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Uncovering ovarian reserve: Insights into hormones, lipids, and essential metals

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ABSTRACT

Background: Polycystic Ovary Syndrome (PCOS) is an endocrine disorder prevalent in women of reproductive age group. The intricate nature of PCOS development is highlighted by fluctuations in various sex hormones, such as luteinizing hormone(LH), follicle-stimulating hormone and prolactin.

Objective: This study aims to assess the lipid profile levels, vital metals and sex hormones in the serum of patients with PCOS and contrast these findings with normal controls.

Materials and Methods: The study analyzed health metrics between 57 women with PCOS and 57 normal controls. The lipid profile was assessed using Beckman Coulter AU480. Sex hormones were estimated using Roche Diagnostics Cobas 6000. Inductively coupled plasma mass spectrometry (ICPMS) was used to measure the essential trace metals, including magnesium (Mg), copper (Cu) Selenium (Se) and zinc (Zn).

Results: The PCOS patients exhibited significantly elevated levels of LH (9.52 vs 3.48, p<0.0001), FSH (9.91 vs. 6.12, p<0.0001) and prolactin (24.71 vs. 16.41, p=0.03) mIU/ml. The correlation study showed a significant positive correlation between AMH and Se levels (R=0.27, p=0.045) and between LH and HDL (R=0.76, p=0.030).

Conclusion: In patients with PCOS, LH, FSH, and prolactin levels were found to be increased. A correlation was observed between Se and AMH, and a relationship was noted between LH and HDL. Selenium appears to have a significant influence on the ovarian reserve.

1. Introduction

Polycystic Ovary Syndrome (PCOS) is increasingly recognized as a prevalent endocrine disorder among women in their reproductive years.[1] The intricate pathogenesis of PCOS is believed to be influenced by variations in several hormone levels, such as Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH), and prolactin. Anti-Müllerian hormone (AMH), a glycoprotein part of the transforming growth factor-beta (TGF-β) family, has been spotlighted.[2] This hormone plays a crucial role in controlling the development and function of ovarian follicles. The levels of AMH in the serum indicate the quantity and quality of the ovarian reserve, thus serving as a valuable biomarker for evaluating a woman's reproductive capacity.[3] Conversely, diminished serum AMH levels could suggest a decreased ovarian reserve. In some instances, increased levels of Anti-Müllerian Hormone in the follicular fluid (FF AMH) have been associated with positive results in In Vitro Fertilization (IVF) cycles.[4] However, this correlation has not been confirmed in all studies.[4–6] It is crucial to mention that AMH levels are not only significant in assessing reproductive potential but also in understanding various gynecological conditions. For example, PCOS is frequently linked with elevated AMH levels and irregular folliculogenesis.

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The functionality of the ovaries and fertility can also be impacted by the levels of crucial trace elements such as copper (Cu), magnesium (Mg), zinc (Zn), and Selenium (Se).[7–9] These elements participate in numerous biological functions, including antioxidant protection, enzymatic activities, hormone production and metabolism, as well as inflammatory responses. Certain elements might interact with AMH, potentially influencing its expression and activity within the ovary.

An imbalance in these elements could potentially lead to oxidative stress and inflammation, often seen in PCOS.[10] Moreover, the deficiency of vital trace elements such as Zn and Cu, as reported in Indian soils,[11] could potentially affect the nutritional status of the population. It is hypothesized that changes in AMH levels are linked to ovarian function, fertility, and the severity of PCOS. Additionally, it is proposed that a correlation exists between AMH and essential trace elements in determining ovarian reserve in women experiencing infertility.

This study aimed to see the relationship between sex hormones (AMH, LH, FSH and prolactin), lipid markers and essential trace elements in women with PCOS.

