

Vitamin D status and its relationship with oxidative stress markers in infertile women with polycystic ovary syndrome

Arfa Azhar¹, Syed Mahboob Alam², Mussarat Ashraf¹,
Ayesha Malick³, Sumaira Riffat³ and Rehana Rehman¹

¹Department of Biological and Biomedical Sciences, The Aga Khan University, Karachi, Pakistan

²Department of Pharmacology, BMSI, JPMC, Karachi, Pakistan

³Department of Physiology, JSMU, Karachi, Pakistan

Abstract: To compare the effect of vitamin D treatment on total antioxidant capacity (TAOC), paraoxonase 1 (PON1) and anti-müllerian hormone (AMH) in infertile females with and without polycystic ovary syndrome (PCOS) were studied. It was a case-control study involving a total of 180 participants; 114 (63 %) PCOS and 66 (37 %) non-PCOS infertile female subjects from 2020-2022. Study participants received a single injection of vitamin D cholecalciferol (300,000 IU) once in the study period. Serum levels of vitamin D, AMH, TAOC and PON1 were determined before and after treatment. The quantitative variables were calculated by means and standard error of the mean (SE), compared by paired t test; significance at $p < 0.05$. The mean age was comparable in both groups whereas the body mass index (BMI) of PCOS females was higher ($p < 0.001$). Vitamin D and AMH were significantly low in PCOS vs. non-PCOS females ($p < 0.001$). TAOC and PON1 mean levels in both groups were comparable. The biomarker levels improved in both groups after vitamin D treatment more in PCOS females ($p < 0.001$). Vitamin D elicited improvement of oxidative stress in infertile females with and without PCOS, however, the dominant and significant role in PCOS is the one highlighted in our study.

Keywords: TAOC, PON1, AMH, vitamin D, PCOS, infertility.

INTRODUCTION

Polycystic ovary syndrome (PCOS) is a multifactorial, autoimmune, and polygenic condition involving females of reproductive age (Parsamanesh et al., 2018). With a prevalence of somewhere between 6-20%, the diagnosis of PCOS itself remains largely controversial due to its wide range of clinical presentations and misunderstood etiology (Deswal et al., 2020). Since PCOS is such a compound pathology, it causes numerous pathologies including hormonal imbalances (Sidra, Tariq, Farrukh, & Mohsin, 2019). In Pakistan alone, it is estimated that 52% of women suffer from PCOS, which hinders their quality of life (Azhar et al, 2020). In a country where infertility is considered taboo and disrespectful, and where most of the blame falls on women, along with hormonal conditions associated with PCOS like weight gain and hirsutism, the high prevalence of PCOS puts a lot of pressure and mental stress on women causing body dysmorphia, depression and low self-esteem (Naheed et al., 2022).

Vitamin D is a steroid hormone contained in calcium homeostasis and bone management. 67-85% of women with PCOS are expected to be deficient of vitamin D, the average serum density 25 (OH) D < 20 ng/mL (Azhar et al., 2021). The purpose of vitamin D within female physiology plays a complex role, reduces serum androgen and serum anti-müllerian hormones (AMH) (Mu et al., 2021).

AMH is expressed by the Granulosa cells of the preantral and antral ovarian follicles, and contributes to ovulatory disturbances by inhibiting primordial follicle recruitment from the oocyte pool and is also believed to interfere with the action of follicle-stimulating hormone (FSH) (Rudnicka et al., 2021). A meta-analysis declared that women without PCOS taking vitamin D resulted in increased AMH but women suffering from PCOS with vitamin D supplements showed low AMH levels and other clinical symptoms (Moridi et al., 2020).

