, testosterone in PCOS patients and to correlate its significance with the insulin resistance to understand and prevent long term complications like cardiovascular disease, metabolic syndrome and diabetes mellitus in these patients.

2. Materials and Methods

2.1. Study population

This case-control study was conducted for the period of one year in the Department of Biochemistry, Rajarajeswari Medical College and Hospital, Bengaluru, Karnataka, India. The study was approved by the institutional ethical clearance committee. Written informed consent was obtained from all subjects included in the study. Complete medical history was recorded and physical examination of each individual was done.

2.2. Inclusion criteria

Clinically diagnosed 50 PCOS patients attending Obstetrics and Gynecology OPD, aged 15 to 35 years were included as cases. Age-matched 50 apparently healthy women were included as controls.

2.3. Exclusion criteria

Women with a history of diabetes mellitus, hypertension, thyroid disorders, any evidence of kidney, liver disease, CAH, Cushing's syndrome, adrenal tumors, pregnancy, anemia were excluded.

Fasting venous blood samples (5 mL) were collected from antecubital vein in clot activator tubes and 2 ml in fluoride tube while taking all aseptic precautions. The samples were allowed to clot and then centrifuged at 2000 rpm for 10 minutes. The serum was separated and stored at -20°C until further analysis.

Serum LH, FSH, testosterone, serum insulin were analysed by chemiluminescence immunoassay (CLIA) on Maglumi 1000. Mindray BS 300, fully automated analyser was used for estimation of serum Lipid profile such as Total cholesterol by CHOD - POD method, HDL by phosphotungstate precipitation, followed by enzymatic method, Triglyceride by GPO method, LDL was calculated using Friedewald's formula [TC-(HDLc+VLDLc)]. Fasting Blood Glucose (FBG) was estimated by Glucose Oxidase-Peroxidase (GOD-POD) method. Insulin resistance was assessed by HOMA IR, calculated as fasting insulin*FBG/405. Height and weight measured for Body mass index (BMI). Blood samples were drawn randomly irrespective of the menstrual cycle.

2.4. Statistical analysis

The data were analysed using descriptive statistics. The results were presented as Mean±SD. Unpaired Student’s
t-test (two tailed, independent) was used to analyse the clinical parameters between cases and control group. A p value <0.05 is considered statistically significant. Chi-square/ Fisher Exact test has been used to find the significance of study parameters on categorical scale between two or more groups. Pearson’s correlation coefficient was applied to analyse the correlation between study variables.

3. Results

In our study the LH/FSH ratio was <1.5 in 46%, 1.5-2.5 in 38% and >2.5 in 16% in cases whereas 98% of controls had <1.5 and none had > 2.5 in controls (Table 1). Testosterone levels were <0.5 in 70% and > 0.5 in 30% of cases whereas none of controls had value ≥0.5 (Table 2). The age was matched in both the group. (Fasting blood glucose) FBG was 91.6±11.9 in cases and 90 ± 11.2 in controls which was not statistically significant p=0.5. The LH /FSH ratio when taken it was found to be 4.6±4.5 and 0.8±0.26 in cases and controls respectively with p 0.01. Testosterone was 0.58±0.92 and 0.13±0.04 in cases and controls respectively with p 0.0 0 1. HOMA IR was found to be 6±7.4 and 2.9±1.3 in cases and controls which was found statistically significant with P < 0.0 1. Lipid profile such as HDL showed 40.4±7.3 and 48±11.2 (p<0.001) which was found to be higher in controls compared to cases and Triglyceride 157.7±26.4 and 140.3±34.2 (p<0.0 1)which was higher in cases compared to controls in our study. However LDL and Total cholesterol were 92.±2±27 and 160±34 in cases, 89±30 and 156±30.3 in controls respectively where we can find that LDL was not statistically significant but still an increased LDL was seen in cases. To see the effect of BMI we compared BMI between the study group and found that the average BMI was within the normal range in cases (23.7±3.8) when compared to controls(25.3±4.6) which was on a higher side p= 0.06 (Table 3).