2. Materials and Methods

2.1. Chemicals and reagents

To analyze micronutrients, nitric acid (60 – 70%) for trace metal analysis (PS-500ML), methanol for trace metal analysis (PS-2.5L), and triton X (PS-500ML) were obtained from Thermo Scientific. Standards of magnesium (CGMG1-125ML), copper (CGCU1-125ML), zinc (CGZN1-125ML), and selenium (CGSE1-125ML) were procured from Inorganic ventures. Commercially available close system pack kits were procured for the analysis of AMH, LH, FSH, and prolactin were procured from Roche Diagnostics.

2.2. Study setting and participants

This study is observational research conducted at a tertiary care hospital in North India. The sample size was determined by a prior study that noted a mean difference of 1 ng/ml in AMH levels between women with PCOS and controls, with a SD ±2 ng/mL. With an assumed power of 80% and an alpha level of 0.05,[12] it was estimated that each group should consist of 54 subjects.

2.3. Sample size calculation

The sample size was calculated using the sample size calculator (http://statulator.com/SampleSize/ss2M.html). The statulator used the input values of a power of 80%, a one-sided level of significance of 5%, and a ratio between the reference and test group sizes of 1.1 for sample size calculation and adjusted the sample size for t-distribution. Assuming a pooled standard deviation of 2 units, the study would require a sample size was 48 in each group, after adding 10% of dropout the sample size was 53 in each group.[13]

2.4. Subject selection criteria

The study encompassed a total of 114 women with suspected gynecological issues and who sought medical advice at the Obstetrics and Gynecology outpatient department of the Ram Prakash Gupta Mother and Child State Referral Hospital in Lucknow. Clinical hyperandrogenism was evaluated based on symptoms such as hirsutism, acne, or alopecia. Biochemical hyperandrogenism was determined by assessing serum testosterone levels, the free androgen index, or levels of sex hormone-binding globulin.

The Rotterdam criteria for PCOS state that two out of the following three criteria must be present: 1) oligo- or anovulation (OA) is a necessary condition for diagnosis; plus another one of the following two criteria: 2) clinical or biochemical hyperandrogenemia; or 3) PCOM (presence of 12 follicles measuring 2–9 mm in diameter in each ovary and/or increased ovarian volume [>10 mL]) under ultrasound. These women (n=57) were clinically diagnosed with PCOS.

The exclusion criteria were: 1) endocrine diseases other than PCOS that cause ovulation disorders, such as hyperprolactinemia, congenital dysfunction of the adrenal cortex, thyroid disorders, Cushing syndrome and disease, and tumors of the pelvic organs; 2) total testosterone (TT) <0.1 ng/mL or AMH ≥23.5 ng/mL; 3) pregnancy; 4) over 30 years old; 5) a history of using drugs such as insulin sensitizers and oral contraceptives within 3 months.

2.5. Collection of blood sample

Patients were invited for blood sample collection on the second day of their menstrual cycle. 03ml of venous blood was drawn into a plain vial from women with PCOS and control group. Blood samples were centrifuged at 3500 rpm for 15 min. and to separate the serum. The serum samples were subsequently used to assess various parameters, including the lipid profile, Hormones and essential trace metals.

2.5.1. Estimation of lipid markers

The lipid profile, which includes high-density lipoprotein (HDL), low-density lipoprotein (LDL), very low-density lipoprotein (VLDL), and total cholesterol (TC), was determined using a commercially available kit. This analysis was conducted on a Beckman Coulter (AU480) instrument, following the standard protocol provided with the commercially available close system kit.

2.5.2. Estimation of essential metals (Mg, Zn, Cu, and Se)

The levels of essential metals (Mg, Zn, Cu, and Se) were determined using Inductively Coupled Plasma Mass Spectrometry (ICPMS) from Perkin Elmer. The process involved the combination of 100 μL of serum with an equal volume of an internal standard (IS). This mixture was then diluted to a volume of 5 ml. A diluent composed of 15 mL methanol, 0.005% Triton X solution, and 10 ml nitric acid was added to the mixture and then further diluted to a total volume of 1000 ml with double-distilled water. The metal concentrations were then analyzed and compared with a standard using a linearity curve.

