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The Complex Dynamics of Hyperandrogenism, Organokines and Total Antioxidant Stress in Polycystic Ovary Syndrome.

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KEYWORDS

Adiponectin, Fetuin-A, Hyperandrogenism, Interleukin-10, PCOS, Phenotype.

ABSTRACT:

Background: Polycystic Ovary Syndrome (PCOS) is a multifaceted endocrine, metabolic, and reproductive disorder with a complex etiology. It is widely recognized that hyperandrogenism is the key characteristic of PCOS, linked to various physiological dysfunctions. Elevated levels of androgens, such as Testosterone, may correlate with issues like cytokine hypersecretion, increased adipocyte proliferation, and dysregulation of signalling pathways. The roles of organokines and total antioxidant status (TAS) across different PCOS phenotypes remain unclear, and some contributions are still debated. This study aimed to explore the relationships between androgens, organokines, and TAS in various phenotypes of PCOS.

Methods: The research involved a cross-sectional study with 90 newly diagnosed PCOS patients, who were classified into four phenotypes according to the Rotterdam criteria, alongside a control group of 90 healthy women. Fasting blood samples were collected to analyze levels of Serum Testosterone, Serum Adiponectin, Serum Fetuin-A, Serum Interleukin (IL)-10, and Serum Total Antioxidant Status (TAS). To investigate potential associations, ANOVA tests and Spearman correlation analyses were performed using MedCalc 22.009 software.

Results: All 4 PCOS phenotype groups presented a significant difference (p-value < 0.05) in comparison with the control group. The diverse and appreciable correlation was observed between serum testosterone and other organokines as well as TAS across all four PCOS phenotypes; with the highest values observed in phenotype A.

Conclusion: A notable correlation was found between testosterone and various organokines as well as total antioxidant status (TAS), emphasizing its critical role in the etiopathology of PCOS. This connection has implications for diagnosis and could help guide effective treatment strategies, while also enabling better prognostication for patients facing infertility related to PCOS, tailored to their individual phenotypes.

Background:

Having a complex multifactorial origin, Polycystic Ovary Syndrome (PCOS) is a common endocrine, reproductive and metabolic condition, affecting approximately 8-13% of women of reproductive age.[1] PCOS presents with a spectrum of clinical symptoms, including irregular menstrual cycles, infertility, hirsutism, and metabolic disturbances such as obesity and insulin resistance.[2] Among the myriad features of PCOS, hyperandrogenism is widely regarded as the primary hallmark, contributing significantly to the disorder's reproductive and metabolic dysfunctions.[3]

Hyperandrogenism is the term used to describe elevated levels of androgens, specifically testosterone, which is associated with insulin resistance and plays a critical role in the pathogenesis of both reproductive and metabolic

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complications in PCOS.[4] Organokines are bioactive hormone-like molecules secreted by various organs that play crucial roles in metabolic regulation and inter-organ communication, organokines initially characterized as factors from adipose tissue, now encompass a range of signalling molecules produced by the liver, muscle, gut, and other tissues.[5] Key types of organokines are adipokines (e.g., Leptin, Adiponectin, Resistin), myokines (e.g., IL-10, IL-6, Irisin, Myostatin), and hepatokines (e.g., Fetuin-A, Ghrelin, FGF21).[6] Excess androgens promote cytokine hypersecretion, resulting in chronic low-grade inflammation, which further exacerbates insulin resistance, metabolic and ovarian dysfunction.[7] Additionally, elevated androgen levels stimulate adipocyte proliferation and alter fat distribution, contributing to obesity, which is thought to be a significant risk factor for the severity of PCOS symptoms.[8] Total antioxidant stress (TAS) is also crucial marker in the context of PCOS as oxidative stress has been implicated in the pathogenesis of numerous metabolic disorders, including PCOS through cellular damage and inflammation, further reinforcing the cycle of hyperandrogenism and insulin resistance.

While hyperandrogenism has been extensively studied, the contributions of organokines and TAS in different phenotypes of PCOS remain less understood. Understanding the interplay between TAS, organokines, and hyperandrogenism may provide new insights into the management of PCOS. This study aims to investigate the association between androgen levels, organokines, and TAS across different phenotypes of PCOS. By elucidating these relationships, we hope to provide deeper insights into the underlying mechanisms of PCOS, identify potential biomarkers for diagnosis and prognosis, and contribute to the development of more targeted therapeutic approaches. Ultimately, a better understanding of these interrelationships could improve clinical outcomes for women suffering from this complex syndrome.

