# Insight into Total Antioxidant Capacity and its Relationship with Insulin Resistance among Polycystic Ovary Syndrome Patients

**Dr Murk Fatima[*], Dr Mehreen Inam Illahi[*], Dr Ome Kulsoom[**], Dr Huma Salahuddin[*], Dr Sofia Amjad[***]**

[*] Physiology, Ziauddin University,
[**] Gynecology and Obstetrics, Dr. Ziauddin Hospital,
[***]Physiology, Superior University,

***Abstract-*** Total antioxidant capacity (TAC) is a property of serum to overcome the oxidative stress to retain the cell structure healthy from the adverse effect of oxidative material. To investigate the Total antioxidant capacity and its association with insulin resistance in women with PCOS. The present study involved two groups, IR (n 59=) and NON-IR (n = 31) of PCOS patients. TAC was measured using spectrophotometric assays. HOMA-IR method was used to assess the insulin resistance. Results were analyzed to compare and correlate IR with TAC. The median levels of serum insulin, HOMA-IR and TSH, were significantly higher among IR groups as compared to NON-IR groups (p-value =.01, p-value =.000, p-value =.000) respectively. Moreover, the median of TAC was significantly lower among the IR group as compared to the NON-IR group (p-value =.005). A significant negative correlation of TAC found with BMI (r=-.222 $p$ < 0.036), WHR (r=-.246 $p$ < 0.019), Insulin (r=-.388 p & $p$ < 0.000) and HOMA-IR (r=-.403 $p$ <.000). However, a significant positive correlation of TAC was found with LH (r=.337 $p$ < 0.001). Insulin was found the major significant predictor of oxidative stress, as determined by a reduction in TAC levels, after adjusting for all study parameters (β=-2.861, P,0.001). Our study has highlighted the role of TAC and HOMA-IR in the pathogenesis of PCOS which supports the role of oxidative stress in the pathogenesis of IR which leads to PCOS. Therefore, these important biomarkers TAC and HOMA-IR help the clinicians in the treatment and management of the condition

***Index Terms***- Basal metabolic index, hyperglycemia, insulin resistance, Oxidative stress, Total antioxidant capacity

## I. INTRODUCTION

The oxidative stress (OS) is variation between pro-oxidative agents and antioxidant defense mechanisms, which have a role in reproductive phases in physiological levels for the (Prasad, Tiwari et al. 2016, Wang, He et al. 2017). The OS has been stated in pathogenesis of many conditions including PCOS (Wang, He et al. 2017). The OS causes damage to DNA, lipids, and proteins thus interfering with the normal cellular functions (Kumar, Abbas et al. 2014). The OS seems to be involved in PCOS by transformed steroidogenesis in the ovaries, which leads to increased androgen levels, distressing follicular development and insulin resistance (IR) (Papalou, M Victor et al. 2016). In a vicious cycle, obesity and IR cause local and systemic OS in PCOS which further exacerbate the metabolic and hormonal signals in PCOS (Ruan, Song et al. 2018).

While the pathophysiology of PCOS has not been well-defined, former studies exposed IR to have a central role in the pathogenesis of PCOS. It has been reported that 50-90% of women with PCOS manifest IR(González, Considine et al. 2020). Majid et al reported that 69% of PCOS patients in Pakistan have IR (Majid, Masood et al. 2017). As a compensatory response to IR, hyperinsulinemia develops which in turn interacts synergistically with LH, as a co-gonadotropin within ovarian theca cells to increase androgen production. (Maqbool, Dar et al. 2019). In the ovary, insulin also promotes arrest of pre-antral follicle development and increased proliferation of thecal cells leads to cysts formation in the ovary (Hussain, Niaz et al. 2018). Hyperinsulinemia has extra-ovarian pleiotropic properties that include enhancement of LH pulse amplitude and decreased hepatic production of sex hormone binding globulin, thus causing increase in free androgen levels in serum (Barber, Dimitriadis et al. 2016). IR leads to OS due to increased levels of free fatty acids and glucose in blood (Mahjoub and Masrour-Roudsari 2012).