Oxidative stress, known as oxidant/antioxidant imbalance, arises when the net content of an oxidant exceeds its antioxidant capacity. (Demirci-Çekiç et al., 2022). Therefore, OS can occur due to an upsurge of 'reactive oxygen species (ROS)', a decline of the antioxidant systems or both (Umare et al., 2022). Paraoxonase 1 (PON1) is a 'high-density lipoprotein (HDL)' related antioxidant enzyme with phospholipase activity (Sucato et al., 2022). PON1 can hydrolyze the accumulation of lipid peroxides. Furthermore, human PON1 takes part in the antiatherogenic activity of HDL and its action has been presented to be contrarywise related to oxidative stress (OS) in human serum (Otocka-Kmieciak, 2022). The PON1 gene has a strong link with increased incidence of PCOS. It is considered that insulin resistance in PCOS might be due to low serum levels of PON1 normally expressed in the liver. Increased OS has also been suggested as a critical factor in the pathogenesis of PCOS-associated infertility (Kulik-Kupka et al., 2022).

*Corresponding author: e-mail: rehana.rehman@aku.edu

Total antioxidant capacity (TAOC) refers to the serum's ability to limit the generation of free radicals, hence protecting the cell structure from molecular damage. There is increasing attention on the antioxidant defense mechanisms in inflammatory diseases (Lv *et al.*, 2022). Although it is known that PCOS is linked to metabolic syndrome, new markers showing this symptom are desperately needed in clinical practice. To the best of our knowledge, the TAOC and PON1 activities have not been studied in PCOS in Pakistan, presuming that the activities of TAOC and PON1 may be reduced and that this antioxidant enzyme plays a role in the PCOS pathogen. Therefore, this study aimed to compare the impact of vitamin D treatment on TAOC, PON1 and AMH in infertile females with and without polycystic ovary syndrome (PCOS).

MATERIALS AND METHODS

This study was a case-control study performed during 2020-2022. The sample size was calculated by Open Epi software, taking the prevalence of infertility as 18%-22% (Gupta, Dere, & Ghildiyal, 2022), assuming a confidence level of 95%, statistical power of $\geq 80\%$. About 180 infertile females from AKUH and Australian Concept Infertility Medical Centre (ACIMC) centers were enrolled for this study once taking a transcribed notified agreement at the time of induction in the study. Infertile PCOS females were recruited based on the following criteria: Confirmation of PCOS: The existence of any standards in 3, that is, Oligo and/or anovulation, additional androgen activity and/or polycystic ovarian appearance in Ultrasound" (15) Out of these 180, 114 (63 %) females were in PCOS group, and 66 (37 %) females were in the non-PCOS group. The cases were infertile for more than two years, aged 15-45 years, with body mass index: Low weight, (BMI <18 kg/m²), normal weight (BMI 18-22.9 kg/m²), overweight (BMI 23-25.9 kg/m²) and obesity (BMI ≥ 26 kg/m²) and Vitamin D deficiency (VDD) serum levels <20 ng/ml from all ethnic backgrounds. Age-matched healthy females with regular menstrual cycles without any evidence of polycystic ovary by ultrasound were included as controls. Women diagnosed as infertile due to sperm defects in males, with a history of secondary fertility, Women with gynecological tumors, diabetes, hypertension, thyroid problems, and common health conditions are excluded. It is also excluded from practicing females on oral contraceptive pills and hormonal treatments or any contraceptive measures.

10 ml of venous blood were collected by all participants during proliferative phase preferably on day 2 of the menstrual cycle. Serum was obtained from centrifugation of blood at 3000rpm for 20 min and then the serum of samples was instantly frozen and kept at -80°C for the detection of vitamin D, AMH, TAOC and PON1.

Vitamin D levels were determined using the available ELISA kit (Cat# VD315B, Calbiotech). Human AMH was analyzed in serum by using Human Mullerian Inhibiting Substance/AMH, MIS/AMH ELISA kit (Ca t# E1052Hu, BT-LAB). TAOC was measured by using TAOC ELISA kit (Cat # E2199Hu, BT-LAB) and PON1 was analyzed by using Human PON1 ELISA kit (Cat# E2157Hu, BT-LAB).

