Potential Pathophysiology Through High Gonadal Hormone Profile and Peripheral Insulin Resistance, Circumventing IFN γ Effects in Women With Polycystic Ovary Syndrome

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Heterogeneous Polycystic ovary syndrome (PCOS) is a low grade inflammatory diseases common with many of the young female and strongly associated with peripheral insulin resistance. Our study focused on investigating the effects of interferon gamma (IFN γ) in control of IR in obese PCOS patients with matched control. PCOS patients diagnosed by Rotterdam criteria and BMI matched controls were checked for Fasting insulin and IFNγ, FSH, LH, prolactin, testosterone, triglycerides and glucose levels and IR was calculated by Homeostasis Model Assessment (HOMA-IR), Quantitative Insulin Sensitivity Check Index (QUICKI) and McAuley (McA) index. Fasting glucose, insulin, and IR by QUICKI, HOMA-IR and McA is higher in obese PCOS in compared to control. High serum testosterone, LH, and FSH levels were also observed in women with PCOS. But IFNγ is not statistically significant and not correlated significantly with fasting insulin (ρ = -0.004), HOMA-IR (ρ = -0.02), QUICKI (ρ = -0.11) or McA (ρ = -0.15) in obese women with PCOS. We suggest that development of peripheral insulin resistance in PCOS is not through the mediation of IFNγ. Further studies are needed to prove the suggested mechanism and to develop drugs for reversing PCOS pathology.

Key words Pro-inflammatory cytokines, PCOS syndrome and peripheral Insulin resistance

I. INTRODUCTION

PCOS is one of the most common endocrine disorders in women of reproductive age and cause of and leading cause for infertility and hirsutism (1). In the latest finging in 2020, central obesity has been identified as a one of the risk factor in PCOS patient (2). Azziz *et al* found the exponential growth of the PCOS prevalence in population of reproductive aged women in United States (3). Population statistics in Sri Lankan patients had also found out in 3030 individuals, that the prevalence of PCOS is similar to above finding among women aged 15-39 years (4). PCOS is strong and it has been identified that the obese PCOS patients showing high insulin resistance (IR) and fasting insulin (FI) levels (5). It has been identified that the obese women with PCOS show greater IR and higher FI levels (6). It has been observed that hyperinsulinemia associated with IR linked to all the features of this syndrome like hyperandrogenism, reproductive disorders (irregular cycles, infertility), acne, hirsutism and metabolic disturbances (7).

There is no standard treatment plan to treat these individuals. Various options have been tried to improve the signs and symptoms. It has been hypothesized that Insulin sensitizing agents like metformin have impact on IR and improve endocrine, metabolic and reproductive abnormalities of women with PCOS and have numerous beneficial effects (6). Another research group had identified the relationship between IR and PCOS syndrome But the practice of treating all women with PCOS with these agents has not been justified (8). At the same time the mechanism underlying the beneficial effects of such medications in the treatment of PCOS remains incompletely understood.

Studies have revealed that the serum levels of C reactive protein (CRP), interleukin 6 (IL- 6) and tumor necrosis factor (TNF α) are increased due to inflammatory mediation in PCOS (9,10). This important finding reflects a chronic low grade inflammatory process in this syndrome. The current study is based on Interferon gamma (IFN-γ) which is a pro – inflammatory cytokine. It is reported that IFN-γ is actively involved in almost all phases of immune and chronic inflammatory disorders (11). In addition, this cytokine is related to the development of IR in obesity (12). Another research team that the stimulated production of IFNγ is significantly decreased in women with PCOS, irrespective of BMI n-vitro study, (13).

Our research study focus on the potential mechanism or a role of pro-inflammatory marker, IFNγ in the development process of IR which may be used in the new drug leads in designing and development for PCOS. Therefore this study was

designed to see the potential relationship between IFNγ and peripheral IR in obese women with polycystic ovary syndrome.

II. MATERIALS AND METHODS

A descriptive cross sectional study was carried out for 12 months and the research was approved by the accredited Ethical Review Committee. Consecutive affected obese women (BMI≥ 30kg/m^2) with PCOS, enrolled at specialist clinics in two Teaching Hospitals, from April to August, were invited for the study. Concurrently, BMI matched asymptomatic, un-medicated, menstruating women were recruited as controls from gynaecology clinics and among the staff members of two hospitals. PCOS was excluded among control individuals by doing biochemical and ultrasound testing. Written informed consent was obtained from all volunteers. Women who were diagnosed to have PCOS by the clinicians using the 2003 Rotterdam criteria were recruited (Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2004). Diagnosis was established clinically, biochemically and radiologically, and confirmed when two out three of the following features were present: Oligomenorrhea (Cycles lasting longer than 35 days) or amenorrhea (Less than two cycles in the past six months), clinical signs of hyperandrogenism with a Ferryman – Gallway (FG) score ≥ 8 and polycystic ovaries. (at least one ovary with at least 12 follicles of a diameter of 2-9mm or a volume>10ml). woman with hyperprolactinaemia and those taking corticosteroid, antiepileptic or antipsychotic drugs or hormonal contraception or suffering from acute illness and those currently pregnant were excluded.