The OS can be measured by antioxidant capacity (Hu, Dong et al. 2020) which is the capacity of serum to decrease the free radical's formation and protect the harmful effects of cell (Kanafchian, Esmaeilzadeh et al. 2020). Oxidative damage observed due to disruption of redox homeostasis caused by reduced antioxidants and simultaneous upsurges in oxidants Abudawood, Alnuaimet al. 2023). In serum antioxidants are proficient in destroying the formation of oxidants (Yilmaz, Inal et al. 2016). All consecutive and synchronized events like folliculogenesis, ovulation and others are accompanied by reactive oxygen species (ROS) production and scavenging. ROS include radical species as reactive nitrogen species, superoxide anion radical, hydrogen peroxide. Antioxidants are strongly modulated during formation of ROS production. There are two two types of antioxidants. One is non-enzymatic such as β-carotene, ascorbate (vitamin C), α-tocopherol (vitamin E) and glutathione (GSH), Other type is enzymatic like glutathione

peroxidase (GPx), catalase, and glutathione reductase (GR) (Murray, Bender et al. 2012) . As exposed by some recent studies, in PCOS patients' lower levels of serum TAC have been found, this may be due to increased oxidative stress (Sulaiman, Al-Farsi et al. 2018) Kanafchian, Esmaeilzadeh et al. 2020). Jabbar et al also reported decreased serum levels of total antioxidant capacity in the PCOS patients as compared to control (Jabbar, Ahmed et al. 2019).

While the elaborate connection between OS and PCOS has been investigated numerous times, their causal relationship, especially concerning IR, has not been elucidated, particularly in Pakistan. Moreover, the literature also lacks association between TAC and IR so the present study has been planned to bridge this gap and to evaluate TAC levels in PCOS patients having IR and Non-IR. Moreover, investigated the impact of IR on TAC and other variables in PCOS.

## II. METHODS

This was a cross-sectional study; 90 participants were steered after taking informed consent from OPD of gynecology at Ziauddin Hospital Karachi Pakistan from 2019 to March 2020. Ziauddin University gave reference number (MFPHY:0910319) to the Institutional Ethics Review Committee of ZU. The diagnosis was based on Rotterdam criteria in PCOS (Fauser, Tarlatzis et al. 2012). The information about the demographic data, menstrual cycle, obesity, infertility, history of PCOS or any other illnesses, obstetrics and gynecological history and surgical history had been taken individually. The patients who received metformin treatment for PCOS within 6 months of the start of the study and had any chronic diseases were excluded from study.
Furthermore, weight and height were measured manually by using weighing machine with a height scale. The BMI of participants was measured by using equation weight (kg) /height (m)2. It is divided in four categories as follows: >27.5 for obese, 23.0-27.5 for overweight, 18.5-22.9 for normal-weight and <18.5 for underweight according to Asian criteria-based (Liabsuetrakul 2011). Waist hip ratio (WHR) indicates the ratio of your hip circumference to your waist circumference. It governs how much fat is stored on your waist, hips, and buttocks. Waist circumference was measured in standing position, at the half of the distance between lower ribs and the crest of the pelvis. Hip circumference was restrained as the widest gluteal circumference (Bhatnager, Nanda et al. 2018). WHR was set at a cutoff ≤ 0.85 by taking the waist circumference in centimeters divided by hip circumference in centimeters (Organization 2011).

The abdominal ultra-sonographic findings, done by experienced sonologist, of participants were recorded. The hormonal profile, advised by the gynecologist, including LH, Free testosterone, FSH, serum insulin levels, TSH and Serum prolactin were also taken. Along with hormonal profile, fasting blood sugar (FBS) was also done. IR was assessed by formula: HOMA = (fasting insulin mIU/mL * fasting glucose mM)/22.5. according to the homeostasis model assessment (HOMA). The patients are divided into two groups into IR and Non-IR groups on the basis of HOMA-IR. IR was diagnosed when HOMA was > 2.5(Wallace, Levy et al. 2004), and Non-IR was diagnosed when HOMA was < 2.5. The 5ml venous blood was drawn by the trained phlebotomist after getting consent. The sample was centrifuged at 2200-2500 RPM for at least 15 minutes then plasma was transferred to the sterilized Eppendorf and stored at -80 °C in multidisciplinary research laboratory (MDRL) in ZMU until the procedure was done. TAC was measured by the Assay Kit of total antioxidant capacity (A015-2, Cloud Clone, USA) by spectrophotometry by the ABTS method. By using traditional standard for TAC measurement assays with Trolox and results were stated in mmol Trolox equivalent.

