Original Article

Tumor Necrosis

Tumor Necrosis Factor Alpha, Obesity and Polycystic Ovarian Syndrome

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ABSTRACT

Objective: To determine the level of tumor necrosis factor alpha in polycystic ovarian syndrome and to find out correlation of TNF- α with BMI in obese and non-obese females.

Study Design: Cross sectional study

Place and Duration of Study: This study was conducted at the outpatient Department of Lady Aitcheson Hospital Lahore 1st July 2016 to 31st December 2016.

Materials and Methods: Eighty female patients diagnosed with PCOS were selected. Their height in meters (m) and weight in kg was determined and they were divided into cases with BMI> 25kg/m^2 and controls with BMI<25kg/m^2. 5ml of blood was taken from antecubital vein to measure serum TNF-alpha by ELISA kit.

Results: The mean age of cases and controls was 25.42±4.16 years and 22.98±3.35 years, with significantly lower mean age in controls, p-value < 0.001. The mean BMI was 27.59±5.72 kg/m2 in cases and 22.74±4.44 kg/m2 in controls with significantly higher mean BMI in cases, p-value < 0.001. Mean TNF-α was statistically higher in cases (66.14±122.37 pg/ml) when compared to controls (19.98±37.10 pg/ml), p-value < 0.05.

Conclusion: Serum TNF-alpha was increased in females with PCOS in both cases and controls but levels are significantly higher in cases. No significant correlation was found between TNF-alpha and BMI in either cases and controls. TNF- α level can be used as a molecular marker of disease.

Key Words: Tumor necrosis factor alpha (TNF-α), Body mass index (BM)I, Polycystic Ovarian syndrome (PCOS)

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INTRODUCTION

TNF-α, also named as cachexin, is a 157 amino acid unglycosylated polypeptide cytokine that is synthesized as a trans-membranous monomeric form with molecular weight of 26 kDa (m-TNF-α).[1-,3] It is produced majorly by activated macrophages (monocytes). On its cleavage by enzyme (TACE) a 17 kDa soluble TNF-α is obtained.[4] Both the 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.[4,5] For mediation of endocrine effects high concentration of s TNF-α must be maintained in blood.[6]

Levels of TNF α are raised in conditions septic shock, graft rejection, parasitic infection cancer, post-hemofiltration, during in vivo cytokine therapy. Its role is both diagnostic as well as prognostic in systemic diseases.[6]

Scientific literature proves that both chronic inflammation and PCOS are associated with each other.[7]

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Pro-inflammatory mediators, e.g. tumor necrosis factor α (TNF-α), interleukin (IL)-6, and IL-1, mediate inflammation and are found to be elevated in women with PCOS.[8-10] Women with PCOS have increased levels of inflammatory cells as well as inflammatory markers. This oxidative stress and chronic inflammation contributes to insulin resistance and ovarian dysfunction.[11]

The corpus luteum secretes TNF-α and its levels changes with different phases of menstrual cycle.[12] The inflammatory mediators especially TNF-α, are 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.[13,14] PCOS being a pro-inflammatory condition, promotes chronic low-grade inflammation which also leads to metabolic disturbances and ovarian dysfunctions.[15] In addition to this, genes which code pro-inflammatory mediators or their ligands are also linked with the genes for obesity, insulin resistance, diabetes and PCOS.[16,17] TNF–α performs several functions such as regulation of the ovulation, fertilization, and implantation, which are usually affected in females with PCOS.[18]

Females with PCOS show hyperandrogenism, infertility, anovulation, obesity, insulin resistance and high levels of TNF-α in their serum. The levels of TNF-α is raised in all the chronic inflammatory diseases and as discussed earlier PCOS is one such disease.[17]

It is commonly seen that that women diagnosed to have PCOS are usually overweight or have high BMI. With

the increase in worldwide prevalence of obesity the incidence of PCOS is also increasing in susceptible individuals.[19]