The degree of hirsutism was determined by the modified FG score. Body mass index (BMI) was calculated as weight/ (height) ^{2} in (kg/m^2). Presence of acanthosis nigricans was determined by examining the neck, axillae, face, chest and knuckles. Groin and vulva were excluded due to common difficulty in clinical differentiation from intertrigo.Transabdominal ultrasound examinations, in unmarried women, and transvaginal ultrasounds, in married women, were performed either by a V.O.G/Senior registrar or Registrar to measure number of follicles and the ovarian volume in the amenorrhoeic phase of each individual.

Serum samples were analyzed for fasting sugar (enzymatic colorimetric assay), insulin by ELISA, triglycerides (enzymatic colorimetric assay, GPO-POD method) and IFNγ (ELISA). Hormonal analysis (FSH, LH, prolactin done by Radio immunoassay & testosterone, by ELISA) of each individual was done. Radio-immuno assay was used to measure the FSH, LH, Prolactin levels and ELISA technique for the assessment of testosterone level

Insulin resistance.

IR was calculated using indirect methods for the assessment of insulin resistance. The indices used were Homeostasis Model Assessment (28), Quantitative Insulin Sensitivity Check Index (29) and McAuley index (30).

Patients were considered as insulin resistant when McA ≤ 5.8, HOMA ≥ 2.6, QUICKI ≤ 0.33 and fasting insulin ≥ 12μIU/ml (Hettihewa *et al*., 2006). The equations used for calculation are listed below.

Homeostasis Model Assessment-:

(HOMA-IR) = Fasting serum insulin (μU/ml) x Fasting serum glucose (mmol/L)22.5

Quantitative insulin sensitivity check index-:

QUICKI = 1/[log (fasting insulin) + log (fasting glucose in mg/dl)]

McAuley index-(McA)-:

McA = exp[2.63-- 0.28 In (insulin in mu/L)-- 0.31 In (triglycerides in mmol/L)]

III STATISTICAL ANALYSIS

Data were analyzed using the Statistical Package for Social Sciences (SPSS), v for windows and Microcal Origin, version 6.0. Pattern of distribution was checked with Prism graph pad-4. Results were expressed as mean ± SD or median with Inter Quartile Range (IQR). Parametric continuous data such as age, BMI, fasting insulin, triglycerides, LH, FSH, prolactin, testosterone, LH/FSH ratio and QUICKI of women with PCOS and matched controls were tested using unpaired t-test. Non parametric continuous data such as interferon gamma, fasting serum glucose, HOMA, McAuley and non parametric ordinal data such as FG score were analyzed with the use of Mann-Whitney U – test. Correlation between IR and serum IFNγ level among cases was analyzed with Spearman`s rank correlation test and coefficient (ρ) was calculated. It gave the numerical expression for the measure of correlation. The value indicated the magnitude of correlation and the sign denoted its direction. Multiple linear regression analysis was used to see the effects of covariates. P-values < 0.05 were considered significant in all data.

IV RESULTS

68.4% of study group were primarily sub-fertile. 24 (85.7%) had polycystic ovaries (PCO) by the Rotterdam criteria

Table 1
Basic Characteristics of The Study Group

Character	Women with PCOS	Controls	p-value
Age	27.7 ± 5.9 *	34.2 ± 5.8 *	< **0.001**
BMI (kg/m^2)	32.0 ± 1.9 *	31.7 ± 1.5 *	NS
FG score	9.5 (6.5-12)**	3.0 (2-3)**	< **0.0001**

Table 1 Basic clinical characteristics of women with PCOS and the controls. There was no significant difference in mean BMI between the two groups. Mean ± SD, analyzed with unpaired t-test and the Median (IQR), analyzed with Mann-Whitney U-test

As per the above findings, acanthosis nigricans was present in 20 (71.4%) out of 28 women with PCOS but none of the control women had this clinical feature. Table 1 shows the basic clinical characteristics of group with PCOS and the control

The mean fasting insulin concentration in women with PCOS was more than double the value of controls ($p < 0.0001$), but the difference in fasting blood glucose was not statistically significant. Significantly high serum levels of both LH and FSH hormones were observed in PCOS group but the LH/FSH ratios were not significantly different. Obese PCOS women were found to have significantly higher total serum testosterone levels than appropriate controls ($p < 0.0001$). The median IFN γ values were not statistically different between the two groups (table 2).

