

## ORIGINAL ARTICLE

OXIDATIVE CAPACITY IN POLYCYSTIC OVARY SYNDROME:  
EXPLORATION AND WAY FORWARDArfa Azhar, Sumaira Riffat\*, Rabiya Ali\*\*, Mussarat Ashraf\*\*\*, Haq Nawaz Khan<sup>†</sup>  
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**Background:** Polycystic ovarian syndrome (PCOS) is a complex disorder characterized by elevated androgen levels, ovarian cysts, chronic anovulation, and infertility, with insulin resistance and hyperinsulinemia as key contributors. The study aims to explore the role of oxidative capacity in presentation of PCOS by estimating Total Oxidant Status (TOS), Alarin, and Fetuin in PCOS and non-PCOS infertile women. **Methods:** This cross-sectional study was conducted from Feb 2021 to Feb 2023. The study recruited 89 women with primary infertility aged 15–49 years. Participants were matched based on age and BMI, including women with a BMI ranging from normal to overweight/obese, while excluding those with secondary infertility, major health conditions, or oral contraceptive use. Healthy infertile women with regular cycles served as controls. Serum levels of TOS, Alarin, and Fetuin-A were measured using ELISA kits. Statistical analysis was performed using SPSS-23. **Results:** Forty-five (51%) of the participants had PCOS, with significantly higher BMI ( $p=0.002$ ). Follicular Stimulating Hormone (FSH) and Luteinizing Hormone (LH) levels were significantly elevated ( $p<0.001$ ) in the PCOS group, while Anti-Müllerian Hormone (AMH) was significantly lower ( $p<0.001$ ). Prolactin, vitamin D, and oxidative stress markers were comparable in both groups and were not significantly different as compared to the fertile females. The Spearman's correlation computed that Prolactin significantly and negatively correlated with Fetuin-A and TOS, while positive correlations were found between Fetuin-A, Alarin, and TOS ( $p<0.001$ ). **Conclusion:** Our findings suggest a potential link between oxidative stress and PCOS, highlighting its complexity.

**Keywords:** Alarin, Fetuin-A, Polycystic ovarian syndrome, PCOS, Total Oxidant Status, TOS

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## INTRODUCTION

Polycystic ovarian syndrome (PCOS) is marked by excessive androgen levels, bilateral cystic enlargement of the ovaries<sup>1</sup>, chronic anovulation, and infertility. Insulin resistance (IR) and hyperinsulinemia are the primary pathogenic factors behind PCOS<sup>2</sup>.

Fetuin-A, a 64 kDa glycoprotein secreted by the liver and adipose tissue, acts as a hepatokine and adipokine. It blocks insulin signalling and induces insulin resistance (IR) by auto-phosphorylating insulin receptors in muscle and liver. IR, often associated with PCOS, is linked to elevated Fetuin-A levels in PCOS cases, suggesting it is a potential marker for PCOS screening.<sup>2-4</sup> Adipose tissues secrete Fetuin-A, an adipokine with anti-inflammatory effects. Elevated Fetuin-A levels may reflect inflammation in unexplained infertility and contribute to its pathophysiology, suggesting its potential use as a biomarker for assessing infertility.<sup>3</sup>

Alarin, part of the 'galanin peptides' family, influences neuronal processing and female reproduction. Elevated Luteinizing Hormone (LH) levels in PCOS play a key role in its onset.<sup>1</sup> Research endeavours are presently directed towards examining the potential correlation among serum 'Alarin' and 'PCOS' in

infertile female cohorts, finding greater serum Alarin levels and a significant positive association with LH in the PCOS group afflicted with infertility. Moreover, infertile females with increased Alarin levels in serum exhibited a higher propensity for PCOS development.<sup>5</sup> Alarin injections in animal models increase LH secretion. Interestingly, PCOS individuals show lower Alarin levels compared to healthy controls.<sup>5</sup> Alarin may serve as a predictive marker for PCOS risk.<sup>6</sup>