Obesity is now considered as a condition in which there is systemic sub-clinical inflammation. There is increased infiltration of CD-4 and CD-8 cells into adipose tissue that further causes release of mediators of inflammation including CRP and TNF-α. There is evidence of visceral adiposity in PCOS patients.[20-22] The fat cell increase in size and produce many hormones namely TNF-α, IL-6, resistin and leptin. It has also been noted that in obesity related disorders the levels of both MTNF-α as well as STNF-α are raised especially in adipose tissues .This adipose tissue derived TNF-α is also involved in regulation of biochemical processes e.g. glucose homeostasis in adipocytes, promotes lipolysis in cultured adipocytes and potently inhibits adipocyte differentiation and lipogenesis.[6]

To summarize PCOS patients have hormonal disturbances that results in insulin resistance and chronic inflammation that results in infertility in young females. Taking into account the role of TNF-α in chronic inflammatory conditions it can be regarded as a diagnostic and therapeutic marker whose levels must be regulated in order to get long term benefits especially in impaired glucose tolerance and dyslipidemia.[23] Thus, obesity can also flare up pre-existing clinical, endocrinological, and metabolic features in women with PCOS.[19]

MATERIALS AND METHODS

This cross-sectional study was done at outpatient department of Lady Aitcheson Hospital Lahore from 1st July 2016 to 31st December 2016. Eighty patients with diagnosis of PCOS based on the operational definition were selected. Patients with co-morbid conditions, other endocrinological abnormalities, menstrual abnormalities, history of smoking or NSAIDs consumption were excluded. Informed and written consent was taken and a complete history and general physical examination was performed. Height in meters and weight in kg was recorded. Body mass index was calculated by formula weight (kg)/height(m^2). On the basis of BMI they were divided into Controls (BMI< 25kg/ m^2) and Cases (overweight: BMI>25kg/ m^2). Patients were called again after an overnight fast on day 3 of menstrual cycle. Fasting glucose was determined by the glucometer. For laboratory parameters TNF-alpha, testosterone, and fasting insulin levels 5ml of blood was taken from antecubital vein under aseptic conditions and after centrifugation stored in 3 aliquots in the chemical Pathology laboratory K.E.M.U at -40 degree celcius. All information collected was entered in a specially designed performa. Serum insulin was detected by Diasourse Insulin Elisa Kit (Kap 125). Diasourse TNF-alpha kit was used for determination of TNF-alpha levels. It is a solid phase enzyme amplified sensitivity immunoassay performed on microtiter plate. Monoclonal antibodiies against the specific epitopes of TNF-alpha are used. Calibrators and samples react with antibodies coated on microtiter wells (Mab2). After incubation a sandwich of MAb-1MAb2-HRP is formed. Microtiter plate is washed and enzyme labelled antibodies is measured by chromogenic reaction by adding chromogenic solution. Incubation done and stop solution added. Microtiter plate is than read at appropriate wavelength. the substrate turnover is measured calorimetrically and by finding absorbance which is proportional to TNF-alpha concentration.

All data was entered and analyzed using SPSS version 20. Mean ± S.D was used to present quantitative data. Normality of data was checked by one sample Kolmogorov Simonov test. Independent sample t-test was applied to compare quantitative data if assumption fulfilled otherwise we used Mann-Whitney U test. Pearson correlation was applied to see relationship between insulin and TNF-α. P-value ≤ 0.05 was considered as significant.

RESULTS

The mean age of cases and controls was 25.42 ± 4.16 years and 22.98 ± 3.35 years, with significantly lower mean age in controls, p-value < 0.001 (Table 1).

Table No.1: Comparison of age in both cases and controls

Group	Mean±SD	P value
Case	25.42±4.16	<0.001
Control	22.98±3.35	

Table No.2: Comparison of BMI in both cases and controls

Group	Mean±SD	P value
Case	27.59±5.72	<0.001
Control	22.74±4.44	

Table No.3: Comparison of TNF- α in both cases and controls

Group	Mean±SD	P value
Case	66.14±122.37	0.001
Control	19.98±37.10	

Table No.4: Correlation between TNF-α with BMI in cases and controls

		Cases	Controls	Overall
TNF-α with BMI	Correlation (r)	-0.104	0.278	0.095
	p-value	0.525	0.082	0.403

The mean BMI was 27.59 ± 5.72 kg/m2 in cases and 22.74 ± 4.44 kg/m2 in controls with significantly higher mean BMI in cases, p-value < 0.001 (Table 2). Mean TNF-α was statistically higher in cases (66.14 ± 122.37 pg/ml) when compared to controls (19.98 ± 37.10 pg/ml), p-value < 0.05 (Table 3).

