

Polycystic Ovary Syndrome under TNF and TAC Regulation

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Abstract— Background: Polycystic Ovary Syndrome (PCOS) is a hormonal syndrome. One of an important disorder in women under physiological and immunological regulation. Affected by many factors and can diagnosis by many serological and immunological markers. **Aim of study:** Goal of present study target on tumor necrosis factor (TNF) and total antioxidant capacity (TAC) investigation. **Methods:** Current investigation involved 80 women (patients and control) with different ages from JANUARY 2022 to MARCH 2023 after recording information about patients. The cupric ion reduction antioxidant capacity (CUPRAC) method has been used to estimate TAC. ELISA test performed to evaluate TNF. **Results:** The highest percentage of disease was found in the age group [19-22]. It was the highest rate of 58%, followed by the age group [23-26] the lowest incidence rate in the age group [27-30] was 5%. According to location of patients in cities, varied between four cities included (Babylon, Karbala, Najaf and Baghdad) with a highest frequent showed in Babylon as (60%). Physiological study showed there are non-significant different between blood groups and PCOS under ($P \leq 0.05$), although varied the percentage in patients in four types (A+, B+, AB+ and O+) with highest percent in A+ about (38%). On other manner when measured total antioxidant capacity (TAC) also showed non-significant different between patients and control according to age group with a highest concentration in age group (19-22 y) in patients and control ($3.7033 \pm 0.8835 \mu\text{m/l}$) ($3.106 \pm 0.427 \mu\text{m/l}$) respectively. Immunological study, TNF concentration showed statistically significant difference ($P \leq 0.05$) in all age group compared to control with a highest level in (27-30y) as ($170.207 \pm 3.68 \text{ pg/ml}$) for patients and ($45.479 \pm 1.247 \text{ pg/ml}$) to control. **Conclusion:** The current study pointed about the correlation subject between TNF and TAC, results find there is an inverse correlation between them in patients' group with present revers relationship in control group, that ensure an important of immune regulation. Those achieve the aim of our study.

Index Terms— PCOS, TNF, TAC, Immune regulation, physiological regulation

I. INTRODUCTION

PCOS is a common endocrinopathy related to gynecological that impacts 8–13% of women who are of reproductive age, which is a frequent gynecological endocrinopathy that accounts for 44% of first-trimester miscarriages in pregnant women (Kakoly *et al.*, 2019). While the exact cause of PCOS remains unknown, there are a number of potential causes. Studies have demonstrated that oxidative stress may play a role in the emergence of PCOS, infertility, and hyperandrogenism (Mohammadi, 2019; uckan *et al.*, 2022). Oxidative stress is a

state where oxidative powers exceed the antioxidant systems, serum's ability to reduce the free radical's formation and protect the cell from oxidative stress (Faris *et al.*, 2023).

Total oxidative stress (TAC) is linked to weight loss and an increase in belly fat (Besagil *et al.*, 2020). Moreover, a better improvement in the heart disease risk factors and a lower risk of pancreatic cancer are linked to higher dietary TAC (Zhong *et al.*, 2020; Al-Musawi *et al.*, 2021). The essential defect that give rise PCOS remains elusive however it is thought to have a complex origin that includes environmental variables, genetics, and a wide range of reproduction-related disorders and metabolic abnormalities (Al-Musawi, 2015; Al-Hindi, 2019b). The involvement of physiological and immunological regulation in PCOS has been the subject of conflicting research. However, macrophages, are crucial antigen-presenting cells (APCs) in particular immunity and anti-infective cells in the body's normal immunological response, which makes them vital players in the immune control of PCOS (Feng *et al.*, 2023). Adipose tissue from PCOS patients contained higher levels of pro-inflammatory factors and macrophages. Macrophages have the ability to produce cytokines and chemokines into the bloodstream, including TNF- α , MIF, IL-6, IL-10, and IL-18. Immune cells secrete pro- or inhibitory cytokines, which can either trigger or reduce host inflammation. Immune cell dysfunction or an imbalance in variables related to immunity might result from abnormal immune cell activity. Additionally, several immunological molecules have a direct bearing on immune response and are strongly associated with the pathological alterations of PCOS (Ying-yi *et al.*, 2022). Regretfully, there is a dearth of information regarding the assessment of immunological markers and physiological parameters, particularly in Iraqi patients with PCOS. This resulted in us designing a case-control research to investigate TAC and TNF levels in Iraqi PCOS patients

II. METHOD

The study included 80 women (patients and control) with different ages from JANUARY 2022 to MARCH 2023 after recording information about patients. 5ml of venous blood samples were drawn from study population and isolated sera were stored at -4°C to freeze for immunological and physiological study. The cupric ion reduction antioxidant capacity (CUPRAC) method has been used to estimate TAC which is based on the ability of an antioxidant in the reduction of an oxidant (Apak *et al.*, 2005). The concentration of TNF was

determined by ELISA test according to the manufacturer's instructions for the kit.