The Statistical Package for Social Sciences (SPSS) version 22 used for statistical analysis. Median and interquartile ranges were calculated for numeric variables. frequencies and percentages were calculated for categorical variables. For comparing two groups Mann-Whitney test was applied. Spearman's correlation was applied for finding a correlation, the univariate and multivariate analysis were used. At *p*-value <0.05 was considered statistical significance.

## III. RESULT

The baseline characteristics of PCOS having IR and Non-IR are given in Table 1. The median weight, BMI and WHR were significantly lower among the Non-IR group as compared to the IR group (p-value =.005, p-value =.00 and p-value =.00) respectively. The median levels of serum insulin, HOMA-IR and TSH, were significantly higher among the IR group as compared to the Non-IR group (p-value =.01, p-value =.000, p-value =.000) respectively. Moreover, median of TAC was significantly lower among the IR group as compared to the Non-IR group

(p-value.005).

Table 1: Properties of IR and Non-IR PCOS groups in clinical, biochemical and hormonal features:

| PARAMETER | IR(n=59) | Non-IR (n=31) | P value |
|---|---|---|---|
| **Demographics** | | | |
| AGE (yrs) | 24.00(6) | 23.00(7) | .378 |
| WEIGHT (kg) | 78.00(18.0) | 68.00(18.0) | .005** |
| HEIGHT (ft,in) | 5.4(.40) | 5.4(.70) | .478 |
| WHR (cm) | 0.92(.06) | 0.86(.10) | 0.00** |
| **Metabolic Stress Markers** | | | |
| BMI (kg/m$^2$) | 29.25(6.50) | 25(4.70) | .000** |
| **Glycemic Profile** | | | |
| FBS (mg/dl) | 100(10) | 95.0(10.0) | .197 |
| SERUM INUSLIN (mIU/L) | 20.0(8.10) | 17.4(11.8) | .01** |
| HOMA.IR | 4.5(2.30) | 2.2(.40) | .000** |
| **Hormone Levels** | | | |
| TESTOSTERONE (ng/dl) | 93(52.9) | 91.0(78.50) | .785 |
| FSH (mIU/ml) | 5.1(5.60) | 6.5(1.70) | .028 |
| LH (mIU/ml) | 15.80 (6.69) | 13.5(6.3) | .169 |
| TSH (mIU/ml) | 2.4(1.30) | 1.74(.60) | .000** |
| **Oxidative Stress Markers** | | | |
| TAC (mmol Trolox E/L) | .17(.10) | .22(.94) | .005* |

IR= Insulin resistance Non-IR= No Insulin resistance, BMI: body mass index FBS: fasting blood sugar FSH: follicle stimulating hormone LH: Luteinizing Hormone; TSH: Thyroid Stimulating Hormone; WHR: Waist hip ratio; HOMA.IR: homeostasis model assessment insulin resistance; TAC: Total anti-oxidant capacity.

Table 2 Correlation of TAC and HOMA-IR with other variables

| FACTORS | TAC<br>***r* (*p*-value)** | HOMA-IR<br>***r* (*p*-value)** |
|---|---|---|
| AGE | -.121(.257) | .051(.634) |
| BMI (kg/m$^2$) | -.222(.036**) | .411(.000**) |
| WHR (cm) | -.246(.019**) | .342(.001**) |
| FBS (mg/dl) | .002(.982) | .311(.003**) |
| LH (mIU/ml) | .337(.001**) | .123(.247) |
| FSH (mIU/ml) | .095(.375) | -.062(.563) |
| INSULIN (mIU/L) | -.388(.000**) | .472(.000**) |
| TSH (mIU/ml) | -.130(.223) | .500(.000**) |
| TESTOSTERONE (ng/dl) | .105(.323) | .073(.494) |
| TAC (mmol Trolox E/L) | 1 | -.403(.000**) |
| HOMA-IR | -.403(.000**) | 1 |

r= Correlation coefficient, FBS: fasting blood sugar; FBS: fasting blood sugar; FSH: Follicle Stimulating Hormone; LH: Luteinizing Hormone; TSH: Thyroid Stimulating Hormone; WHR: Waist hip ratio; HOMA.IR: homeostasis model assessment insulin resistance; TAC: total anti-oxidant capacity