Total oxidant status (TOS) reflects the body's overall oxidation state.<sup>7</sup> Oxidative stress results from an imbalance between oxidants and antioxidants, disrupting cellular redox. Oxidants include reactive oxygen species (ROS) and reactive nitrogen species (RNS).<sup>8</sup> ROS examples are hydrogen peroxide, hydroxyl radical, and superoxide, while RNS includes nitric oxide and its metabolites. ROS contributes to 95% of total oxidants.<sup>9,10</sup>

Multiple techniques measure individual oxidants, but assessing oxidative stress in disease requires more than measuring single oxidants. TOS measurement offers a comprehensive view of oxidative stress in disease pathophysiology.<sup>11,12</sup> Evaluating biomarkers like Fetuin-A, Alarin, and TOS is clinically essential. No studies have compared these biomarkers between infertile PCOS and non-PCOS women. We

hypothesize that oxidative status, as measured by TOS, Alarin, and Fetuin, is impaired in PCOS compared to non-PCOS infertile women. We aim to explore oxidative capacity in PCOS by estimating TOS, Alarin, and Fetuin levels.

## MATERIAL AND METHODS

This cross-section study was carried out from Feb 2021 to Feb 2023. The sample size was calculated using OpenEpi, with infertility prevalence as 18–22%<sup>13</sup>. The calculated sample size was 44 participants per group, but 45 were included in the PCOS group and 44 in the non-PCOS group.

The study was vetted and approved by The Aga Khan University Ethical Review Committee (ERC# 2022-4812-20408). Informed consent was obtained from all participants.

The infertile women (n=89), aged 15–49 years, were recruited from Aga Khan University Hospital and Australian Concept Infertility Medical Centre (ACIMC) for the current study. The control group included healthy infertile women with regular menstrual cycles and no signs or ultrasound evidence of PCOS. Participants were recruited by the gynaecologist (Co-PI) and ACIMC collaborator.

The control and cases were matched based on age and BMI. Participants had primary infertility, defined by WHO as failure to conceive for over two years, and BMI classified as normal (18.5–22.9 Kg/m<sup>2</sup>) or overweight/obese ( $\geq 23$  Kg/m<sup>2</sup>). PCOS diagnosis followed ASRM (American Society for Reproductive Medicine) criteria requiring 2 of 3 conditions: 1) oligo/ anovulation, 2) clinical/biochemical hyperandrogenism, and 3) ultrasound-confirmed polycystic ovaries with  $\geq 12$  follicles of 2–9 mm.<sup>14</sup> Women with secondary infertility, age >45, oral contraceptive use, major health conditions, congenital adrenal hyperplasia, or inherited disorders were excluded.

Ten milliliters of venous blood were collected during the proliferative phase, ideally on day two of the menstrual cycle. Sera were isolated at 4,000 rpm for 10 minutes and stored at -80°C. Serum total oxidant status, Alarin, and Fetuin-A levels were measured using commercial ELISA kits.

Statistical analysis was performed using SPSS-23. Descriptive statistics, paired *t*-tests, and the Mann-Whitney U-test compared PCOS and non-PCOS groups. Relationships between serum total oxidant status, Alarin, and Fetuin-A levels were assessed using Rapid Linear Progression and Pearson correlation tests, and *p*<0.05 was considered statistically significant.

## RESULTS

There were 89 infertile female subjects in the study, out of which 45 (51%) infertile females had PCOS and 44 (49%) were without PCOS.

Table-1 contains the analysis of the descriptive variables of the study. The mean age of infertile females with PCOS was not significantly different as compared to females without PCOS. However, BMI was significantly raised in women with PCOS (*p*=0.002). The mean serum values of Follicle-stimulating hormone (FSH) and Luteinizing hormone (LH) are significantly raised in women with PCOS (*p*<0.001). The Anti-Müllerian Hormone (AMH) of infertile women with PCOS was significantly decreased (*p*<0.001). Prolactin and oxidative stress markers were comparable in both groups and were not significantly different as compared to the fertile female subjects (*p*=0.001).