Study participants were assessed for their vitamin D levels. Those having vitamin D deficiency serum concentrations <20ng/ml (Hanif *et al.*, 2019) received vitamin D Cholecalciferol (300,000 IU) single injection for 12 weeks.

Ethical approval

Ethical approval was acquired from Ethical Review Committee, Aga Khan University through ERC No.2022-4812-20408.

STATISTICAL ANALYSIS

Statistical evaluation was carried out with the Statistical Package for Social Sciences (SPSS) version 20 software system. Descriptive statistics and paired t-test was accomplished to find out the mean and standard deviation and differences in PCOS and Non-PCOS females. Spearman's rank correlation tests were performed to determine the association. Statistical significance was assumed at $p < 0.05$.

RESULTS

Table 1 describes descriptive attributes of the infertile females into both PCOS and non-PCOS groups. The mean age was comparable in both groups and was not found to be significantly different, however, body mass index (BMI) was considerably greater in females with PCOS as compared to non-PCOS ($p < 0.001$). The mean serum values of vitamin D and AMH were significantly lower in PCOS females as matched to non-PCOS ($p < 0.001$). No significant variations were revealed in the mean quantities of TAOC and PON1 in both groupings.

Table 2 shows the comparison of vitamin D, AMH, TAOC and PON1 before and after the medicine in both PCOS and non-PCOS females. The mean values of these variables improved after treatment in each of the two groups PCOS and non-PCOS, being significantly higher in PCOS ($p < 0.001$).

DISCUSSION

The findings of our study observed that vitamin D treatment led to considerable increase in TAOC, PON1, and AMH levels in both PCOS and non-PCOS infertile females. The mean age of participants in both groups was

Table 1: Descriptive characteristics of the infertile females with and without PCOS

	Non-PCOs (n=66)	PCOs (n=114)	p-value
Age (Years)	32.86 ± 5.03	33.81 ± 5.57	0.252
BMI (Kg/m ²)	26.42 ± 4.09	29.66 ± 4.91	<0.001**
Vitamin D (ng/ml)	25.98 ± 10.37	21.17 ± 9.84	0.055
AMH (ng/ml)	2.81 ± 1.41	1.58 ± 1.17	<0.001**
TAOC (U/ml)	15.57 ± 11.04	18.49 ± 13.3	0.099
PON1 (ng/ml)	113.64 ± 41.93	136.29 ± 91.19	0.056

Data shown as mean and standard error, *(p<0.05) is assumed as significant difference. **(p<0.01) is considered highly significant, Mann-Whitney U test was utilized.

Table 2: Comparison of biochemical variables before and after vitamin D supplementation

	PCOS (n=114)			Non-PCOS (n=66)		
	Before treatment	After treatment	p-value	Before treatment	After treatment	p-value
AMH (ng/ml)	1.58 ± 1.17	2.29 ± 1.75	<0.001*	2.81 ± 1.41	3.59 ± 1.76	<0.001*
TAOC (U/ml)	18.49 ± 13.30	117.38 ± 44.92	<0.001*	15.57 ± 11.04	100.42 ± 40.99	<0.001*
PON1 (ng/ml)	136.29 ± 91.19	260.76 ± 114.28	<0.001*	113.64 ± 41.93	213.83 ± 61.92	<0.001*

*Data described as average and standard deviation, paired t test was used, p-value <0.01 was believed highly meaningful.

found to be similar and not significantly different. Our results are in accordance with the findings of another research (Mahboobifard *et al.*, 2022) stating that there are no significant differences in age, infertility and BMI between the two groups. However, a significant difference in women's BMI has been observed in both groups of our study. The females with PCOS had significantly higher BMI values as compared to non-PCOS females, which were comparable with the findings of (Gürsu *et al.*, 2022).

