ORIGINAL ARTICLE

Significance of TNF-Alpha and Insulin Resistance in Women with Polycystic Ovarian Syndrome

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ABSTRACT

Background: Polycystic ovarian syndrome PCOS is known to be a common gynaecological disease affecting the women in reproductive age. The increased prevalence of insulin resistance is related with this disorder, along with chronic low grade inflammation.
Aim: To investigate the Insulin resistance TNF-alpha levels and their relationship with BMI in PCOS.
Methods: So PCOS women were included in the study. The patients were divided into two groups--group 1 BMI<25kg/m^2 and group 2 BMI>25kg/m^2.
Results: Mean insulin resistance in Controls (Group 1) (4.25± 3.53) and Cases (Group 2) (5.37± 4.96) with no statistically significant difference. p value >0.05. Mean TNF- alpha was higher in Group 2 (66.14±122.37 pg/ml) when compared to Group1 (19.98±37.10 pg/ml), p-value < 0.05. Mean Insulin in Group 2 was statistically higher, (26.35±25.78 µU/ml) than group1 (16.63±13.58 µU/ml), p-value < 0.05. There was no significant co-relation with Insulin Resistance and BMI. Also, no significant correlation was found between TNF-alpha and BMI in both cases and controls.
Conclusion: In conclusion, insulin resistance and chronic low-grade inflammation play a significant role in pathogenesis of PCOS.
Keywords: Polycystic ovarian syndrome, inflammatory marker, IR, BMI.

INTRODUCTION

PCOS is a multifarious disorder and one of the leading cause of infertility[1,2]. The prevalence of PCOS is 5-10 percent in females of reproductive age group[3,4,5]. It is now recognized as a part of metabolic syndrome and presents with raised BMI, increase insulin levels, raised androgen levels and altered lipid profile along with cardiac and gynecological abnormalities[4]. In addition to this patients also present with cosmetic problems and anovulation, infertility, menstrual disturbances, endometrial cancers and obesity secondary to above mentioned metabolic disorders[4,6,3].

Transvaginal ultrasound is used to diagnose PCOS. This syndrome can be identified by the presence of polycystic ovaries with one ovary having size more than 10 cm^3 and has 12 or more follicles having diameter 2 to 9 nm or ovaries contain 8 or more sub capsular follicular cysts≤ 10nm with increased ovarian stroma. These findings are present in more than 80 percent of females with PCOS[7,8].

Symptoms of PCOS appear at menarche when hypothalamic pituitary axis is activated. At that time hormones come into play and results in redistribution of body fat[9]. At the same time insulin level increases which results in raising the levels of androgens in blood by stimulating the process of ovarian steroidogenesis as well as decreasing the concentration of SHBG[10]. As a result of these affects there is hyperinsulinemia, insulin resistance, hyper androgenemia leading to anovulatory cycles.

Molecular and genetic studies show that PCOS is a chronic inflammatory process resulting in release of many cytokines and inflammatory molecules e.g. IL-6, TNF-α, CRP etc.

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TNF-α,also named as cachexin, is a 157 aminoacid unglycosylated polypeptide cytokine that is synthesized as a trans-membranous monomeric form with molecular weight of 26 kDa (m-TNF-α)[11,12,13]. It is produced majorly by activated macrophages (monocytes). On its cleavage by enzyme (TACE) a 17 kDa soluble TNF-α is obtained[14]. Both these forms have biological activity but activity of m TNF-α is different as it mediates paracrine and autocrine activity and STNF-α produces endocrine effects[14,15].

TNF-α, is believed to play crucial role in reproductive physiology. Biosynthesis of steroid in ovaries, maturation of ovarian follicles, ovulation, fertilization, and implantation etc. are influenced by TNF-α[15,16].The corpus luteum also secretes TNF-α[17].

TNF-α plays a role in causing obesity and insulin resistance in PCOS. It is associated with hyperandrogenesim, IR and obesity and was found to be elevated in women with PCOS[18,9,7]. This is responsible for decrease sensitivity of cells to insulin. They also confirm the fact that TNF-α reduces the tyrosine kinase activity of insulin receptor[19].