The Spearman’s rank correlation between study variables and oxidative stress markers is shown in Table-2. A significant negative correlation was found between prolactin and Fetuin-A (*r*= -0.223, *p*<0.001) and prolactin and total oxidant status (*r*= -0.223, *p*<0.001). A significant positive correlation was found between Fetuin-A and Alarin (*r*=0.434, *p*<0.001) and Fetuin-A and TOS (*r*=0.454, *p*<0.001). A positive correlation was observed between Alarin and TOS (*r*=0.423, *p*<0.001). There was no correlation among age, BMI, FSH, LH, and AMH with oxidative stress markers.

Figure-1 describes the correlation of oxidative stress markers Fetuin-A, Alarin, and TOS. These markers have a significant positive correlation among them (*p*<0.001). Prolactin has a significant negative correlation with all OS markers (*p*<0.005). All other variables such as age, BMI, FSH, and LH do not show any correlation with oxidative stress markers.

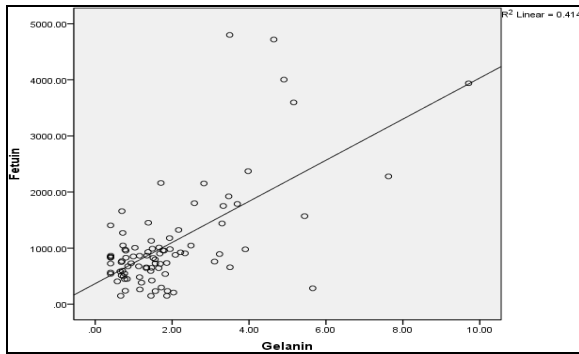
**Table-1: Demographic characteristics of females with PCOS and Non-PCOS**

	PCOS (n=45)	Non-PCOS (n=44)	<i>p</i>
Age (Years)	34.29±5.51	33.27±5.01	0.482
BMI (kg/m <sup>2</sup> )	29.43±4.8	26.03±4.05	0.002
FSH (IU/ml)	12.09±15.96	6.47±2.81	<0.001
LH (IU/L)	9.28±11.07	4.78±2.12	<0.001
AMH (ng/ml)	1.65±1.19	2.94±1.42	<0.001
Prolactin (µg/L)	15.62±10.39	12.59±6.71	0.208
Fetuin-A	944.33±654.34	1188.70±1121.42	0.765
Alarin	2.01±1.63	1.79±1.6	0.164
TOS	3.81±3.49	4.14±2.99	0.135

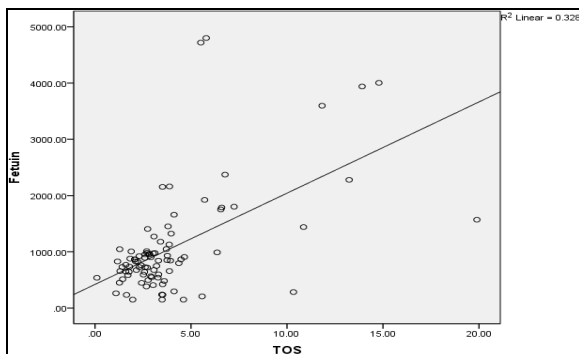
**Table-2: Correlation of study variables with oxidative stress markers**

	Fetuin-A	Alarin	TOS
Age	0.041	0.018	0.142
BMI	0.030	0.043	-0.142
FSH	0.066	0.059	-0.166
LH	-0.130	-0.066	-0.174
AMH	-0.015	0.027	0.046
Prolactin	-0.223*	-0.116	-0.223*
Fetuin-A	-	0.434**	0.454**
Alarin	0.434**	-	0.423**
TOS	0.454**	0.423**	-