2.5.3. Estimation of (LH, FSH, AMH, and prolactin) sex hormones

The hormonal parameters, including Luteinising Hormone (LH), Follicle Stimulating Hormone (FSH), Anti-Mullerian Hormone (AMH), and Prolactin, were assessed using an immunochemiluminence method. This analysis was performed on a Roche Diagnostics instrument (Cobas-6000), following the protocol provided by the manufacturer.

2.6. Statistical analysis

The data was recorded in mean and standard deviations. The two groups were compared using a student t-test. The Pearson correlation coefficient was used to see the relationship between the two variables. The p-value <0.05 was taken as statistically significant. The data was analyzed using SPSS 21 software (Chicago, IL, USA).

3. Results

As evident from Table 1, variables TC, TG, LDL, and VLDL, as well as magnesium, copper, zinc, and selenium levels were significantly elevated (p<0.05). However, HDL(p=0.002) and zinc(p=0.029) levels were significantly lower in women with PCOS.

There was a significant difference in the serum levels of hormones such as LH, FSH, and prolactin between the PCOS cases and the control group. The with PCOS exhibited elevated levels of LH (9.52±7.19 vs 3.48±1.93,p<0.0001), FSH (9.91±3.66 vs 6.12±2.56,p<0.0001) mIU/ml and prolactin (24.71±19.87 vs 16.41±7.98 ng/mL, p=0.033). Furthermore, the difference in AMH levels was statistically significant, with higher levels observed in the PCOS group (5.69±3.43 ng/ml) compared to the control group (2.29±1.96 ng/ml, p<0.0001) (Table 2).

The correlation analysis demonstrated a significant positive relationship between LH and HDL cholesterol (r=0.756, p=0.030). However, no significant relationships were observed between AMH, FSH, prolactin, and any of the lipid profile parameters (TC, TG, HDL, LDL, VLDL), nor between LH and the other lipid parameters (TC, TG, LDL, VLDL) (p<0.05) (Table 3). There was no correlation was observed in non-PCOS women (Table 4).

Furthermore, the analysis revealed significant correlation between AMH (ng/ml) and Selenium (Se) levels (r=0.271, p=0.045). However, no significant correlations were observed between AMH and the levels of Magnesium (Mg), Copper (Cu), and Zinc (Zn). Similarly, LH, FSH, and Prolactin showed no significant correlations with any trace elements. All other variables showed no correlations (p< 0.05) (Table 5). There were no correlation was found in non-PCOS women (Table 6).

4. Discussion

The present study delved into the lipid profile, hormone levels, and essential metals in patients with Polycystic Ovary Syndrome (PCOS) and a control group. The findings revealed no significant differences in the lipid profile and essential metal levels between the two groups, aligning with previous research suggesting these parameters may not play a crucial role in PCOS pathogenesis. However, the slight trend towards significance in zinc levels calls for further exploration.

The lack of significant differences in lipid profile and essential metal levels between PCOS patients and controls aligns with a study conducted on a large cohort of Chinese women with PCOS, which reported a high prevalence of dyslipidemia among PCOS patients.[14] Another study also suggested that a substantial proportion of women with PCOS may experience elevated cholesterol and/or triglyceride levels.[15] The observed trend towards significance in zinc levels is consistent with the understanding that zinc plays a vital role in various physiological processes. However, more extensive studies are needed to establish a definitive link between zinc levels and PCOS.

Significant differences were observed in the levels of Luteinizing Hormone (LH), Follicle Stimulating Hormone (FSH), and prolactin, with higher levels in the PCOS group. This aligns with previous research indicating that these hormones are often elevated in PCOS patients.[16–18] The correlation analysis revealed a significant positive correlation between LH and HDL, suggesting a potential link between these parameters in PCOS patients. Anti-Müllerian Hormone (AMH) and Selenium levels showed a significant positive correlation. This novel finding suggests that selenium could potentially regulate AMH levels, which might influence our current understanding of PCOS pathophysiology. However, further studies are needed to confirm this relationship and elucidate the underlying mechanisms.