III. STATISTICAL ANALYSIS

The results of the statistical analysis were expressed as (mean ±SD) compared between females with PCOS and controls using a One-Way ANOVA table under probability 0.05 significance using the U.S. Census statistical package of social science (SPSS) version 26 (Niazi, 2004).

IV. RESULTS AND DISCUSSION

The current study was conducted on 40 women suffering from polycystic ovary syndrome and 40 women as a control for all ages from different cities for the period from January 2022 to march 2023. The highest percentage of disease was found in the age group [19-22] years. It was the highest rate of 58%, followed by the age group [23-26] the lowest incidence rate in the age group [27-30] was 5% (Fig.1).

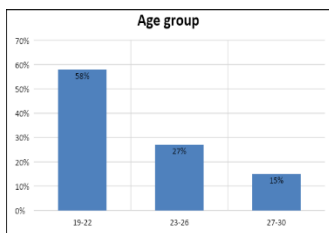


Figure (1): Demographic of study population according to Age group

Among women of reproductive age, polycystic ovary syndrome is one of the most prevalent endocrine disorders; its reported prevalence ranging from 4% to 21% depending on the population studied and diagnostic criteria applied (Lizneva et al., 2016). The results of the current study showed that the percentage of ovarian cysts was in the younger age groups compared to the older ones, and this is consistent with what was mentioned by other studies that showed that PCOS manifests typically during early reproductive years (Conway et al., 2014). The health implications of PCOS are thought to be lifelong, however, it is unclear if they continue into reproductive age and add to the overall burden of peri- and post menopause health issues (Helvaci and Yildiz, 2021).

In (Fig. 2) it shows the relationship of blood group (A+, AB+, B+ and O+) to patient with polycystic ovary disease. The highest percentage appeared with A+ group about (38%), while the lowest in B+.

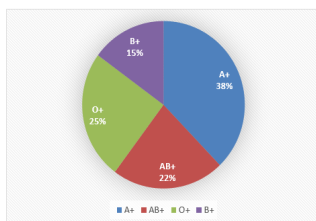


Figure (2): Distribution of study population according to Blood group

The result of the current study showed that women with blood group 'A+' had the highest risk of PCOS followed by those who

were 'O+' positive. Additionally, our research indicates that there was no correlation between Rh negative individuals and PCOS. According to the current study finding 'A+' females, are more prone to PCOS.

There are studies that say that people with blood group O+ are more likely to be infected than others. But in the current research, it was shown that there is no relationship between blood types and polycystic ovary syndrome, as polycystic ovaries is caused by an increase in fats and sugars in the body As a result of the wrong diet, gaining excess weight, and some hormonal disorders, as this existing research is not compatible with these studies (Dogan, 2023).

The study population recorded from many cities in middle of Iraq. Four cities included (Babylon, Karbala, Najaf and Baghdad) with the highest percent showed in Babylon (60%), Figure (3).

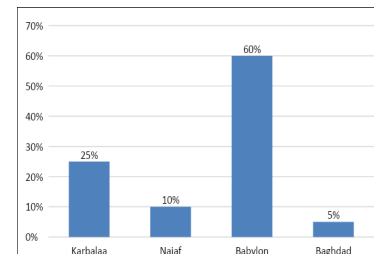


Figure (3): Distribution of study population according to city

Conventional estimates place the prevalence of PCOS at 4% to 8% among all women of reproductive age, based on research conducted in Spain, Greece and the USA (March et al., 2010). According to the most recent community-based prevalence survey, the prevalence of PCOS worldwide is 18% using the Rotterdam diagnostic criteria (Azziz et al., 2005), whereas the prevalence of PCOS in Iraqi women in AL-Hilla city was estimated in our study almost 60%, higher than the percentage in the world.