Another important observation was that serum values of vitamin D and AMH were significantly less in PCOS females as matched to non-PCOS. These findings indicate that women with PCOS are at greater probability for vitamin D and AMH deficiency, which can further impact their reproductive health. This is in line with previous research that has reported the advantageous impacts of vitamin D supplements on reproductive well-being in females along with infertility. A study by Cheng, Mu *et al.* (2021) found that vitamin D supplement in PCOS patients managed to considerable improvements within AMH levels, and ovulation rates. These results support our findings and highlight the prospective function of vitamin D in improving reproductive health in PCOS patients (Mu *et al.*, 2021). In comparable research by Alifarja *et al.* (2020), the authors reported that vitamin D supplementation in infertile females take the lead to valuable rise in AMH levels and improved endocrine parameters. These findings are coherent with our research and support the possible role of vitamin D in enhancing fertility outcomes in infertile females (Aramesh *et al.*, 2021). Finally, the results suggest that vitamin D treatment led to a noteworthy increase in AMH levels in both PCOS and non-PCOS female groups.

AMH is an indicator of the ovarian reserve, and lower AMH levels have been associated with decreased fertility (Cedars, 2022). Thus, the findings suggest that vitamin D treatment may have a positive impact on fertility by reducing AMH levels in both PCOS and non-PCOS infertile women. Nevertheless, variations in the mean values of TAOC and PON1 in both groups were not significant. This result is constant with the outcomes of other research articles, which suggest that there is no clear association between these biomarkers and infertility in females with PCOS (Zhao *et al.*, 2023).

Furthermore, a study by Chehsmaza *et al.* (2020) found that vitamin D supplementation led to significant increases in PON1 levels in obese females with PCOS. This finding supports our results and implies that vitamin D supplementation might perform a role in improving oxidative stress in PCOS patients, which is a known contributing factor to infertility in this population. Results with preceding researches reported a positive effect of vitamin D supplement on OS levels in women with PCOS (Kyei *et al.*, 2020 and Zhao *et al.*, 2021). One study by Y Muhammad *et al.* (2021) observed that vitamin D supplement significantly reduced OS markers, including malondialdehyde (MDA), a marker of lipid peroxidation, and enhanced antioxidant enzymes like superoxide dismutase (SOD) and glutathione peroxidase (GPx) in PCOS females (Muhammad *et al.*, 2021). These results recommend that vitamin D treatment can perform a starring role in reducing OS and in improving the antioxidant capacity in women with PCOS.

A study by Dubey *et al.* (2021) however, documented that vitamin D supplementation did not significantly augment OS markers in women with PCOS. The discrepancy between our study and by Dubey *et al.* (2021) could be

expected to variations in the study design, sample size, and the length of vitamin D supplementation (Dubey *et al.*, 2021). The results also indicate that vitamin D treatment headed to a substantial increase in PON1 levels in both groups of women. PON1 is a key enzyme involved in antioxidant defense, and increased levels of PON1 have been linked to improved fertility. Thus, the findings suggest that vitamin D treatment may have a positive impact on fertility by improving the levels of PON1 in both PCOS and non-PCOS infertile women. Overall, the results of this study provide important insights into the effects of vitamin D treatment on TAOC, PON1, and AMH levels in PCOS and non-PCOS infertile females. The inconsistency among what we observed in the recent research and other experimental assessments viewing the negative relationship between vitamin D in PCOS females improved oxidative stress due to its antioxidant activities (Renke *et al.*, 2023) and reduced ROS and pro-inflammatory cytokines (Sharma *et al.*, 2023).

Vitamin D supplement in women with Vitamin D - deficiency with PCOS caused a significant increase in plasma TAOC and PON1 levels is in agreement with our study, in which Cammisotto *et al.* (2021) examined enhanced TAOC activity and PON1 levels after vitamin D supplementation in rats (Cammisotto *et al.*, 2021). In addition, our previous study suggested that taking 400 IU/Day vitamin D administration for 9 weeks has developed enhanced plasma taoq and total GSH level in healthy pregnant women (Cammisotto *et al.*, 2021).

CONCLUSION

Vitamin D administration improved antioxidant defense and ovarian reserve in all infertile females with and without PCOS as evident by improvement in levels of TAOC, PON1 and AMH. The role of vitamin D is elicited in our study, in the improvement of oxidative stress in infertile females with and without PCOS, however, the dominant and significant role in PCOS is the one highlighted in our study.