Materials and Methods

This cross-sectional study was conducted on 180 adult females aged between 18-35 years, in the Department of Biochemistry, Kiran Medical College, Surat to evaluate the association between androgen levels, organokines, and TAS across different phenotypes of PCOS compared to healthy controls. The study included five groups: One control group with 90 healthy subjects and four PCOS phenotypes (A, B, C, and D) comprising 44, 11, 14, and 21 subjects, respectively; diagnosed as per the Rotterdam criteria[10], which requires the presence of combination of the following three features: irregular menstrual cycles or ovulatory disfunction (OD), clinical or biochemical signs of hyperandrogenism (HA), and polycystic ovaries (PO) on ultrasound.

Subjects with previous or current or use of medication like OC pills, ovulation induction agents, estrogenic or anti-androgenic Drugs, antidiabetic drugs (Metformin), glucocorticoids, anti-obesity drugs, history of hormone therapy, insulin sensitizers, Vitamin-D or calcium supplements in last 6 months, diagnosed cases of any chronic illness like cardiovascular disease, hyperprolactinemia, diabetes mellitus, thyroid disorders and recent history of Pregnancy/Lactation (6 months) were excluded from the study.

Clinical Assessments

After being informed about the purpose and procedure of the study, voluntary written informed consent and detailed medical history along with anthropometric data, including age, weight, height, waist and hip circumference were recorded. Fasting blood samples were collected in the morning after an overnight fast of 10-12 hours for biochemical analyses. Blood samples were processed to obtain serum for the quantification of Serum Testosterone, Serum adiponectin, Fetuin A, Interleukin-10 (IL-10), Luteinizing hormone (LH) and follicle-stimulating hormone (FSH) and Total Antioxidant Status (TAS) levels using enzyme-linked immunosorbent assay (ELISA) kits.

Statistical Analysis:

Body Mass Index (BMI), Waist-to-hip Ration (WHR), and LH:FSH Ratio were calculated. Continuous variables were denoted as mean with standard deviation (Mean $\pm$ SD). Difference between study groups were assessed using one-way ANOVA, with a p-value of < 0.05 deemed statistically significant. Correlations between parameters were evaluated using Pearson's correlation coefficient as r-value. Data were analyzed using MedCalc 22.009 software.

Result:

This cross-sectional study included 180 adult females, comprising 90 healthy controls and 90 subjects diagnosed with Polycystic Ovary Syndrome (PCOS) categorized into four phenotypes based on Rotterdam criteria. (Table 1)

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Table 1: Phenotypic distribution of PCOS subjects.

PCOS Phenotype	Clinical Features	PCOS Subjects (n=90)	Percentage
Phenotype A	Ovulatory dysfunction + Hyperandrogenism + Polycystic ovaries	44	48.9 %
Phenotype B	Ovulatory dysfunction + Hyperandrogenism	11	12.2 %
Phenotype C	Hyperandrogenism + Polycystic ovaries	14	15.6 %
Phenotype D	Ovulatory dysfunction + Polycystic ovaries	21	23.3 %

Table 2: Demographic and Anthropometric Data

Parameter	Control (n=90)	PCOS Phenotype A (n=44)	PCOS Phenotype B (n=11)	PCOS Phenotype C (n=14)	PCOS Phenotype D (n=21)	p-value
Age (years)	25.3 ± 4.5	26.1 ± 4.2	25.7 ± 4.0	24.8 ± 4.6	25.6 ± 4.3	**0.467**
Weight (kgs)	60.2 ± 8.3	70.1 ± 9.2	65.4 ± 7.8	68.0 ± 8.1	72.3 ± 10.0	**<0.01**
Height (cm)	162.5 ± 5.4	160.8 ± 6.1	161.2 ± 5.9	161.5 ± 5.6	161.7 ± 5.5	**0.841**
BMI (kg/m²)	22.9 ± 3.2	27.1 ± 3.6	25.2 ± 2.8	26.0 ± 3.0	27.7 ± 4.1	**<0.01**
Waist Circumference (cm)	70.5 ± 6.2	84.7 ± 8.1	78.5 ± 7.4	82.1 ± 8.0	85.0 ± 7.9	**<0.01**
Hip Circumference (cm)	95.2 ± 5.8	102.3 ± 6.4	98.5 ± 5.9	100.0 ± 6.0	103.1 ± 6.2	**<0.01**
Waist-Hip Ratio (WHR)	0.74 ± 0.05	0.83 ± 0.07	0.79 ± 0.04	0.82 ± 0.06	0.82 ± 0.07	**<0.01**