A significant negative correlation of TAC found with BMI (r=-.222 $p < 0.036$), WHR (r=-.246 $p < 0.019$), Insulin (r=-.388 $p < 0.000$) and HOMA-IR (r=-.403 $p < .000$). However, positive significant correlation of TAC was observed with LH (r=.337 $p < 0.001$). While HOMA-IR had significant positive correlation with BMI (r=.411 $p < 0.000$), WHR (r=.342 $p < 0.001$), FBS (r=.311 $p < 0.003$), Insulin (r=472 $p < 0.000$) and TSH (r=.500 $p < 0.00$) (Table 2).

In univariate and multivariate tests showed significant association between insulin and TAC. Moreover, it was observed that with one factor rise in BMI and WHR leads to increased risk of IR in PCOS by 9.9% and 11.2%. While one factor rise in insulin leads to an increased risk of IR in PCOS by 19.8%. It was also observed that with one-unit decrease in TAC chances of IR in PCOS increased by 13.4%. (Table 3).

Table 3 Univariate analysis for clinical and biochemical factors associated with insulin resistance in PCOS

| FACTORS | Unadjusted Prevalence Ratio (PR) | 95% CI | P VALUE |
|---|---|---|---|
| AGE (yrs) | -.172 | .684-1036 | .104 |
| BMI (kg/m$^2$) | 1.198 | .721-1.23 | .001* |
| WHR (cm) | 1.535 | 703.1-1.58 | .009* |
| FBS (mg/dl) | .021 | .944-1.102 | .607 |
| LH (mIU/ml) | -.030 | 1.96-45.2 | .514 |
| FSH (mIU/ml) | .068 | .806-1.42 | .639 |
| TESTOSTERONE (ng/dl) | -.006 | .967-1.022 | .665 |
| TSH (mIU/ml) | 2.245 | 1.9-45.8 | .005* |
| INSULIN (mIU/L) | .198 | .96-1.54 | .001* |
| TAC (mmol Trolox E/L) | -4.198 | .001-.220 | .002* |

FBS: fasting blood sugar; FBS: fasting blood sugar; FSH: Follicle Stimulating Hormone; LH: Luteinizing Hormone; TSH: Thyroid Stimulating Hormone; WHR: Waist hip ratio; HOMA.IR: homeostasis model assessment insulin resistance; TAC: total anti-oxidant capacity

The Table 4 shows association between TAC levels with insulin and BMI by the multivariate analysis modeling. The BMI and insulin are included as the predictor variables in multiple regression models for TAC. The major significant predictor of oxidative stress was insulin, as determined by a reduction in TAC levels, after adjusting for all study parameters (β=-2.861, P,0.001). While this model showed strong link between decrease TAC levels and increasing obesity parameters (i.e. BMI), it found association was statistically significant (P=0.015). 38.5% of the variability in TAC among Pakistani women, as indicated by the R2 value of the model was demonstrated in this model.

Table 4 Biochemical factors association in IR by Multivariate analysis.

| Factors | Wald | Beta | PR Adjusted | 95% CI | p value |
|---|---|---|---|---|---|
| BMI (kg/m$^2$) | .196 | 5.887 | 1.217 | 1.038-1.426 | .015* |
| INSULIN (mIU/L) | .143 | 3.821 | 1.154 | 1.000-1.332 | .051* |
| TAC (mmol Trolox E/L) | -2.861 | 11.088 | .057 | .011-.308 | .001* |

*Significant at *p* value <0.1.

## IV. DISCUSSION

Antioxidants function as a protection against the harmful effects of free radical injury in the body. Mostly, the measurement of total antioxidant capacity (TAC) indicates the ability of tissue or biofluid's oxidant-buffering potential (Marques, Magalhães et al. 2014). The changes in plasma antioxidants will lead to OS, it would be reflected in women with PCOS (Moti, Amini et al. 2015).