Future research is required to explore the cause-effect mechanism and determine the optimal dosing and duration of vitamin D supplementation for fertility enhancement and explore the relationship between TAOC and PON1 with infertility in women with PCOS.

REFERENCES

Aramesh S, Alifarja T, Jannesar R, Ghaffari P, Vanda R, and Bazarganipour F (2021). Does vitamin D supplementation improve ovarian reserve in women with diminished ovarian reserve and vitamin D deficiency: a before-and-after intervention study. *BMC Endocr. Disord.*, **21**(1): 1-5.

- Azhar A, Abid F and Rehman R (2020). Polycystic ovary syndrome, subfertility and vitamin d deficiency. *J. Coll. Physicians Surg. Pak.*, **30**(5): 545.
- Azhar A, Haider G, Naseem Z, Farooqui N, Farooqui MU and Rehman R (2021). Morphological changes in the experimental model of polycystic ovary syndrome and effects of vitamin D treatment. *J. Obstet. Gynaecol. Res.*, **47**(3): 1164-1171.
- Cammisotto V, Nocella C, Bartimoccia S, Sanguigni V, Francomano D, Sciarretta S and D'Amico A (2021). The role of antioxidants supplementation in clinical practice: Focus on cardiovascular risk factors. *Antioxidants*, **10**(2): 146.
- Cedars MI (2022). Evaluation of female fertility AMH and ovarian reserve testing. *J. Clin. Endocrinol. Metab.*, **107**(6): 1510-1519.
- Demirci-Çekiç S, Ozkan G, Avan AN, Uzunboy S, Çapanoğlu E and Apak R (2022). Biomarkers of oxidative stress and antioxidant defense. *J. Pharm. Biomed. Anal.*, **209**: 114477.
- Deswal R, Narwal V, Dang A and Pundir CS (2020). The prevalence of polycystic ovary syndrome: A brief systematic review. *J. Hum. Reprod. Sci.*, **13**(4): 261.
- Dubey P, Reddy S, Boyd S, Bracamontes C, Sanchez S, Chattopadhyay M and Dwivedi A (2021). Effect of nutritional supplementation on oxidative stress and hormonal and lipid profiles in PCOS-affected females. *Nutrients*, **13**(9): 2938.
- Gupta A, Dere SS and Ghildiyal PR (2022). To assess prevalence of anxiety, depression and its association with coping in females suffering from infertility. *Telangana J. Psychiatry*, **8**(1): 34.
- Gursu T, Eraslan A and Angun B (2022). Comparison of body mass index, anti-müllerian hormone and insulin resistance parameters among different phenotypes of polycystic ovary syndrome. *Obstet. Gynecol. Clin. Med.*, **2**(4): 164-170.
- Hanif QA, Qamar S, Aslam P, Omar H, Mustafa N and Masood S (2019). Association of vitamin D deficiency with polycystic ovarian syndrome. *Pak. Armed Forces Med. J.*, **69**(2): 241-244.
- Kulik-Kupka K, Jabczyk M, Nowak J, Jagielski P, Hudzik B and Zubelewicz-Szkodzinska B (2022). Fetuin-A and its association with anthropometric, atherogenic, and biochemical parameters and indices among women with polycystic ovary syndrome. *Nutrients*, **14**(19): 4034.
- Kyei G, Sobhani A, Nekonam S, Shabani M, Ebrahimi F., Qasemi M and Fardin A (2020). Assessing the effect of MitoQ10 and Vitamin D3 on ovarian oxidative stress, steroidogenesis and histomorphology in DHEA induced PCOS mouse model. *Heliyon*, **6**(7): e04279.
- Lv R, Dong Y, Bao Z, Zhang S, Lin S and Sun N (2022). Advances in the activity evaluation and cellular regulation pathways of food-derived antioxidant peptides. *Trends Food Sci. Technol.*, **122**: 171-186.
- Mahboobifard F, Rahmati M, Amiri M, Azizi F and