The demographic and anthropometric data indicate significant differences in weight, waist and hip circumference, BMI, and waist-hip ratio between all four phenotypes of PCOS and healthy controls. This analysis highlights the association of PCOS with obesity and altered body composition, which can have implications for the management and treatment of the condition. Further research may explore the underlying mechanisms linking these anthropometric features with hormonal and metabolic profiles in PCOS. (Table 2)

Table 3: Biochemical Parameters

Parameter	Control (n=90)	PCOS Phenotype A (n=44)	PCOS Phenotype B (n=11)	PCOS Phenotype C (n=14)	PCOS Phenotype D (n=21)	p-value
Serum Testosterone (ng/mL)	24.97 ± 2.37	83.63 ± 2.01	79.79 ± 2.02	76.58 ±1.99	24.89 ± 3.20	**<0.01**
Serum Adiponectin (µg/mL)	18.85 ± 2.89	2.06 ± 1.25	2.19 ± 0.97	3.64 ± 2.22	3.91 ± 1.23	**<0.01**
Fetuin-A (ng/mL)	65.09 ± 7.65	229.75 ± 17.01	152.70 ± 8.59	194.18 ± 22.56	101.64 ± 13.86	**<0.01**
IL-10 (pg/mL)	16.94 ± 0.56	2.37 ± 0.76	2.42 ± 1.27	3.45 ± 1.88	3.90 ± 1.27	**<0.01**
LH (mIU/mL)	7.93 ± 0.57	21.50 ± 3.00	16.85 ± 2.39	12.05 ± 2.01	14.58 ± 0.89	**<0.01**

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FSH (mIU/mL)	9.40 ± 1.06	7.24 ± 1.57	5.65 ± 0.94	7.04 ± 1.19	6.92 ± 1.80	**<0.01**
LH:FSH Ratio	1.23 ± 0.19	2.75 ± 0.80	2.53 ± 0.08	1.71 ± 0.01	2.13 ± 0.52	**<0.01**
TAS (µmol/L)	779.01 ± 53.34	242.11 ± 15.95	481.18 ± 24.50	389.14 ± 31.81	491.57 ± 30.48	**<0.01**

The mean testosterone levels were significantly higher and among the phenotypes, Phenotype A showed the highest levels, while Phenotype D had the lowest. Serum Adiponectin levels were significantly lower in all PCOS groups compared to controls ($p < 0.01$). Fetuin A levels were elevated in the PCOS groups, particularly in Phenotype A. IL-10 levels were significantly lower in all PCOS groups compared to controls ($p < 0.01$). LH and FSH levels were significantly elevated and reduced respectively in all PCOS groups ($p < 0.01$) with Phenotype A showing the highest levels and phenotype C being lowest levels of LH:FSH ratio. TAS levels were significantly lower in all PCOS phenotypes compared to the control group ($p < 0.01$). The lowest TAS was observed in Phenotype A. (Table 3)

Table 4: Correlation Analysis with Testosterone Levels

Correlation with Testosterone Levels (r value)					
Parameter	**Control (n=90)**	**PCOS Phenotype A (n=44)**	**PCOS Phenotype B (n=11)**	**PCOS Phenotype C (n=14)**	**PCOS Phenotype D (n=21)**
Serum Adiponectin (µg/mL)	-0.0465	-0.9511	-0.8044	-0.9688	-0.8784
Fetuin-A (g/mL)	0.0416	0.9081	0.9551	0.9554	0.8441
IL-10 (pg/mL)	-0.0449	-0.9034	-0.8357	-0.8939	-0.8743
LH (mIU/mL)	0.1125	0.9201	0.9164	0.9184	0.8816
FSH (mIU/mL)	0.1276	-0.9079	-0.9031	-0.9209	-0.8536
LH:FSH Ratio	0.1183	0.9302	0.9176	0.7733	0.7425
TAS (µmol/L)	-0.0801	-0.9694	-0.8980	-0.8292	-0.7255