Table 2 Insulin Resistance By HOMA –IR, QUICKI And McAuley Indices In PCOS And Control Group

	HOMA-IR	QUICKI	McAuley	
Women with PCOS	11.2(6.2-16.1)**	0.28 ± 0.02 *	3.7(3.5-4.4)**	
Controls	4.6(3.5-7.0)**	0.30 ± 0.02 *	5.1(4.5-5.4)**	
p value	< 0.001	< 0.0001	< 0.0001	

Table 2 Obese Lankan PCOS women had significantly greater IR when compared with the controls. This difference was evident in all three insulin resistant indices. Test was repeated in three times.

Difference in fasting blood glucose was not statistically significant. Serum levels of Testosterone, LH and FSH hormones were significantly high in PCOS group but the LH/FSH ratios were not significantly different ($p < 0.0001$). The mean IFNγ values were not statistically different between the two groups (table 3)

Table 3

parameter	PCOS	Controls	p value
Fasting serum glucose (mmol/l)	89.4(85.2-98.2)**	90.8(85.8-100.5)**	NS
Fasting insulin (μU/ml)	49.7 ± 23.2 *	23.2 ± 9.8 *	< **0.0001**
Triglycerides (mmol/l)	2.0 ± 0.7 *	1.8 ± 0.8 *	NS
IFN γ (pg/ml)	197(142-239)**	180(170-191)**	NS
LH (IU/L)	12.2 ± 5.8 *	6.8 ± 4.3 *	< **0.001**
FSH (IU/L)	8.1 ± 2.4 *	5.9 ± 2.6 *	< **0.005**
Prolactin (ng/ml)	9.3 ± 3.0 *	7.6 ± 4.3 *	NS
Testosterone (ng/ml)	2.1 ± 0.6 *	1.4 ± 0.7 *	< **0.0001**
LH:FSH ratio	1.6 ± 0.9 *	1.2 ± 0.9 *	NS

Table 3 Bio-chemical results of women with and without PCOS. Mean ± SD, analyzed with unpaired t-test and the Median (IQR), analyzed with Mann-Whitney U-test

Correlation between IFNγ and insulin resistance by three different indices were analyzed. For the QUICKI index, Spearman`s correlation coefficient (ρ) was – 0.11 and the p value was 0.54. There was no significant positive

or negative correlation between IFN γ and QUICKI index. There was no significant correlation between IFN γ and fasting insulin, Spearman`s correlation coefficient (ρ) was – 0.004 and the p value was 0.98. For McA, Spearman`s correlation coefficient (ρ) was – 0.16 and the p value was 0.41 and for HOMA-R, it was – 0.02 and the p value was 0.9. There was no significant correlation between IFN γ and McAuley index or HOMA-IR index in obese women with PCOS.

V DISCUSSION

This study was decided to detect any potential relationship of IFNγ in the development of IR and clinical symptoms of PCOS patients. In normal female, oestrogen upregulates the expression of TNF-α, and IFN-γ, which is important in endometrium receptivity and had been changed in in PCOS (31). In our study we found that there is no significant difference in IFNγ in test and control group of patients and couldn't find any significant relationship of IFN-γ with insulin level or IR either. This shows that IR in PCOS pathophysiology could be related by IFN-γ free pathway and it is strongly

backed up by another latest research done by Juan *et al* about the importance of other marker like leptin taking part in process of PCOS highlighting the importance of the connection between leptin and inflammation in PCOS providing new insights therapeutic strategy for this disease(34). Several studies have proposed many pro-inflammatory molecules play a role in the complex inflammatory cascade that is associated with PCOS (31,33). With this data, we suggest that PCOS pathophysiology may be related to the IR in IFN-γ bypassing pathway.

Menstrual irregularity (oligomenorrhoea or amenorrhoea) was the most common phenotype among women with PCOS (27, 96.4%, 14). The median FG score was more than eight in PCOS, but this was less than the previous value observed in Sri Lanka (14). However, next study by the same researchers had found Lankan women with PCOS to be less hirsute (median FG of 10, IQR= 5) than originally found (15). We found that the FG score of this latter study is closer to the value observed in the present study (15).