In our study we observed statistically significant decreased levels of TAC among the PCOS groups (IR and Non-IR). It was found that the most decreased level was found in the IR group as compared to Non-IR. Kanafachianet al has supported the same findings between IR and Non-IR groups (Kanafchian, Esmaeilzadeh et al. 2020). Other studies have also reported lower levels of TAC in PCOS as compared to normal control significantly. (Sulaiman, Al-Farsi et al. 2018, Jabbar, Ahmed et al. 2019, (Artimani, Karimi et al. 2018)) (Abudawood, Alnuaim et al. 2023, Faris, El Nashar et al. 2023). Hilali et al similarly reported decreased TAC levels among PCOS women compared to the control group (Hilali, Vural et al. 2013). However, meta-analysis done by Murri et al., found no significant difference in TAC between the PCOS women and the controls (Murri, Luque-Ramírez et al. 2013). This controversial finding is due to heterogeneity in the studies included in the meta-analysis as well as the differences in the criteria used for the definition of PCOS, differences in race and ethnicity of the subjects and the use of different assays with different sample sizes in these studies. Decreased TAC occurs due to change in the antioxidant levels of plasma or increased oxidative stress which occur due to IR and hyperandrogenism in PCOS (Maleedhu, Vijayabhaskar et al. 2014). The high oxidant levels lead to apoptosis and proliferation of granulosa and theca cells, resulting in multiple unregressed follicles and anovulation leading to cysts formation that develops PCOS (Yildirim, Turkyilmaz et al. 2017).

In our study we found high levels of FBS among the IR group in PCOS. As well as statistically significant high insulin and HOMA-IR among the groups with the highest levels in IR group

as also supported by (Kanafchian, Esmaeilzadeh et al. 2020) . High insulin and HOMA-IR were also reported in PCOS by many studies (Amini, Omani-Samani et al. 2018 Wiweko, Indra et al. 2018). IR occur in PCOS due to genetic variations in insulin signaling as in single-nucleotide polymorphisms (SNPs) of genes involved in the insulin pathway that SNPs concern the gene encoding the insulin receptor substrate-1 and the gene encoding the plasma cell membrane glycoprotein-1(Pappalardo, Vita et al. 2017). Much evidence has suggested that oxidative stress induced by oxygen species (ROS) may contribute to the development of IR and hyperandrogenism which are key features of PCOS (Deepika, Nalini et al. 2014).

We explored BMI and waist circumference among all study participants and found that both were significantly higher among those with IR of >2.5 compared to those with IR of <2.5 as also reported by (Al-Jefout, Alnawaiseh et al. 2017).

We also observed TAC had negative correlation with BMI, WHR, insulin and HOMA-IR. Egeonu et al also found a weak TAC and BMI (Egeonu, Eleje et al. 2023). Victor et al also concluded an increase in ROS positively correlated with HOMA-IR (Victor, Rovira-Llopis et al. 2016). Kanafchaian et al also found that TAC with HOMA-IR in obese PCOS had negative correlation (Kanafchian, Esmaeilzadeh et al. 2020). Hyperglycemia and higher levels of free fatty acids in IR leads to an increase in oxidative stress (Lakshmi and Malini 2023) which increases the production of reactive oxygen species (ROS) and decreases TAC (Mahjoub and Masrour-Roudsari 2012).

In our study we also found HOMA-IR is positively correlated with BMI, WHR, FBS and insulin in PCOS while negative correlation with TAC. Dahan et al have also shown increased magnitude of IR in obese PCOS as compared to lean subjects (Dahan and Reaven 2019). Dahan et al further stated that a BMI of at least 30 kg/m2 is the prime predictor of IR with increased risk for metabolic irregularities in PCOS (Dahan and Reaven 2019). Reason behind it is the abnormal metabolism of lipids causing high production and secretion of free fatty acids (FFAs) in obese subjects. Raised FFA levels can be a source of abnormal pro-inflammatory response and consequent development of IR. It has also been reported that the down regulation of intracellular antioxidant systems as well as increased production of ROS in the adipose tissue causes oxidative stress thus leading to development of IR (Dludla, Nkambule et al. 2019). While Sulaiman et al also found possible associations between increasing obesity parameters and decreasing TAC levels. (Sulaiman, Al-Farsi et al. 2018).