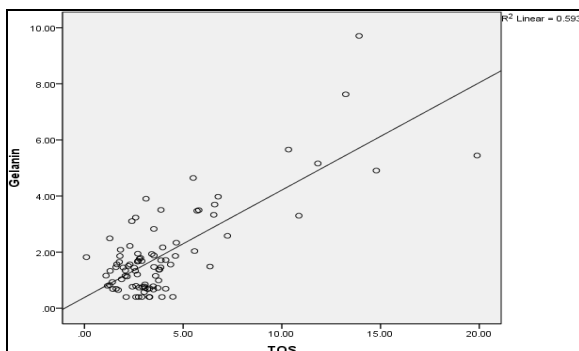
\**p*<0.05, \*\**p*<0.01



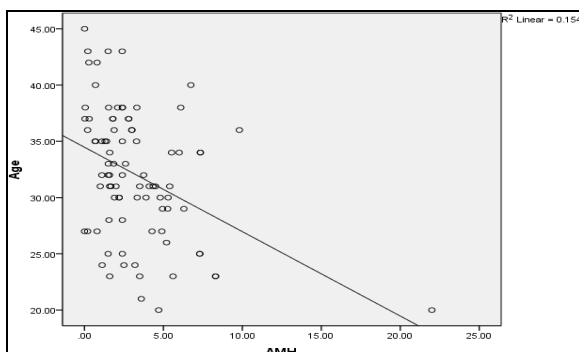
**Figure-1a: Correlation of Fetuin with Gelatin in infertile women**



**Figure-1b: Correlation of Fetuin with Total Oxidant Status in infertile women**



**Figure-1c: Correlation of Gelatin with Total Oxidant Status in infertile women**



**Figure-1d: Correlation of Age with Anti-Müllerian Hormone in infertile women**

## DISCUSSION

Polycystic ovarian syndrome is a conglomeration of metabolic disorders and hormonal disturbances resulting in female infertility.<sup>15</sup> PCOS is usually characterized by neuroendocrine dysfunction with deregulated gonadotropin secretion, indicating disrupted gonadotropin-releasing hormone (GnRH) secretion. It is assumed that Fetuin and Alarin have distinct causative roles for PCOS by regulating GnRH-secreting neurons.<sup>16</sup>

While providing demographic characteristics, the current research compared infertile women with PCOS to non-PCOS. The average age of infertile females with PCOS did not significantly differ from that of infertile females without PCOS; this indicates that age is not a distinguishing factor between the two groups according to current findings. Some studies explored that the mean age in PCOS was less than the controls. However, the research discovered that women with PCOS who are infertile exhibit a notably greater BMI compared to those without PCOS. This suggests that higher BMI may be associated with PCOS, which aligns with existing literature linking obesity to PCOS.<sup>16</sup> According to a meta-analysis, women classified as obese exhibited an odds ratio of 2.77 for developing PCOS in comparison to their non-obese counterparts. Another study supports the current finding and reported that the median BMI measurements exhibit markedly greater in 'PCOS' group versus controls.<sup>17</sup> However, in recent years, 20–50% of thin and lean women were diagnosed with PCOS.<sup>18,19</sup>

Our study found FSH levels to be notably increased in infertile PCOS females compared to non-PCOS counterparts. Elevated FSH levels in infertile PCOS women in current findings may reflect disturbances in ovarian function, such as anovulation. In contrast to present discoveries, the scientists mainly agreed on the point that PCOS patients have high LH and low FSH, thus raising the 'LH: FSH' ratio, linked to infertility.<sup>19</sup> Another study reported reduced FSH concentration in 'PCOS' patients versus control counterparts. The present investigation revealed that the serum LH concentrations are noticeably elevated in infertile females with 'PCOS' than in 'non-PCOS' counterparts. Heightened levels of LH serve as a distinctive characteristic of PCOS, correlating with hyperandrogenism and irregular menstrual patterns, thereby contributing to infertility.<sup>18</sup> LH release increases because of an abnormal feedback mechanism brought on by ovarian estrogen.<sup>18</sup> Corroborated with our findings, scientists reported that mean LH concentrations were noticeably elevated in infertile cases diagnosed with 'PCOS' than those of control counterparts.<sup>2</sup> Likewise, it is also noticed that in several subcategories of PCOS, serum LH and LH:FSH ratios