No significant correlation was found between TNF-α with BMI in cases, controls nor overall, p-value > 0.05 (Fig.1).

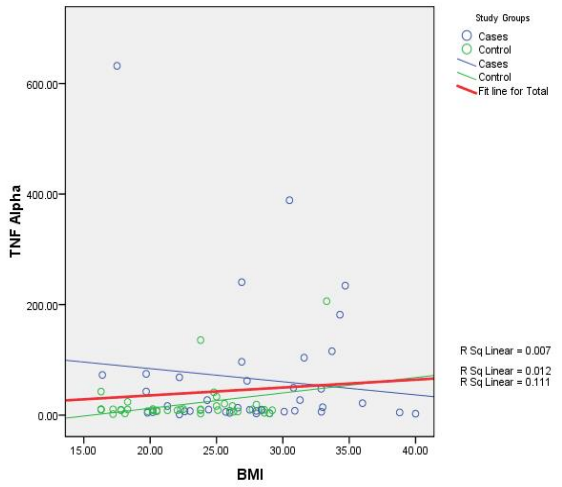

Figure No. 1: Scatter diagram of TNF-α and BMI

DISCUSSION

In our study, mean BMI was 27.59±5.72 kg/m2 in cases and 22.74±4.44 kg/m2 in controls with significantly higher mean BMI in cases, p-value < 0.001 (Table 2) Mean TNF-α was statistically higher in cases (66.14±122.37 pg/ml) when compared to controls (19.98±37.10 pg/ml), p-value < 0.05 (Table 3).

Victor et al[24] found that level of TNF–α is more 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. Gonalez et al[25] also reported raised TNF-α in females with PCOS. Gonalez et al[25] further developed a positive correlation between TNF-α and BMI in females with PCOS. They 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.

Cristiano et al[17] reported that no significant difference in TNF-α was observed in females of PCOS with normal BMI or high BMI. Randeva et al[26] also found no difference in levels of circulating cytokines including TNF-α in females with PCOS, whether thin lean or obese.

A meta-analysis study of women with PCOS reported no difference in TNF-α levels in women with PCOS and controls.[27] Another study found that no difference in levels of TNF-α exist in normal weight and overweight females.[28]

Seyam et al[29] in their analysis related TNF–α with BMI in PCOS patients and found that as BMI increases so does the level of TNF-α. In comparison to his study Mohlig et al[30] proved that PCOS is not associated with chronic inflammation. He in his studies argued that BMI is the strongest parameter related to chronic inflammation in in females with PCOS. The endocrinological parameters which are important in relation to PCOS do not result in low grade chronic inflammation and are not the risk factor for type II diabetes or metabolic syndrome. They also related BMI and IR with chronic inflammation not the disease itself. The risk of diabetes is linked to obesity and metabolic alterations and is encountered in such patients only.

Araya et al reported raised TNF–α levels in PCOS and found a positive correlation with BMI. TNF-α is implicated in affecting ovarian function and producing hyperandogeneamia.[31]

Several studies have reported raised TNF–α levels in PCOS but what is triggering this increase is not confirmed.[29-31] If only obesity is regarded as a key factor taking part in producing PCOS in females than lean individual should never suffer from the syndrome. It is established that PCOS is affecting both lean and obese subjects although obese individuals are more likely to have disease. So overweight and obesity are a risk factor for PCOS. Literature is also available showing contrary results. Possible explanation to contrary results is that not only BMI matters but also the pattern of obesity as well.[32,33]

Last but not the least is role of inflammation particularly TNF-α in PCOS and its relation with BMI. As mentioned level of TNF–α rises as BMI increases in females with PCOS. So inflammation also 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.