Pearson’s correlation coefficient analysis was used in cases between HOMA IR and BMI with other study variables. FSH showed a negligible negative correlation with HOMA IR and mild negative correlation with BMI. LH showed a positive correlation with both HOMA IR (r 0.45, p<0.01) and BMI(r 0.61, p<0.01). Whereas testosterone showed no correlation. LH/FSH ratio was positively correlated with HOMA IR(r 0.42, p<0.01) and also BMI(r 0.53, p <0.001). TC(r 0.31,p 0.02) showed significant positive correlation whereas LDL(r 0.18p 0.21), TG(r 0.16p 0.26) though positive showed negligible correlation with HOMA IR. BMI showed a significant positive correlation with HOMA IR (r 0.43, p <0.01)(Table 4)

4. Discussion

PCOS is the most common heterogenous gynecological disorder of reproductive age. The diagnostic criteria is not fully understood yet because of its varied pathophysiology and clinical presentation. The altered gonadotropin pulse amplitude is considered the major pathogenic mechanism involved which leads to excess LH in circulation with decreased FSH. The hypothalamic, pituitary and ovarian signals are reflected by the FSH and LH release pattern.11

In our study the cases had lower BMI in line with the study by Padnis but however the LH levels were positively correlated with LH in our study as opposed to Padnis et al. Padnis noted that non obese PCOS women had significantly high LH than obese women with PCOS which is contradictory to our findings.12 Hyperandrogenism is the pre dominant biochemical abnormality in women with PCOS. However, the cause of this hyperandrogenism and its relation to the central clinical component of PCOS, chronic anovulation, are only partly understood.13 Increased androgen production is associated with elevated LH concentration which is peripherally accentuated with their inefficient aromatization to estrogen. This being related to low FSH levels in PCOS, with local androgen excess and estrogen deficit within ovary. The above features creates a potent androgenic environment for follicle leading to cessation of follicular growth. As there is an inappropriate LH levels there is an elevated testosterone levels.14 In clinical setup serum total testosterone levels is the most common measure for investigation of hyperandrogenism. In our study testosterone levels were high in cases compared to controls and was statistically significant (p= 0.0 0 1). (Table 2)

Table 1) In our study the LH/FSH ratio was <1.5 in 46 %, 1.5-2.5 in 36% and >2.5 in 18% in cases whereas 98% of controls had <1.5 and none had > 2.5 in controls. Eissas et al concluded in their study that, polycystic ovary syndrome (PCOS) is characterized by increased frequency of hypothalamic GnRH pulses leading to a relative increase in LH synthesis by the pituitary.15 Women with PCOS have higher GnRH, which in turn results in an increase in LH/FSH ratio.16 Their results revealed significant increase in serum LH and significant decrease in FSH/LH ratio. The LH/FSH ratio was statistically significant and high in cases(p 0.01)(Table 3) in our study similar to the above study. We further did correlation between LH/FSH ratio and found LH/FSH ratio was positively correlated with HOMA IR(r 0.42, p<0.01) and also BMI(r 0.53, p <0.001)(Table 4).

On the other hand, Cho LW et al found that, LH/FSH ratio did not differ significantly between the PCOS cases and non affected group.17 Khalifa et al showed in his studies that there was a significant raise in LH/FSH ratio with increasing weight in PCOS patients. Similarly in our study we found
Table 1: Distribution of LH/FSH Ratio in two groups studied

| LH/FSH Ratio | Cases     | Controls   |
|--------------|-----------|------------|
|              | No  %     | No %       |
| <1.5         | 23 46.0   | 49 98.0    |
| 1.5-2.5      | 19 38.0   | 1 2.0      |
| >2.5         | 8 16.0    | 0 0.0      |
| Total        | 50 100.0  | 50 100.0   |

P<0.001**, Significant, Fisher Exact test

Table 2: Distribution of Testosterone (ng/ml) in two groups studied

| Testosterone (ng/ml) | Cases     | Controls   |
|----------------------|-----------|------------|
|                     | No  %     | No %       |
| <0.5                 | 35 70.0   | 50 100.0   |
| 0.5-1                | 12 24.0   | 0 0.0      |
| >1                   | 3 6.0     | 0 0.0      |
| Total                | 50 100.0  | 50 100.0   |

P<0.001**, Significant, Fisher Exact test

Table 3: Comparison of study variables in two groups studied

|                          | Cases n=50 (Mean±SD) | Control n=50 (Mean±SD) | P value |
|--------------------------|-----------------------|------------------------|---------|
| Age(years)               | 28.5±5.2              | 22±4.1                 | 0.15    |
| FBG(mg/dL)               | 91.6±11.9             | 90±11.2                | 0.5     |
| FSH (mIU/mL)             | 4.6±2.3               | 5.8±3                  | 0.02*   |
| LH (mIU/mL)              | 7.8±5.2               | 4.14±1.4               | <0.001* |
| LH/FSH ratio             | 2.3±4.36              | 0.8±0.26               | 0.01*   |
| Testosterone (ng/ml)     | 0.58±0.92             | 0.13±0.04              | 0.001*  |
| Insulin(μIU/ml)          | 25.9±31.4             | 13.36±6.5              | 0.001*  |
| HOMA IR                  | 6±7.4                 | 2.9±1.3                | <0.01*  |
| TC(mg/dL)                | 160±34                | 156.4±30.3             | 0.55    |
| HDL(mg/dL)               | 40.4±7.3              | 48±11.2                | <0.001* |
| LDL(mg/dL)               | 92.2±27               | 89±30                  | 0.57    |
| Triglyceride(mg/d)       | 157.7±26.4            | 140.3±34.2             | <0.01*  |
| BMI(kg/m2)               | 23.7±3.8              | 25.3±4.6               | 0.06    |