were observed to increase as the levels of other hormones declined.<sup>20</sup>

According to current findings, non-PCOS females have significantly greater concentrations of AMH compared to infertile females with PCOS. The greater AMH in the serum of non-PCOS females generally suggests a larger pool of developing follicles, which is usually considered advantageous for fertility. In contrast to the current findings, a study highlighted that median values of AMH were expressively dominant in PCOS cases.<sup>17</sup> In a study involving infertile women, the mean levels of AMH within the PCOS cohort exhibited notable elevation among lean individuals (BMI $\leq$ 25) compared to their overweight counterparts. In a retrospective analysis encompassing a substantial cohort of young infertile women, a little but notable decline was observed in AMH concentration in serum among overweight individuals diagnosed with PCOS.<sup>21,22</sup>

The lower levels of Fetuin-A in the PCOS group could be explained because Fetuin-A is a proinflammatory cytokine that produce low-grade inflammation along with C-reactive protein which is usually increased in PCOS and also suppresses Fetuin-A expression in the liver.<sup>2</sup> This would also explain the negative association between Fetuin-A concentrations and hepatic fat. ElSirgany *et al*, signifies higher Fetuin-A levels in PCOS than the control group establishing a correlation between Fetuin-A levels and other hormones measured in infertile women with PCOS.<sup>2</sup> A direct relation between BMI has been found with different features of PCOS signified by research which also delineates an association between Fetuin-A and insulin resistance in the PCOS population.<sup>23</sup> Our results are similar to study, where insignificant differences in Fetuin-A levels were found in both groups. Alarin was found to be a significant factor in GnRH secretion and for GnRH modulated LH secretion. That study showed higher Alarin and LH levels in women with PCOS group than controls compared with our results.<sup>5</sup> The increased prolactin level is due to the stimulating effect of estradiol assuming that hyperprolactinemia is due to increased oestrogen secretion in PCOS females.<sup>24</sup> It is suggested that insulin resistance in PCOS could also lead to increased prolactin levels. We have observed higher LH levels in PCOS patients who also had high serum levels of prolactin but it is the shortcoming of our study that serum oestrogen levels were not done however, increased serum oestrogen and prolactin levels are found in PCOS patients with increased LH in a study done by Zahra Davoudi *et al*, in 2021.<sup>24</sup>

Various researches demonstrate elevated TOS in the serum and follicular fluid of women with PCOS.<sup>25</sup> Interestingly, a study pointed to lower TOS levels after both oral glucose tolerance and mixed meal tests in PCOS patients.<sup>26</sup> Our study demonstrates a non-significant value for TOS measured in PCOS and normal

female group. But TOS shows a significant positive relation with Fetuin-A and Alarin. This demonstrates a combating/compensatory mechanism for increased low-grade inflammation associated with PCOS.

In summary, our study found decreased Fetuin-A levels, likely due to the presence of different isoforms and glycosylation defects. Alarin levels were higher in the PCOS group, consistent with previous studies. Prolactin was elevated in PCOS, likely due to increased oestrogen. TOS showed no significant difference, possibly influenced by confounding oxidants.

## CONCLUSION

The study demonstrates a pronounced oxidative imbalance in the PCOS group. Oxidative stress plays a more substantial role in the pathophysiology of PCOS.

## LIMITATIONS

Study limitations include small sample size, cross-sectional design, and lack of stratification of obese PCOS and control groups to assess the effects of obesity, PCOS, and insulin levels. Strengths include a homogeneous population and evaluation of two biomolecules with hormonal parameters on oxidative stress in PCOS. Larger studies comparing infertile and fertile women are needed for definitive conclusions.

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**AA:** Reviewed, edited, and supervised manuscript writing

**SR:** Did manuscript writing

**RA:** Did manuscript writing

**MA:** Did a manuscript statistical analysis

**HNK:** Did manuscript writing

**RR:** Conceived, designed, and supervised the manuscript

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