TNF-α acts through autocrine-paracrine manner in muscular and fatty tissue. Hyperglycemia stimulates TNF-α release in obese female with PCOS. TNF-α gene decreases tyrosine phosphorylation of insulin receptor and enhances the phosphorylation of insulin receptor substrate. Both these effects results in inhibition of insulin signaling and glucose uptake[20]. This is responsible for decrease sensitivity of cells to insulin[19].
This study was designed to find out the significance of TNF-alpha and insulin resistance in PCOS.

The objective of the study was to assess the insulin resistance and TNF-alpha levels in normal weight and overweight PCOS and to correlate the insulin resistance and TNF-alpha with BMI in normal weight and overweight PCOS patients.

MATERIALS AND METHODS

Eighty women with PCOS participated in this study. The medical ethical committee of University (K.E.M.U) approved the study and written informed consent was obtained from patients. They were divided according to BMI into two groups; controls (group I) (BMI < 25 kg/m2, age 28.7 ± 5.2 years), cases (group II) (BMI > 25 Kg/m2, age 28.3 ± 4.2 years). All women were euthyroid and not taking any medication or hormones for six months (including oral contraceptive agents) known to affect sex hormones or carbohydrate metabolism. All women were examined clinically (general, gynecologic examination, weight, height) and examined by transvaginal ultrasonography. Patients with overt diabetes mellitus and impaired glucose tolerance were excluded from the study.

The diagnosis of PCOS was made on the basis of the ESHRE/ASRM diagnostic criteria[21] , women with PCOS meet two of the following three criteria after exclusion of other etiologies (pituitary insufficiency, persistent hyperprolactinemia, congenital adrenal hyperplasia): (1) oligomenorrhea or anovulation; (2) clinical and/or biochemical signs of hyperandrogenism; (3) polycystic ovaries.

Body mass index (BMI) was calculated for all groups as body weight (kg) divided by body height squared (m2). Insulin resistance was assessed by means of the homeostasis model assessment (HOMA), which was measured by multiplying fasting serum insulin (microunits per milliliter) and fasting plasma glucose (micromoles per liter) divided by 22.5 [22].

1. Plasma glucose level was estimated using glucometer[23].
2. Serum insulin was detected by Diasourse Insulin Elisa Kit (Kap 125)[24].
3. Determination of serum tumor necrosis factor α (TNF-α) was done by photometric enzyme-linked immunosorbent assay (ELISA).

RESULTS

Table 1: Comparison of BMI in both cases and controls

Study Groups	Mean	S.D	Median	I.Q.R
Cases	27.59	5.72	28.40	6.57
Control	22.74	4.44	20.90	6.68

P value <0.001

Table 2: Comparison of Insulin Resistance in both cases and controls

Study Groups	Mean	S.D	Median	I.Q.R
Cases	5.37	4.96	4.35	4.25
Control	4.25	3.53	3.25	2.95

P value 0.249

Table3: Comparison of TNF- α in both cases and controls

Study Groups	Mean	S.D	Median	I.Q.R
Cases	66.14	122.37	11.66	65.54
Control	19.98	37.10	10.10	11.82