- Ramezani Tehrani F (2022). To what extent does polycystic ovary syndrome influence the cut-off value of prolactin? Findings of a community-based study. *Adv. Med. Sci.*, **67**(1): 79-86.
- Moridi I, Chen A, Tal O and Tal R (2020). The association between vitamin D and anti-mullerian hormone: A systematic review and meta-analysis. *Nutrients*, **12**(6): 1567
- Mu Y, Cheng D, Yin TL and Yang J (2021). Vitamin D and polycystic ovary syndrome: A narrative review. *Reprod. Sci.*, **28**(8): 2110-2117.
- Muhammad Y, Kani YA, Iliya S, Muhammad JB, Binji A, El-Fulaty Ahmad A and Ahmed AU (2021). Deficiency of antioxidants and increased oxidative stress in COVID-19 patients: A cross-sectional comparative study in Jigawa, Northwestern Nigeria. *SAGE Open Medicine*, **9**: 2050312121991246.
- Naheed A, Sadia S, Naila I, Muhammad Adnan A, Abu H and Zafaar S (2022). Polycystic ovary syndrome: It's not just infertility. In: W. Zhengchao (Ed.), *Polycystic Ovary Syndrome* (pp. Ch. 3). Rijeka: IntechOpen.
- Otocka-Kmiecik A (2022). Effect of carotenoids on Paraoxonase-1 activity and gene expression. *Nutrients*, **14**(14): 2842.
- Parsamanesh N, Moossavi M, Bahrami A, Butler AE and Sahebkar A (2018). Therapeutic potential of curcumin in diabetic complications. *Pharmacol. Res.*, **136**: 181-193.
- Renke G, Starling-Soares B, Baesso T, Petronio R, Aguiar D and Paes R (2023). Effects of vitamin D on cardiovascular risk and oxidative stress. *Nutrients*, **15**(3): 769.
- Rudnicka E, Kunicki M, Calik-Ksepka A, Suchta K, Duszewska A, Smolarczyk K and Smolarczyk R (2021). Anti-müllerian hormone in pathogenesis, diagnostic and treatment of PCOS. *Int. J. Mol. Sci.*, **22**(22): doi:10.3390/ijms222212507
- Sharma K, Kumar S, Prakash R, Khanka S, Mishra T, Rathur R and Narender T (2023). Chebulinic acid alleviates LPS-induced inflammatory bone loss by targeting the crosstalk between reactive oxygen species/NFκB signaling in osteoblast cells. *Free Radic. Biol. Med.*, **194**: 99-113.
- Sidra S, Tariq MH, Farrukh MJ and Mohsin M (2019). Evaluation of clinical manifestations, health risks and quality of life among women with polycystic ovary syndrome. *Plos one*, **14**(10): e0223329.
- Sucato V, Corrado E, Manno G, Amata F, Testa G, Novo G and Galassi AR (2022). Biomarkers of coronary microvascular dysfunction in patients with microvascular angina: A narrative review. *Angiology*, **73**(5): 395-406.
- Umare M, Wankhede N, Bajaj K, Trivedi R, Taksande B, Umekar M and Kale M (2022). Interweaving of reactive oxygen species and major neurological and psychiatric disorders. Paper presented at the *Annales Pharmaceutiques Françaises*. **80**(4): 409-425.
- Zhao JF, Li BX and Zhang Q (2021). Vitamin D improves levels of hormonal, oxidative stress and inflammatory parameters in polycystic ovary syndrome: A meta-analysis study. *Ann. Palliat. Med.*, **10**(1): 169-183.
- Zhao J, Dong L, Lin Z, Sui X, Wang Y, Li L and Liu J (2023). Effects of selenium supplementation on Polycystic ovarian syndrome: A systematic review and meta-analysis on randomized clinical trials. *BMC Endocr. Disord.*, **23**(1): 33.