This study had some limitations as a single-centered study and cross-sectional study. We need to perform TAC levels on a large sample size from multiple centers. In addition to this a case control study design is required to establish the role of TAC in PCOS pathology. However, in the future to assess levels of TAC before and after antioxidant supplementation to establish the temporality role of TAC in PCOS follow-up studies should be done.Analyze and understand all the provided review comments thoroughly. Now make the required amendments in your paper. If you are not confident about any review comment, then don't forget to get clarity about that comment. And in some cases there could be chances where your paper receives number of critical remarks. In that cases don't get disheartened and try to improvise the maximum.

## V. CONCLUSION

This study has concluded that significantly lower levels of TAC was found in PCOS specifically in the IR group so it concluded that oxidative stress has a part in pathogenesis of PCOS. Furthermore, these two parameters (TAC and HOMA-IR) can be used as prognostic biomarkers together or individually which may help the clinicians in the treatment and management of the condition. For future recommendation case-control studies with larger sample sizes should be conducted. The molecular mechanisms of oxidative stress leading to Insulin resistance should be explored in PCOS by laboratory animal studies.

## REFERENCES


1. Abudawood, M., L. Alnuaim, H. Tabassum, H. K. Ghneim, M. A. Alfhili, S. T. Alanazi, N. D. Alenzi and S. Alsobaie (2023). "An Insight into the Impact of Serum Tellurium, Thallium, Osmium and Antimony on the Antioxidant/Redox Status of PCOS Patients: A Comprehensive Study." International Journal of Molecular Sciences 24(3): 2596.
2. Al-Jefout, M., N. Alnawaiseh and A. Al-Qtaitat (2017). "Insulin resistance and obesity among infertile women with different polycystic ovary syndrome phenotypes." Scientific reports 7(1): 1-9.
3. Artimani, T., J. Karimi, M. Mehdizadeh, M. Yavangi, E. Khanlarzadeh, M. Ghorbani, S. Asadi and N. Kheiripour (2018). "Evaluation of pro-oxidant-antioxidant balance (PAB) and its association with inflammatory cytokines in polycystic ovary syndrome (PCOS)." Gynecological endocrinology 34(2): 148-152.
4. Barber, T. M., G. K. Dimitriadis, A. Andreou and S. Franks (2016). "Polycystic ovary syndrome: insight into pathogenesis and a common association with insulin resistance." Clinical Medicine 16(3): 262.
5. Deepika, M., S. Nalini, G. Maruthi, V. Ramchander, K. Ranjith, K. P. Latha, V. U. Rani and P. Jahan (2014). "Analysis of oxidative stress status through MN test and serum MDA levels in PCOS women." Pakistan journal of biological sciences: PJBS 17(4): 574-577.
6. Egeonu, R. O., G. U. Eleje, J. I. Ikechebelu, J. O. Ugboaja, O. S. Umeononihu, C. C. Okoro, C. G. Okafor, E. O. Ugwu, C. I. Enechukwu and A. A. Onwuegbuna (2023). "Comparison of Serum Oxidative Stress Markers of Malondialdehyde and Total Antioxidant Capacity Between Women with Infertility Due to Polycystic Ovary Syndrome and Tubal Factor."
7. Faris, Z. A., A. M. El Nashar, M. A. E. Elgazzar and A. S. Saad (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.
8. Fauser, B. C., B. C. Tarlatzis, R. W. Rebar, R. S. Legro, A. H. Balen, R. Lobo, E. Carmina, J. Chang, B. O. Yildiz and J. S. Laven (2012). "Consensus on women's health aspects of polycystic ovary syndrome (PCOS): the Amsterdam