P value <0.05 is considered statistically significant*

Table 4: Correlation of HOMA IR and BMI with study variables

|                          | Age | FSH | LH | Testosterone | LH/FSH ratio | TC | HDL | LDL | TG | BMI |
|--------------------------|-----|-----|----|--------------|--------------|----|-----|-----|----|-----|
| HOMA IR                  | r   | 0.21| -0.08| 0.45 | 0.01 | 0.42 | 0.31 | -0.01 | 0.18 | 0.16 | 0.43 |
| IR                       | p   | 0.14| 0.58 | <0.01 | 0.9 | <0.01 | 0.02 | 0.9 | 0.21 | 0.26 | <0.01 |
| BMI                      | P   | 0.53| 0.09 | <0.01 | 1 | <0.001 | - | - | - | - | - |

P value <0.05 is considered statistically significant

Insulin is also shown to increase gonadotropin secretion and decreases hepatic synthesis and secretion of SHBG so there is increased free testosterone. The increased ovarian enzymatic activity in synthesis of testosterone precursors are also found. Thus increased insulin and IR are considered to be another key factors for this syndrome. Hence we can presume that hypothalamic dysfunction and IR are factors functioning independently for metabolic dysfunction of PCOS. The mechanism linking PCOS and increased cardiovascular risk profile are not well understood. Nevertheless IR and hyperinsulinemia increases the risk for cardiovascular diseases along with which the hyperandrogenemia could contributes to vascular damage and endothelial dysfunction in women. The insulin regulatory molecules on the theca cells are responsive to insulin, whereas in the muscle and liver it shows resistance. Thus, the metabolic disorders associated with PCOS along with their long-
term sequelae have to be closely evaluated in both lean and obese PCOS patients. In our study HOMA IR was used to analyse the insulin resistance and we found an increased and statistically significant HOMA IR in cases compared to controls. On correlation study of HOMA IR with other biochemical parameters it was found that HOMA IR had correlation with LH and LH/FSH ratio but with FSH it showed negligible negative correlation and with testosterone no correlation. Study by Chan Hong found no significant correlations between the serum LH/FSH ratio and biochemical parameters related to insulin resistance. Metabolic disturbances, viz. impaired GTT, abnormal lipid profile, higher fasting/2-hour insulin levels, and higher insulin resistance, are also witnessed in the PCOS patients. Presence of obesity makes these patients susceptible to deranged lipid profile, a greater degree of insulin resistance.

However, most women with PCOS also have a metabolic abnormality that includes increased levels of low density lipoprotein cholesterol, reduced levels of high-density lipoprotein cholesterol and hypertriglycerideremia, which together are thought to increase the risk for metabolic derangements like cardiovascular disease, and type 2 diabetes mellitus with age. There was a deranged lipid profile in our cases with decreased HDL and increased Triglycerides though in our case the BMI was predominantly within range but their correlation with HOMA IR did not show any significance except for Total cholesterol which showed a positive correlation with HOMA IR. This syndrome, a complex disorder with multiple components, including reproductive, metabolic, and cardiovascular manifestations, has long-term health issues which is now well recognized. Considering the increasing rate of patients with PCOS in modern societies, the importance of studying the effect of endocrine disorders is essential to understand their associated risk for CVD and T2DM which can be followed up for a better outcome.

5. Conclusion

PCOS is much more than just oligomenorrhea, infertility. It encompasses long term health problems like CVD, DM and increased exposure to estrogen can lead to endometrial carcinoma. We must limit complications of PCOS, although not well understood IR seems to underlie many clinical features of PCOS. IR increase risk for T2DM and dyslipidemia. It is imperative that we act to prevent the consequences of this syndrome by indentifying and following the case.

6. Limitations

The study was done on a small sample group. A larger prospective study group would be required to ascertain the effect of BMI and IR for future risk. Blood samples were drawn randomly and only one sample taken throughout the menstrual cycle.

7. Source of funding

None.

8. Conflict of interest

None.

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