Our findings shows that both PCOS and control group of participants shoed high FG score. This observation is compatible with the findings of an early study by Wijeyaratne *et al* (14,15). They showed that the normal Asian women were significantly more hirsute than their Caucasian counterparts. In contrast, USA study conducted in women with PCOS, suggested that the absence of hirsutism in Japanese women was probably due to the difference in dietary, genetic and environmental factors, interestingly, androgen hormone profile parameters were similar (16). In the current PCOS study group, polycystic ovaries (PCO) were present in 85.7% (n=28) and in control group it was 5.3%. Hirsutism is also high in PCOS patients as per the FG score and another group had found out that hirsutism in PCOS patients is not influenced by serum androgen levels, HOMA-IR nor anthropometric measures and more related to the metabolic disorder rather than to hyperandrogenism.

In our study, fasting glucose is normal in both groups and PCOS patient showed significant difference of fasting insulin and IR by all three indices. But BMI and Triglycerides difference is not significant. This finding is compatible with the research finding by kaluza *et al* about the central obesity can be the change of metabolism in PCOS and not related to the BMI (2). This shows us that triglyceride may not involve in the mechanism of IR in PCOS patients. Few more researchers had also shown significant association between IR with obesity and identified it as core mechanism of pathophysiology of PCOS (1,18,19) compatible to our findings. We hypothesized the central obesity would be the most reasonable key factor for this observation. Previous Sri Lankan team had also noted that higher FI and low insulin sensitivity in Sri Lankan patients with PCOS compared with patients with PCOS in UK(14,15). This can be explained with the concept of central obesity and Asians have been identified to have higher central body fat (25). They informed central obesity, not the BMI, is strongly correlated with IR related pathophysiology in PCOS (14,15). We noted that both PCOS patients and controls group were obese and all are insulin resistant in the present study. But the PCOS group shows significant higher IR values than the control group. Dunaif *et al*., (1987) proved that IR is strongly related to the metabolic changes of PCOS (27). Therefore we suggest that PCOS is not directly related to the BMI and there should be other metabolic pathway leading to increased gonadal hormonal secretion ending with PCOS pathology (22,23). We observed significantly high testosterone, FSH and LH levels in PCOS patients when compared to the control group. The hepatic production of sex hormone binding globulin (SHBG) has been found to be inhibited in hyperinsulinaemic states and this has enabled this protein to be used as an indicator in the screening for insulin resistance (23). This inverse relationship was found to be present among insulin resistant South Asian PCOS women and their SHBG levels were significantly reduced (Wijeyaratne *et al*., 2002). Due to economic constrain SHB was not measured in the current study

After comparing with previous research findings, we suggest that correctable etiological pathology in PCOS is mainly related to IFNγ bypassing system in IR. One research had found out that high levels of gonadal hormonal profile- GnRH analogues may lead to insulin resistance and imbalanced body fat distribution (20). Another research group had suggested adiponectin secreted by adipose cells and is related in the pathogenesis of metabolic syndrome (21). Adiponectin can regulate glucose and lipid metabolism, increase insulin, inhibit glycogenesis, anti-inflammation and protect blood vessels. Further adiponectin can control the hypothalamus to decrease appetite and resist obesity, and increase insulin secretion and regulate the sensitivity of insulin in the target tissue(21). Our results is further in favour of these finding indicate that PCOS patients have high IR and high gonadal hormone but not significant IFNγ mediated inflammation, obesity and BMI. Development of PCOS can be strongly related to the mechanisms other than obesity and most possible explanation is adiponectin mediated pathway. Our study showed IFN γ is not elevated in both PCOS and control group. Several *in-vitro* studies have been reported that IFN γ is elevated and associated with the IR. Secondly, there

is no significant correlation between IFN γ and IR in obese women with PCOS. Therefore, the initial hypothesis related to the IFNγ and its possible inflammatory effects in the development of IR in PCOS could not be established.

We further found that fasting serum glucose was not significantly different between women with PCOS and the controls. It is also compatible with the finding of Sasanne *et al* showing that obese women with PCOS showed no difference in fasting glucose levels (27)

Considering all our results after comparing with previous research findings, we suggest that correctable etiological pathology in PCOS is possibly related to common metabolic pathway for

development of peripheral insulin resistance and hypergonadism via the IFNγ bypassing cellular pathway.

Conflict of interest

This work is self-funded and there is no conflict of interest

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