Use of technology calibration absorbance linked immunoassay Enzyme - Linked Immunosorbent Assay (ELISA), the results of the current study rose significantly (P<0.05) in the level of concentration of TNF and for all age groups of patients compared to control its subsidiaries. The age group of 27-30 years old the highest its levels of TNF, Table (1).

Table (1): The level of TNF in patients with polycystic ovary syndrome

Sample type	Age group	Concentration of TNF pg/ml		P value (≤ 0.05)
		Mean	Std. Deviation	
patient	19-22	77.0944691	31.91563347	.002 *
	23-26	95.7244017	36.74988366	
	27-30	170.2070000	30.68169768	
	Total	96.6503285	45.45977013	
control	19-22	100.8305975	28.19935907	.023 *
	23-26	81.5359580	34.56762157	
	27-30	45.4793033	1.24797720	
	Total	87.7042435	33.35355153	

*Significant difference under P value (≤ 0.05) by ANOVA

Present studies pointing to the concentration of TNF in Table (1) refer to the activity of an immune system in the age group (27-30 y) with POCS. TNF play an important protective role agent POCS progress by different pathways, this theory susceptible with current results (Liang et al., 2023).

In general, all results about TNF concentration appear highest level compare to an inverse result with syndrome frequently in Figure (1) at same age group compare to control with significantly difference, that's mean ensure an immune protective by TNF (Rezayat et al.,2023).

The assessment of total antioxidant capacity levels in studied population illustrated in figure (4), the concentration of total antioxidant capacity showed a significant ($P < 0.05$) increase in control 4.06 ± 0.49 as compared with patients $2.28 \pm 0.61 \mu\text{m/l}$.

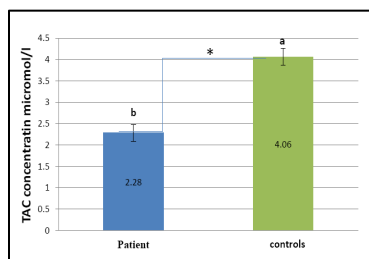


Figure (4): Estimation of total antioxidant capacity in polycystic ovary patients and control

The current study revealed a significant decrease in TAC in PCOS patients compared with control subjects, this may be due to the depletion of antioxidants in the equation of the harmful effect of increased free radicals in PCOS patients. The study's finding supports the findings of Kanafchian et al. (2020), who found that PCOS patients have lower TAC levels. Reduced TAC in these patients is correlated with the cause of PCOS, according to research by Enechukwu et al. (2019) and Ghowsi et al. (2018) discovered, however, that there were no appreciable variations in serum TAC levels between the PCOS patients and control.

The effect of age on total antioxidant capacity levels is summarized in Table (2), the results of the statistical analysis showed that there were non-significant ($P < 0.05$) differences in the distribution of TAC according to the age groups of each of the patients.

Table (2): effect of age on the levels of total antioxidant capacity in patients and control

Sample type	Age group	Concentration of ATC $\mu\text{m/l}$		P value (≤ 0.05)
		Mean	Std. Deviation	
patient	19-22	3.7033	.88352	.077 ^{ns}
	23-26	3.6671	.45808	
	27-30	2.4861	.91713	
	Total	3.5098	.86577	
control	19-22	3.1065	.42755	.062 ^{ns}
	23-26	2.4849	.60821	
	27-30	2.9278	.11134	
	Total	2.9243	.50674	

NS: non-significant difference under P value (≤ 0.05) by ANOVA

In general, there were no significant differences in the levels of TAC among the age groups of patients and control, the reason may be attributed to the high levels of free radicals in the bodies of patients due to infection, regardless of their age. As for the slight decrease in the elder age groups, it is due to chronic diseases that these age groups have, such as Diabetes, which may constitute another burden on the body in the increase of free radicals, which in turn is reflected in the decrease in TAC (Al-Musawi et al., 2021b).

The current study revealed a significant decrease in TAC in PCOS patients compared with control subjects, this may be due

to the depletion of antioxidants in the equation of the harmful effect of increased free radicals in PCOS patients. The study results agree with Kanafchian et al. (2020).

Figure (5): Correlation between TNF and TAC in study population

Figure (5) refer to an inverse significant correlation between TNF and TAC in patients' group, contrariwise relationship in control groups. Interpenetration of immunological and physiological regulation is the goal target of present investigation that reflected by their two opposite pathways under immune regulation specially macrophage dependent on multifactor contributed in balance between TNF and TAC by decrease free radical which affected on total antioxidant capacity (Jakus et al., 2021).