The data reflect correlations with testosterone levels across different PCOS phenotypes compared to controls. Serum adiponectin and IL-10 levels are negatively correlated with testosterone, indicating that lower adiponectin and IL-10 may be associated with higher testosterone levels in PCOS. Conversely, fetuin-A shows a positive correlation, suggesting that elevated fetuin-A levels might align with increased testosterone. Hormonal analysis reveals higher LH and lower FSH levels in PCOS, which correlates with elevated testosterone, further supporting the hormonal imbalance characteristic of the condition. The LH:FSH ratio also correlates positively with testosterone, reinforcing the association between this hormonal profile and elevated testosterone levels. Lastly, lower total antioxidant status (TAS) across phenotypes may suggest that oxidative stress could contribute to the relationship between testosterone and metabolic dysfunction in PCOS. Overall, these correlations highlight the interconnectedness of hormonal and metabolic factors in PCOS.

Discussion

In the present study, phenotype A also known as full-blown PCOS, found to be the unique phenotype having prevalence of 48.9 % followed by phenotype D, C, B, respectively 23.3 %, 15.6 % and 12.2 %. The study conducted by Gluszak et al.[11] had shown different prevalence of 60.2%, 16.1%, 18.3%, and 5.4%, for phenotype A, B, C, and D respectively, which was

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comparable to study conducted by Pehlivanov et al.[12] While phenotype D was found as the most prevalent phenotype by Elasam et al. in Sudanese women with 51.6%, and other phenotypes A, B and C with values 7.6%, 22.6% and 18.2%, respectively.[13]

The hyperandrogenism, characterized by elevated androgen levels, being a defining feature of PCOS, plays a pivotal role in the clinical manifestations of PCOS, including insulin resistance, increased adiposity, and reproductive dysfunction[2-3]. In present study, a significant difference was found in total testosterone levels among the four studied phenotypes. However, in phenotype D, total testosterone concentration (24.89 $\pm$ 3.20 ng/ml) was comparable to those of the control group (24.97 $\pm$ 2.37 ng/ml) but lower when compared to other phenotypes A (83.63 $\pm$ 2.01 ng/ml), B (79.79 $\pm$ 2.02 ng/ml) and C (76.58 $\pm$1.99 ng/ml). Jamil et al. also reported high levels of total testosterone in phenotype A, B and C compared to phenotype D.[14] Yilmaz et al. also found the same with the levels of testosterone in phenotype D group being more similar to control group than the other PCOS phenotypes[15]. These results indicate that individuals with PCOS exhibit variability regarding androgen levels in different phenotypes. Elevated levels of testosterone can exacerbate oxidative stress, leading to a dysregulated antioxidant system.[16] Recent studies have demonstrated that hyperandrogenism not only impacts metabolic pathways but also influences the secretion and function of organokines that play critical roles in inflammation and metabolism.[17] Organokines such as adiponectin and fetuin A are crucial mediators in the pathophysiology of PCOS.

Adiponectin, an adipose tissue-derived adipokine acting as hormone, is known for its insulin-sensitizing and anti-inflammatory properties.[18] In this study lower levels of adiponectin were seen in all four groups compared to control (p value < 0.01), with phenotype A having the lowest values and showed a negative correlation with serum total testosterone concentrations (r = -0.9511) and similar for phenotype D which showed negative correlation (r =-0.8784) despite of having comparatively low testosterone levels; this indicates that adiponectin and total testosterone levels are in inverse correlation of one another. Fux et al. also found that PCOS patients with phenotype A had lower values of adiponectin compared to the rest of the groups and reached statistical significance when compared to groups C and control (P < 0.05). Serum adiponectin levels showed negative correlation with total testosterone level (r = −0.40). The linear regression analysis showed that the levels of total testosterone ($\beta = -0.383$, $P < 0.0001$), contributed to the decrease of serum adiponectin levels.[19] These results support the hypothesis that Ramanand et al, stated in their study that in women with PCOS, hyperandrogenism might indirectly favour insulin resistance, by inducing abdominal adiposity and possibly decreasing adiponectin.[20] Similarly Mirza et al. found that increased testosterone levels have been associated with reduced adiponectin secretion, highlighting a potential feedback loop that exacerbates metabolic syndrome.[21] However, when adiponectin is administered in conjunction with a low-calorie diet to overweight and obese PCOS women, the antiandrogen flutamide decreases visceral fat and reduces total and LDL cholesterol and total testosterone levels[22].