P value 0.025

b. Mann Whitney U test

Fig.1: Scatter diagram of Insulin Resistance and BMI

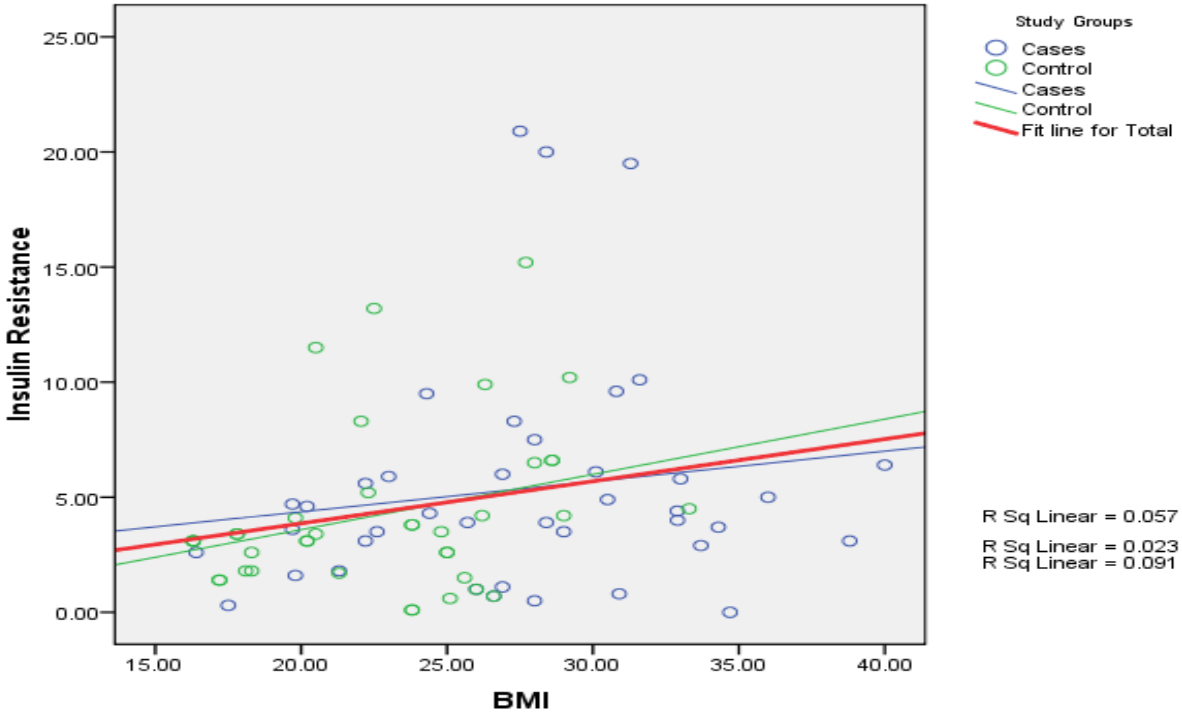
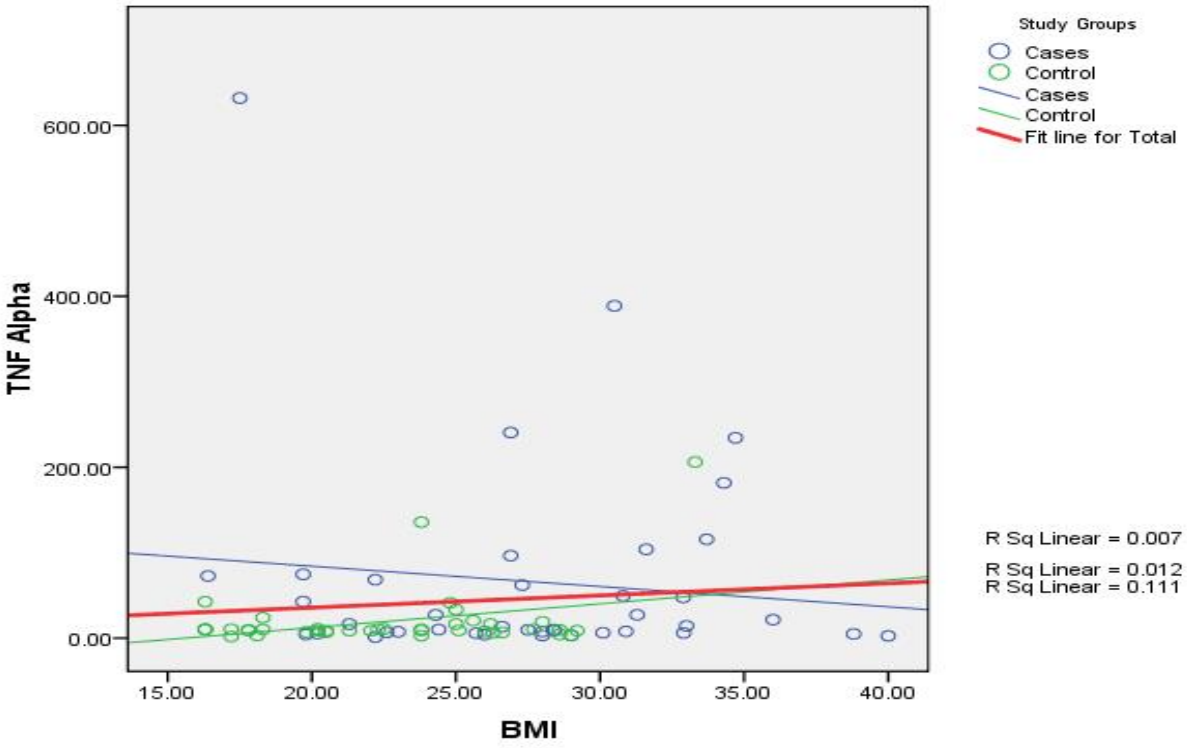


Fig. 2: Scatter diagram of TNF-α and BMI



DISCUSSION

The hallmark of PCOS is insulin resistance and chronic inflammation leading to long term complications[25]. Insulin resistance is a key component of PCOS. All PCOS females have some degree of insulin resistance in addition to other metabolic derangements. Chronic inflammation is also involved in development of disease[26,27]. Serum markers of chronic inflammation, such as TNF-alpha and IL-6 are reported to be raised in PCOS.