The placentas growth, development, and quality can all adversely affected by an imbalance between the ovaries generation of free radicals and antioxidants, abetter prognosis for PCOS is associated with less oxidative stress. Switching processes occur between protective parameters by induce stimulation or inhibition free radical led to an inverse relationship in spite of that unclear how TNF affected on TAC level and vice versa dependent on free radical, present study agree with (Jung et al., 2021).

CONCLUSION

The current study pointed about the correlation subject between TNF and TAC, results find there is an inverse correlation between them in patients' group with present revers relationship in control group, that ensure an important of immune regulation. Those achieve the aim of our study.

REFERENCES

- Al-Hindi, Zeana Shaker. (2019). Correlation of Alu repeat I/D, TPA-Gene polymorphism in women polycystic ovary syndrome and UTI. *Biochem. Cell. Arch.* 19(1): 1939-1942.
- Al-Musawi, H. S. (2015). Association of The T45G Polymorphism of Adiponectin Gene with Polycystic Ovary Syndrome in Women of Babylon Province, Iraq. *Medical Journal of Babylon* .12(4):1020 – 1026.
- Al-Musawi, Hawraa Sabah; Al-Lami1, Makarim Qassim and . Al-Saadi, Ali H. (2021 a). Assessment of Glycemic Control, Renal Function, and Oxidative Stress Parameters in Type 2 Diabetes Mellitus Patients. *Iraqi Journal of Science*, . 62(12): 4628-4638
- Al-Musawi, Hawraa Sabah; Al-Lami1, Makarim Qassim and . Al-Saadi, Ali H. (2021b). Age and gender impact on glycaemic control, renal function and oxidative stress parameters in Iraqi patients type 2 diabetes mellitus. *Biochem. Cell. Arch.* 21:491-499.
- Apak, R; Gu"clu", K.; O" zyu"rek, M.; Karademir, S. E. and Altun, M. (2005). Total antioxidant capacity assay of human serum using copper(II)-neocuproine as chromogenic oxidant: The CUPRAC method. *Free Radical Research*, 39(9): 949.
- Azziz, R.; Marin, C.; Hoq, L.; Badamgarav, E. and Song, P. (2005). Health care-related economic burden of the polycystic ovary syndrome during the reproductive life span. *J Clin Endocrinol Metab.* (90):4650– 4658.
- Besagil, PS.; Calapkorur, S. and Sahin, H. (2020). Determination of the relationship between total antioxidant capacity and dietary antioxidant intake in obese patients. *Niger J Clin Pract.* (23): 481-488.