Fetuin A is a hepatokine, implicated in the regulation of insulin sensitivity and inflammation. In present study, all four groups exhibited higher levels of fetuin A compared to the control group ($p < 0.01$), with phenotype A showing the highest values and phenotype C having a very strong positive correlation with serum total testosterone concentrations ($r = 0.9554$). The higher levels of Fetuin A suggest a possible role in the inflammatory and metabolic pathways associated with PCOS, particularly in Phenotypes A and C. These data were similar to the results of Pal et al. stating that circulating Fetuin-A levels were associated with hyperandrogenism and insulin resistance by the suppression of autophosphorylation and tyrosine kinase activity of insulin receptors in hepatic and muscular tissues. [23] and similarly Liu et al. stated that elevated fetuin A levels in women with PCOS may contribute to the development of insulin resistance and could serve as a marker for metabolic disturbances associated with hyperandrogenism.[24]

Interleukin-10 (IL-10), an anti-inflammatory myokine playing protective role in metabolic homeostasis. In this study, phenotype A showed lowest IL-10 levels, followed by B, C and D phenotype and they were significantly lower compared to controls ($p < 0.01$) showing strong positive correlation with total testosterone levels in all 4 phenotypes (r-value between 0.8357 to 0.9034) Abraham et al. stated that the pathogenesis of PCOS is associated with raised pro-inflammatory cytokines and reduced anti-inflammatory (IL-10) factors, which harms insulin sensitivity and advances the onset of PCOS.[25] The hypothalamus-pituitary-ovarian gonadal axis (HPO axis) is influenced by hyperinsulinemia, which causes the

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pituitary to respond to gonadotropin-releasing hormone (GnRH) by increasing the secretion of testosterone and luteinizing hormone and this ultimately results in follicle development inhibition, follicular atresia, and insulin resistance as a feedback mechanism.[26]

Total antioxidant stress (TAS) is a critical measure of the body's ability to counteract oxidative stress. The significant reduction in TAS levels across all PCOS phenotypes ($p < 0.01$) highlights the oxidative stress often experienced by these patients, possibly contributing to the pathophysiology of the syndrome. This study indicates a strong negative correlation (r value between -0.7255 to -0.9694) of TAS with testosterone concentrations, suggesting that hyperandrogenism may compromise antioxidant defences in all four phenotypes of PCOS. Similarly, Sun Y et al. found that women with PCOS often exhibit elevated oxidative stress levels, which may be led to hyperandrogenism through reducing sex hormone binding globulin levels.[27] Fenkci et al. indicated that lower TAS levels negatively correlate with insulin resistance and not only affects metabolic health but may also impair ovarian function, thereby exacerbating reproductive challenges in PCOS. [28]

According to Malini et al. the most common clinical symptom of women diagnosed with PCOS, is a greater LH-FSH ratio. [29] Present study also showed significant difference in LH:FSH ratio between control and all phenotypes ($p < 0.01$); Phenotype A having highest values followed by B, D and C. This study got positive correlation between Total testosterone levels and LH:FSH ratio among all 4 groups indicating that endocrinal dysregulation are interrelated. Nisenblat et al. stated that the LH-FSH ratio for healthy women should be 1:1 but in PCOS, there is higher level of LH than the FSH level, resulting in increased ovarian androgen production and ovulatory failure results in the growth of ovarian theca cells, which raises steroidogenesis and eventually causes hyperandrogenism in PCOS subjects.[30]

The intricate relationship between hyperandrogenism, organokines, and total antioxidant stress in PCOS emphasizes the multifaceted nature of this prevalent endocrine disorder, revealing how these interconnected factors contribute not only to the clinical manifestations such as irregular menstrual cycles and infertility, but also to the long-term health risks, including metabolic syndrome and cardiovascular diseases, thereby highlighting the need for comprehensive management strategies that address both the hormonal imbalances and the oxidative stress that often accompanies this condition.

Conclusion

The cross-sectional study of PCOS subjects showed unique prevalence of phenotype A. A significant link was discovered between testosterone levels and different organokines, along with TAS, highlighting its important role in the development of PCOS based on different phenotypes. Overall, these findings underscore the complex interplay between hormonal dysregulation, metabolic dysfunction, and oxidative stress in PCOS, suggesting that targeted therapeutic strategies addressing these areas may improve clinical outcomes. Future research should aim to explore longitudinal effects based on these biomarkers for diagnostic implications and intervention on larger scale could significantly assist in shaping effective treatment approaches to improve outcomes for women with PCOS based on phenotypic classification.

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