The significant positive correlation between AMH and Selenium levels found in our study is another novel finding. Although no previous studies have reported this specific correlation, many studies have shown that higher

Table 1: Demographic and biochemical variables of the study population

Variables	PCOS (n=57)	Control (n=57)	p-value
Age (Years)	26.25±4.78	27.75±5.91	0.139
Total cholesterol (mg/dL)	196.31±39.12	159.28±33.87	<0.0001*
Triglyceride (mg/dL)	206.11±74.51	104.90±56.34	<0.0001*
HDL (mg/dL)	40.14±8.34	45.02±8.14	0.002*
LDL (mg/dL)	121.12±28.57	96.88±16.11	<0.0001*
VLDL (mg/dL)	41.2±14.14	20.98±11.20	<0.0001*
Mg (mg/L)	3.37±1.32	3.14±0.67	0.243
Cu (μg/L)	140.34±58.59	133.07±40.52	0.442
Zn (μg/L)	54.24±39.90	69.57±33.98	0.029*
Se (μg/L)	14.30±5.65	13.87±4.76	0.661

Abbreviations: HDL: High-density lipoprotein, LDL: Low-density lipoprotein, VLDL: Very low-density lipoprotein, Mg: Magnesium, Cu: Copper, Zn: Zinc, Se: Selenium. The student t-test was used to calculate p-value. *p-value <0.05 was considered as statistically significant.

Table 2: Comparison of hormonal profiling in PCOS women and normal controls

Variables	PCOS (n=57)	Control (n=57)	p-value
AMH (ng/mL)	5.69±3.43	2.29±2.96	<0.0001*
LH (mIU/mL)	9.52±7.19	3.48±1.93	<0.0001*
FSH (mIU/mL)	9.91±3.66	6.12±2.56	<0.0001*
Prolactin (ng/mL)	24.71±19.87	16.41±7.98	0.033*

Abbreviations: AMH: Anti-Mullerian Hormone, LH: Luteinising Hormone, FSH: Follicle Stimulating Hormone. The student t-test was used to calculate the p-value. *p-value <0.05 was considered as statistically significant

Table 3: Correlation of hormonal profile with biochemical variables in the PCOS group

Variables	TC (mg/dL)	TG (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	VLDL (mg/dL)
AMH (ng/mL)	r=-0.026	r=0.011	r=0.046	r=-0.044	r=0.059
	p=0.894	p=0.954	p=0.812	p=0.820	p=0.761
LH (mIU/mL)	r=0.034	r=-0.161	r=0.756	r=-0.214	r=-0.153
	p=0.937	p=0.703	p=0.030*	p=0.610	p=0.717
FSH (mIU/mL)	r=0.064	r=-0.575	r=0.600	r=0.083	r=-0.574
	p=0.881	p=0.136	p=0.116	p=0.844	p=0.137
Prolactin (ng/mL)	r=-0.162	r=-0.192	r=0.263	r=-0.206	r=-0.203
	p=0.729	p=0.680	p=0.569	p=0.658	p=0.663

Abbreviations: r: Pearson correlation, TC: Total cholesterol, TG: Triglyceride, HDL: High-density lipoprotein, LDL: Low-density lipoprotein, VLDL: Very low-density lipoprotein, AMH: Anti-mullerian Hormone, LH: Luteinising Hormone, FSH: Follicle Stimulating Hormone. Pearson correlation coefficient was used to calculate the correlation between two variables. *p-value <0.05 was considered as statistically significant.