- Conway, G.; Dewailly, D.; Diamanti-Kandarakis, E.; et al. (2014). The polycystic ovary syndrome: a position statement from the European Society of Endocrinology. *Eur J Endocrinol.* (171):1-29.
- Dogan, O. (2023). Are ABO/Rh blood groups A risk factor for polycystic ovary syndrome?. *Medicine* 102(36):p e34944 DOI:10.1097/MD.00000000000034944
- Enechukwu, Cl.; Onuegbu, AJ.; Olisekodiaka, MJ.; Eleje, GU.; Ikechebelu, JI.; Ugboaja, JO.; et al. (2019). Oxidative stress markers and lipid profiles of patients with polycystic ovary syndrome in a Nigerian tertiary hospital. *Obstet Gynecol Sci.* 62(5): 335-343.
- Faris, Z., El Nashar, A., Elgazzar, M. and Saad, A. (2023). Total antioxidant capacity and oxidative stress in Polycystic ovary syndrome, a case-control study. *The Egyptian Journal of Fertility of Sterility.* 27(2): 97-108. doi: 10.21608/egyfs.2023.297466
- Ghowsi, M.; Khazali, H. and Sisakhtnezhad, S. (2018). The effect of resveratrol on oxidative stress in the liver and serum of a rat model of polycystic ovary syndrome: An experimental study. *Int J Reprod Biomed.* 16(3): 149-158.
- Helvacı, N. and Yildiz, BO. (2022). The impact of ageing and menopause in women with polycystic ovary syndrome. *Clin Endocrinol.* 97: 371- 382. <https://doi.org/10.1111/cen.14558>
- Jakus T, Jurdana M, Žibera L, Pražnikar ZJ. (2021). Acute moderate-intensity exercise increases total antioxidant capacity and anti-inflammatory responses in competitive cyclists: The role of adiponectin. *European Journal of Inflammation.* 19. doi:10.1177/2058739221998890
- Jung, S.Y., Koh, J.H., Kim, KJ. et al., (2021). Switching from TNF α inhibitor to tacrolimus as maintenance therapy in rheumatoid arthritis after achieving low disease activity with TNF α inhibitors and methotrexate: 24-week result from a non-randomized, prospective, active-controlled trial. *Arthritis Res Ther.* 23:182 <https://doi.org/10.1186/s13075-021-02566-z>
- Kanafchian, M.; Esmaeilzadeh, S.; Mahjoub, S.; Rahsepar, M. and Ghasemi, M. (2020). Status of serum copper, magnesium, and total antioxidant capacity in patients with polycystic ovary syndrome. *Biol Trace Elem Res.* 193(1): 111-117.
- Liang, Jx., Zhang, Y., Xiao, Ch. et al. (2023). Application value of tumor necrosis factor inhibitors in in vitro fertilization-embryo transfer in infertile women with polycystic ovary syndrome. *BMC Pregnancy Childbirth* 23, 247. <https://doi.org/10.1186/s12884-023-05546-0>
- Lizneva, D.; Kirubakaran, R.; Mykhalchenko, K.; et al. (2016). Phenotypes and body mass in women with polycystic ovary syndrome identified in referral versus unselected populations: systematic review and meta-analysis. *Fertil Steril.* 106:1510-1520.
- March, WA.; Moore, VM.; Willson, KJ.; Phillips, DI.; Norman, RJ. And Davies, MJ. (2010). The prevalence of polycystic ovary syndrome in a community sample assessed under contrasting diagnostic criteria. *Hum Reprod.* (25):544–551.
- Mazloomi, S.; Barartabar, Z. and Pilehvari, S.(2023). The Association Between Increment of Interleukin-1 and Interleukin-6 in Women with Polycystic Ovary Syndrome and Body Mass Index. *J Reprod Infertil.* 24(1):26-34. doi: 10.18502/jri.v24i1.11906. PMID: 36919048
- Mohammadi, M. (2019). Oxidative Stress and Polycystic Ovary Syndrome: A Brief Review. *International journal of preventive medicine,* 10, 86. https://doi.org/10.4103/ijpvm.IJPVM_576_17
- Niazi, A.D. (2004). *Statistical analysis in Medical Research.* 2nd ed. Coll.of Med., Nahrain Univ. Baghdad. P: 73-98.
- Ranglani, S., Hasan, S., Mahfooz, K., Gordon, J., Garcia-Rates, S. and Greenfield, S. (2023). Antagonism of a key peptide 'T14' driving neurodegeneration: Evaluation of a next generation therapeutic. *Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie,* 167, 115498. <https://doi.org/10.1016/j.biopha.2023.115498>
- Rezayat, F., Hajiaghayi, M., Ghasemi, N., Mesdaghi, M., Ramezani Tehrani, F., & Mosaffa, N. (2023). Inflammatory Responses of Women with Polycystic Ovary Syndrome in Vitro Differ from Healthy Women. *International journal of molecular and cellular medicine,* 12(1), 70–80. <https://doi.org/10.22088/IJMCM.BUMS.12.1.70>
- Uçkan, K.; Halit, D.; Kasım ,T.; Eren ,S. and Canan,D. (2022). Role of Oxidative Stress in Obese and Nonobese PCOS Patients. *International Journal of Clinical Practice.* (9). <https://doi.org/10.1155/2022/4579831>
- Ying-yi L.; Lei Z.; Yi-qiu P.; Ying-ying L.; Rui-xia L. and Cheng-hong Y. (2022). Immune regulation in polycystic ovary syndrome. *Clinica Chimica Acta.* 531: 265-272 <https://doi.org/10.1016/j.cca.2022.04.234>.
- Zhong, GC.; Pu, JY.; Wu ,YL.;Yi ZJ, Wan, L.; Wang, K.; et al. (2020). Total antioxidant capacity and pancreatic cancer incidence and mortality in the prostate, lung, colorectal, and ovarian cancer screening trial. *Cancer Epidemiol Biomarkers Prev.* 29(5): 10