CONCLUSION

Serum levels of TNF–α are raised in females with PCOS in both cases as well as controls but levels are significantly higher in cases so it can be regarded as a molecular marker for diagnosis of PCOS. No significant correlation was found between TNF-α and BMI in females with PCOS.

Author's Contribution:

Concept & Design of Study:	Imrana Ihsan
Drafting:	Asma Tehreem Kazi
Data Analysis:	Shafia Rasool Qazi
Revisiting Critically:	Imrana Ihsan, Asma Tehreem Kazi
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Conflict of Interest: The study has no conflict of interest to declare by any author.

REFERENCES

1. Anderson H, Fogel N, Grebe SK, Singh RJ, Taylor RL, Dunaif A. Infants of women with polycystic ovary syndrome have lower cord blood

androstenedione and estradiol levels. J Clin Endocrinol Metab 2010; 95:2180–86.
2. Dumesic DA, Oberfield SE, Stener-Victorin E, Marshall JC, Laven JS, Legro RS. Scientific statement on the diagnostic criteria, epidemiology, pathophysiology, and molecular genetics of polycystic ovary syndrome. Endocr Rev 2015; 36(5): 487–525.
3. Maliqueo M, Lara HE, Sanchez F, Echiburu B, Crisosto N, Sir-Petermann T. Placental steroidogenesis in pregnant women with polycystic ovary syndrome. Eur J Obstet Gynecol Reprod Biol 2013; 166:151–55.
4. Erdogan M, Karadeniz M, Berdeli A, Alper G, CaglayanO, Yilmaz C. The relationship of the interleukin-6-174 G>C gene polymorphism with oxidative stress markers in Turkish polycystic ovary syndrome patients. J Endocrinol Invest 2008; 31: 624-9.
5. Escobar-Morreale HF, Calvo RM, Villuendas G, Sancho J, San Millan JL, Association of polymorphisms in the interleukin 6 receptor complex with obesity and hyperandrogenism. Obes Res 2003;11: 987-96
6. Kriegler M, Perez C, DeFay K, Albert I, Lu SD. A novel form of TNF/cachectin is a cell surface cytotoxic transmembrane protein: ramifications for the complex physiology of TNF. Cell 1988; 53: 45–53.
7. Sathyapalan T, Atkin SL. Mediators of inflammation in Polycystic Ovary Syndrome in Relation to Adiposity. Mediators of inflamm 2010; 1-6. 758565.
8. Rojas J, Chávez M, Olivar L, Rojas M, Morillo J, Mejías J, et al. Polycystic Ovary Syndrome, Insulin Resistance, and Obesity: Navigating the Pathophysiologic Labyrinth. Int J Reproduc Med 2014; 2014(28): 1-17.
9. Legro R. Obesity and PCOS: Implications for Diagnosis and Treatment. Seminars in Reproductive Med 2012; 30(6): 496–506.
10. Reaven GM. Insulin resistance: the link between obesity and cardiovascular disease. Med Clin North Am 2011; 95(5):875–92.
11. Black RA, et al. A metalloproteinase disintegrin that releases tumour-necrosis factor-α from cells. Nature 1997; 385:729–33.
12. Perez C, Albert I, DeFay K, Zachariades N, Gooding L, Kriegler M. A nonsecreteable cell surface mutant of tumor necrosis factor (TNF) kills by cell-to-cell contact. Cell 1990; 63:251–58.
13. Xu H, Sethi JK, Hotamisligil GS. Transmembrane tumor necrosis factor (TNF)-α inhibits adipocyte differentiation by selectively activating TNF receptor 1. J Biol Chem 1999; 274:26287–95.
14. Grell M. Tumor necrosis factor (TNF) receptors in cellular signaling of soluble and membrane-expressed TNF. J Inflamm 1995;47:8–17.
15. Pasquali R, Stener-Victorin E, Yildiz BO, Duleba AJ, Hoeger K, Mason H, et al. SPCOS Forum: research in polycystic ovary syndrome today and tomorrow. Clin Endocrinol 2011; 74: 424–33.
16. Knochenhauer ES, et al. Prevalence of polycystic ovary syndrome in unselected black and white women of the southeastern United States. J Endicrinol Metab 1998; 83: 3078-82.
17. Barcellos CRG, Rocha MP, Hayashida SAY, Dantas WS, Yance VDRV, et al. Obesity, but not polycystic ovary syndrome, affects circulating markers of low-grade inflammation in young women without major cardiovascular risk factors.- Hormones 2015; 14(2):251-7
18. Rosner W, Auchus RJ, Azziz R, et al. Utility, limitations,and pitfalls in measuring testosterone: an endocrine society position statement. J Clin Endocrinol Etabolism 2007; 92:405–13
19. Xu H, Uysal KT, Becherer JD, Arner P, Hotamisligil GS. Altered tumor necrosis factor-α (TNF-α) processing in adipocytes and increased expression of transmembrane TNF-α in obesity. Diabetes 2002; 51:1876–83.
20. Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW. Obesity is associated with macrophage accumulation in adipose tissue. J Clin Investigat 2003;112:1796–1808.
21. Rice S, Pellatt L, Ramanathan K, et al. Metformin inhibits aromatase via an extracellular signal-regulated kinase-mediated pathway. Endocrinol 2009; 150:4794–4801.
22. Driancourt MA, Reynaud K, Cortvrindt R, et al. Roles of KIT and KIT LIGAND in ovarian function. Reviews Reproduct 2000; 5:143–52.
23. Dandona P, Weinstock R, Thusu K, Abdel-Rahman E, Aljada A, Wadden T. Tumor necrosis factor- in sera of obese patients: fall with weight loss.1998.J Clin Endocrinol Metab1998; 83:2907–10.
24. Victor VM, Rovira-Llopis S, Bañuls C, Diaz-Morales N, deMarañon AM, Rios-Navarro C, et al. Insulin resistance in PCOS patients enhances oxidative stress and leukocyte adhesion: role of myeloperoxidase. PLoS ONE 2015; 11(3) :e0151960.
25. Gonzalez F, Thusu K, Abdel-Rahman E, Prabhala A, Tomani M, Dandona P. Elevated serum levels of tumor necrosis factor α in normal-weight women with polycystic ovary syndrome. Metabolism 1999; 48:437–41.
26. Randeva HS, Tan BK, Weickert MO, et al. Cardiometabolic Aspects of the Polycystic Ovary Syndrome. Endocrine Rev 2012;33(5):812-41.
27. Gao L, Gu Y, Yin X. High serum tumor necrosis factor-alpha levels in women with polycystic ovary