Table 4: Correlation of hormonal profile with biochemical variables in non-PCOS group

Variables	TC (mg/dL)	TG (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	VLDL (mg/dL)
AMH (ng/mL)	r=-0.001	r=0.098	r=-0.098	r=0.198	r=0.123
	p=0.234	p=0.120	p=0.234	p=0.978	p=0.190
LH (mIU/mL)	r=0.078	r=0.109	r=0.109	r=0.172	r=0.345
	p=0.987	p=0.095	p=0.091	p=0.071	p=0.081
FSH (mIU/mL)	r=0.098	r=-0.168	r=0.198	r=0.182	r=0.091
	p=0.123	p=0.032	p=0.198	p=0.074	p=0.234
Prolactin (ng/mL)	r=0.122	r=0.091	r=0.012	r=0.012	r=0.018
	p=0.076	p=0.178	p=0.970	p=0.891	p=0.658

Table 5: Correlation of hormonal profile with essential metals in PCOS women

Variables	Mg (mg/L)	Cu (μg/L)	Zn (μg/L)	Se (μg/L)
AMH (ng/mL)	r=0.051	r=-0.016	r=0.230	r=0.271
	p=0.713	p=0.909	p=0.090	**p=0.045***
LH (mIU/mL)	r=-0.056	r=-0.133	r=0.099	r=-0.079
	p=0.814	p=0.577	p=0.679	p=0.741
FSH (mIU/mL)	r=0.199 p=0.400	r=-0.052 p=0.829	r=-0.026 p=0.913	r=-0.018 p=0.938
Prolactin (ng/mL)	r=0.009	r=-0.177	r=-0.104	r=0.043
	p=0.973	p=0.496	p=0.693	p=0.871

Abbreviations: r: Pearson correlation, AMH: Anti-Mullerian Hormone, LH: Luteinising Hormone, FSH: Follicle Stimulating Hormone, Mg: Magnesium, Cu: Copper, Zn: Zinc, Se: Selenium. Pearson correlation coefficient was used to calculate the correlation between two variables. *p-value <0.05 was considered as statistically significant.

Table 6: Correlation of hormonal profile with essential metals in non-PCOS women

Variables	Mg (mg/L)	Cu (μg/L)	Zn (μg/L)	Se (μg/L)
AMH (ng/mL)	r=-0.098	r=0.006	r=0.193	r=0.075
	p=0.876	p=0.268	p=0.366	p=0.916
LH (mIU/mL)	r=-0.129	r=0.087	r=0.098	r=0.135
	p=0.568	p=0.178	p=0.186	p=0.754
FSH (mIU/mL)	r=0.109	r=0.109	r=0.081	r=0.166
	p=0.159	p=0.789	p=0.852	p=0.170
Prolactin (ng/mL)	r=0.123	r=0.012	r=0.174	r=0.158
	p=0.976	p=0.977	p=0.165	p=0.076

hormone levels, gut microbiome composition, and plasma metabolomics are new parameters related to the PCOS phenotypes.[19–22] This suggests that the interplay between hormones and essential metals could be an important area for future research in PCOS.

While this study provides valuable insights into the complex interplay of hormones, lipid profiles, and essential metals in Polycystic Ovary Syndrome (PCOS), it is not without limitations. The study was conducted in a single tertiary care center in North India, which may limit the generalizability of the findings to other populations. The sample size was relatively small, which could affect the statistical power to detect significant differences or correlations. Furthermore, the study was cross-sectional, which prevents establishing causality between the observed variables. Lastly, potential confounding factors such as lifestyle factors, dietary habits, and genetic predispositions were not controlled for in this study. Future research should address these limitations to provide a more comprehensive understanding of PCOS.

5. Conclusion

This study highlights the potential intricate interactions among hormones, lipid profiles, and essential metals in Polycystic Ovary Syndrome (PCOS). While the lipid profile and essential metal levels did not significantly differ between the PCOS and control groups, notable differences were observed in the Luteinizing Hormone (LH), Follicle Stimulating Hormone (FSH), and prolactin levels. Moreover, a significant positive correlation was identified between Anti-Müllerian Hormone (AMH) and Selenium levels. These findings underscore the complex hormonal dynamics in PCOS and highlight the potential role of AMH and Selenium in this condition. Further research is needed to fully understand these relationships and their implications for the pathogenesis of PCOS.

6. Source of Funding

None.

7. Conflict of Interest

None.

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