ESHRE/ASRM-Sponsored 3rd PCOS Consensus Workshop Group." Fertility and sterility 97(1): 28-38. e25.
9. González, F., R. V. Considine, O. A. Abdelhadi and A. J. Acton (2020). "Inflammation triggered by saturated fat ingestion is linked to insulin resistance and hyperandrogenism in PCOS." The Journal of Clinical Endocrinology & Metabolism.
10. Hilali, N., M. Vural, H. Camuzcuoglu, A. Camuzcuoglu and N. Aksoy (2013). "Increased prolidase activity and oxidative stress in PCOS." Clin Endocrinol (Oxf) 79(1): 105-110.
11. Hu, X., D. Dong, M. Xia, Y. Yang, J. Wang, J. Su, L. Sun and H. Yu (2020). "Oxidative stress and antioxidant capacity: development and prospects." New Journal of Chemistry 44(27): 11405-11419.
12. Hussain, S., S. Niaz and S. I. Munir (2018). "Serum Insulin Levels, Insulin Resistance and Type 2 Diabetes in Patients of Polycystic Ovarian Syndrome." PAKISTAN JOURNAL OF MEDICAL & HEALTH SCIENCES 12(2): 474-476.
13. Kanafchian, M., S. Esmaeilzadeh, S. Mahjoub, M. Rahsepar and M. Ghasemi (2020). "Status of serum copper, magnesium, and total antioxidant capacity in patients with polycystic ovary syndrome." Biological Trace Element Research 193(1): 111-117.
14. Kumar, V., A. K. Abbas, N. Fausto and J. C. Aster (2014). Robbins and Cotran pathologic basis of disease, professional edition e-book, elsevier health sciences.
15. Lakshmi, K. and S. S. Malini (2023). "Insulin resistance and Oxidant-Antioxidant Markers in Young Women with Polycystic Ovarian Syndrome." Bulletin of Pure & Applied Sciences-Zoology 42(1): 106-116.
16. Majid, H., Q. Masood and A. H. Khan (2017). "Homeostatic model assessment for insulin resistance (HOMA-IR): A Better marker for evaluating insulin resistance than fasting insulin in women with polycystic ovarian syndrome." J Coll Physicians Surg Pak 27(3): 123-126.
17. Maqbool, M., M. A. Dar, I. Gani and M. I. Geer (2019). "Insulin Resistance and Polycystic ovary Syndrome: A Review." Journal of Drug Delivery and Therapeutics 9(1-s): 433-436.
18. Moti, M., L. Amini, S. S. M. Ardakani, S. Kamalzadeh and M. Masoomikarimi (2015). "Oxidative stress and anti-oxidant defense system in Iranian women with polycystic ovary syndrome." Iranian journal of reproductive medicine 13(6): 373.
19. Murray, R., D. Bender, K. M. Botham, P. J. Kennelly, V. Rodwell and P. A. Weil (2012). "Harpers Illustrated Biochemistry 29/E." Ed Mc Graw Hill LANGE, China.
20. Papalou, O., V. M Victor and E. Diamanti-Kandarakis (2016). "Oxidative stress in polycystic ovary syndrome." Current pharmaceutical design 22(18): 2709-2722.
21. Prasad, S., M. Tiwari, A. N. Pandey, T. G. Shrivastav and S. K. Chaube (2016). "Impact of stress on oocyte quality and reproductive outcome." Journal of biomedical science 23(1): 1-5.
22. Ruan, X., J. Song, M. Gu, L. Wang, H. Wang and A. O. Mueck (2018). "Effect of Diane-35, alone or in combination with orlistat or metformin in Chinese polycystic ovary syndrome patients." Archives of gynecology and obstetrics 297(6): 1557-1563.
23. Sulaiman, M. A., Y. M. Al-Farsi, M. M. Al-Khaduri, J. Saleh and M. I. Waly (2018). "Polycystic ovarian syndrome is linked to increased oxidative stress in Omani women." International Journal of Women's Health 10: 763.
24. Wang, S., G. He, M. Chen, T. Zuo, W. Xu and X. Liu (2017). "The role of antioxidant enzymes in the ovaries." Oxidative medicine and cellular longevity 2017.

## AUTHORS

**First Author** – **Dr Murk Fatima** MPhil Physiology, Department of Physiology, Ziauddin University,

**Second Author** – **Dr Mehreen Inam Illahi:** MPhil Physiology, Department of Physiology, Ziauddin University,

**Third Author** – **Dr Ome Kulsoom**, Department of Gynecology and Obstetrics, Dr. Ziauddin Hospital.

**Fourth Author** – **Dr Huma Salahuddin:** MPhil Physiology, Department of Physiology, Ziauddin University.

**Fifth Author** – **Dr Sofia Amjad:** PhD Physiology, Professor of Department of Physiology, Superior University.

**Correspondence Author** – **Dr Murk Fatima** MPhil Physiology, Assistant Professor, Department of Physiology, Ziauddin University, Karachi