In this study, 80 female patients were selected with the diagnosis of PCOS and were divided into cases (BMI > 25kg/m$^{2)}$ and controls (BMI<25kg/m^2)and their fasting glucose, insulin, TNF- alpha were determined.

The results showed that mean fasting serum insulin levels were raised in cases (BMI>25Kg/m^2) 26.35 ± 25.78 as well as in controls(BMI<25Kg/m^2) was 16.63 ± 12.96, p-value 0.038). However, mean insulin resistance in cases and controls was almost same i.e. 5.37±4.96 and 4.25±3.53 respectively with no statistically significant difference, p-value > 0.05. Although the levels were higher than normal cutoff value but no significant correlation was found between BMI and IR in either cases or controls however, a weak positive correlation was observed overall (combining cases and controls). This shows that, irrespective of BMI, fasting insulin levels as well as insulin resistance by HOMA-IR are high in all patients of PCOS. This suggest that perhaps BMI does not affect insulin sensitivity in cells directly and some other factors play role in hyperinsulinemia and IR.

In our study levels of TNF-α are found to be higher than normal both in cases as well as controls but the value is higher in cases, suggesting that TNF-α levels increase with increasing BMI in PCOS females. However, on correlation analysis no correlation was found between the two variables.

A study published in 2014, showed that serum insulin levels and IR (by HOMA-IR) in PCOS patient was high as compared to healthy controls[28], supporting this study where it was noticed that PCOS females with normal BMI as well as those with high BMI are insulin resistant. This also proves that all patients with PCOS whether thin lean or overweight and obese are insulin resistant and this IR is not solely dependent on BMI. Areej H, Catherine MG (2008) also found IR and hyperinsulineamia in both lean as well as obese females with PCOS.

TNF–α levels are higher in PCOS females as compared to control and a potential correlation exist between IR and inflammatory markers such as TNF-α and IL-6 in females with PCOS[29,30]. Several studies reported raised TNF-α in PCOS females. Gonalez study (1999), developed a positive correlation between TNF-α and BMI in females with PCOS. He also confirmed with his finding that the levels of TNF-α were higher in both normal and raised BMI females with PCOS and value is even higher in high BMI obese patients[31,32].

Two studies done in 2011 reported increase TNF–α levels in PCOS patients[32]. In contrary to this another study reported no significant difference in TNF–α levels in PCOS patients as compared to controls[33]. Results of studies by Lingling Gao and Thathapudi et al. 2014, also showed that TNF-α are more in females with PCOS[34,35]

Studies of Cristiano et al. 2015 showed no significant difference in TNF-α in females of PCOS with normal BMI or

high BMI[36].In year 2016, Orostica et al obtained TNF-α levels in serum and endometrial samples of females with PCOS[37]. Araya et al reported raised TNF–α levels in PCOS and found a positive correlation with BMI and that TNF-α is implicated in affecting ovarian function and producing hyperandogeneamia.

PCOS produce a pro-inflammatory environment which is potentiated by obesity. Both these conditions affect insulin signaling pathways resulting in decrease insulin sensitivity and ultimately leading to insulin resistance and metabolic syndrome.

Several studies have reported raised TNF–α levels in PCOS but what is triggering this increase is not confirmed. Inflammation particularly TNF-α in PCOS and its relation with BMI. As mentioned inflammation induces IR and level of TNF–α rises as BMI increases in females with PCOS.

The evidence gained from these studies suggest that TNF-α is a contributing factor in biochemical and clinical derangements e.g., obesity, hyperandrogenism and hyperinsulinemia.

CONCLUSION AND RECOMMENDATION

Inflammation affects all the aspect of disease pathogenesis as well as its complications. Inflammation, insulin resistance and BMI play hand in hand in PCOS progression and complication but none can be regarded as a causative factor. By limiting and controlling these factors we can decrease the symptoms and eliminate morbid complications but cannot eliminate the disease.

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