syndrome: a meta-analysis. PLoS ONE. 2016;11 (10):e0164021.
28. Baldani DP, Skrgatic L, Ougouag R. Polycystic Ovary Syndrome: Important Underrecognised Cardiometabolic Risk Factor in Reproductive-Age Women. Int J Endocrinol 2015;2015:786362.
29. Seyam E, Hasan M, Khalifa EM, Ramadan A, Hefzy E. Evaluation of tumor necrosis factor alpha serum level in obese and lean women with clomiphene citrate-resistant polycystic ovary disease. Gynecol Endocrinol 2017;33(11):892-8.
30. Möhlig M, Spranger J, Osterhoff M, et al. The polycystic ovary syndrome per se is not associated with increased chronic inflammation. Eur J Endocrinol 2004;150: 525-32
31. Araya AV, Aguirre A, Romero C, Miranda C, Molina MC, Ferreira A. Evaluation of tumor necrosis factor alpha production in ex vivo short term cultured whole blood from women with polycystic ovary syndrome. Eur Cytokine Netw 2002;13(4):419–24.
32. Escobar-Morreale HF, Villuendas G, Botella-Carretero JI, Sancho J. Obesity, and not insulin resistance, is the major determinant of serum inflammatory cardiovascular risk markers in pre-menopausal women. Diabetologia 2003; 46(5): 625–33.
33. Lim SS1, Davies MJ, Norman RJ, Moran LJ. Overweight, obesity and central obesity in women with polycystic ovary syndrome: a systematic review and meta-analysis. Hum Reprod Update 2012;18(6):618-37.