The endocrine disrupting potential of Aluminium and other heavy metals in Poly cystic ovary syndrome (PCOS): Case control study

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Abstract

Toxic metals may play a crucial role in the etiology of polycystic ovary syndrome (PCOS). These are omnipresent in the environment and linked to oxidative stress. n=110 subjects were assigned to case and control groups. Blood samples were analyzed to determine hormone levels, aromatase activity and heavy metals such as Aluminum(Al), Cadmium(Cd), Lead(Pb) and Nickel(Ni). Additionally, oxidative stress markers, malondialdehyde(MDA) and paraoxonase-1(PON-1) were measured. The quantification of heavy metals was carried out using Inductively coupled plasma mass spectrometry(ICPMS/MS). Other parameters were assessed through standard techniques. Statistical analyses including Student's t-test, correlation r, Chi-square tests and Multiple regression models, were executed using SPSS version 19 software.

MDA levels were markedly higher in PCOS patients, measuring 1.53 ± 0.71 µM/L. In the control group, it was 0.88 ± 0.20 µM/L. A significant inverse relationship was identified between MDA and aromatase, whereas a positive correlation was noted with testosterone and lead. Additionally, Al levels were raised in PCOS patients, averaging 74.72 ± 26 ppb, compared to 62.34 ± 20.11 ppb in controls. No significant variations were detected for other metals. Importantly, Al exhibited a positive correlation with insulin in those patients. PCOS patients had higher oxidative stress, mainly induced by lead, which in turn negatively affected aromatase and resulted in hyperandrogenism. In addition, aluminium exhibited a positive relationship with Insulin, implying its contribution to the metabolic disturbances.

Keywords: Aromatase, Heavy metals, Oxidative stress, PCOS, Steroidogenesis.

Introduction

Polycystic ovary syndrome (PCOS) is a common endocrine condition that impacts women of reproductive age. It is defined by the presence of hyperandrogenism, irregular ovulation and the morphological appearance of polycystic ovaries[22]. The causes of PCOS are complex and involve a combination of genetic, metabolic and environmental influences that play a role in its development[18,19]. Recent studies indicate that endocrine-disrupting chemicals (EDCs), particularly heavy metals like Al, Cd, Pb, As and Ni, could significantly impact hormonal equilibrium and worsen the symptoms of PCOS[10,21]. These heavy metals can disrupt the endocrine system by modifying steroidogenesis and influencing inflammatory processes[13]. Aluminium has garnered significant interest due to its prevalent use in food products, cosmetics and pharmaceuticals.

Research has shown that exposure to aluminium may adversely affect ovarian function by inhibiting the activity of aromatase (CYP19A1), an enzyme essential for the synthesis of estrogen[3]. Furthermore, cadmium and lead function as xenoestrogens, interacting with estrogen receptors and causing hormonal imbalances[16]. These metals have the potential to cause oxidative stress and disrupt mitochondrial function in ovarian cells, thereby exacerbating the pathophysiology of PCOS[9].

Although earlier research has established a connection between exposure to endocrine-disrupting chemicals and reproductive disorders, there is a scarcity of studies that have focused specifically on the impact of heavy metals in the context of PCOS[1,4,6,7,14,17]. Comprehending the endocrine-disrupting capabilities of these metals may yield valuable insights into the environmental risk factors linked to PCOS. This research intends to examine the concentrations of aluminium and various heavy metals in women with PCOS in comparison to healthy controls, evaluating their possible influence on the onset and severity of the condition. The results could assist in pinpointing environmental factors contributing to PCOS and guide public health initiatives aimed at reducing exposure risks.

Material and Methods

This observational case-control study was designed to evaluate the impact of heavy metals on endocrine disruption in individuals diagnosed with polycystic ovary syndrome (PCOS). The institutional ethics committee approved the study under reference number PIEC/MLT/01/2022. Participants were sourced from the Saraswathi Speciality Clinic in Nagarabhavi, Bangalore. The study included a total of 110 individuals, comprising of 53 control subjects and 57 cases. Subject selection was carried out based on the criteria

specified below. The sample size was calculated using n Master software version 2, with an effect size of 0.57, a significance level (α) of 5% and a power of 80%.

Selection criteria: Participants between the ages of 18 and 40 years have a diagnosis of PCOS based on the established Rotterdam criteria. A diagnosis is confirmed by the presence of at least two of the following characteristics: i. Clinical or biochemical hyperandrogenism, ii. Oligomenorrhea or amenorrhea, along with the presence of ovarian cysts. Individuals with other hormonal disorders, such as thyroid, adrenal, or pituitary issues, were excluded from the study. Furthermore, participants with occupational exposure to heavy metals, those receiving hormone therapy and individuals using oral contraceptives were also not included. A control group of age-matched, normally menstruating healthy individuals was established for comparison.

Sample collection: Before the collection of samples, informed consent was obtained from all participants. Interviews were performed to assess anthropometric data, clinical presentations and medical histories, employing a validated questionnaire. Following this, an on-duty gynecologist examined the subjects and diagnosed PCOS based on established criteria. Biochemical and radiological tests were conducted to confirm the diagnosis. Approximately 5ml of blood was drawn from each participant, after which the serum was separated, aliquoted and stored at -80°C for future analysis.

Biochemical characterization: It involved the assessment of hormones including total testosterone, insulin and estradiol (E2), utilizing established kit methodologies. The levels of estradiol were determined through an enzyme-linked fluorescent assay, in which 200µl of serum was introduced into a well containing a conjugate labeled with alkaline phosphatase. In this setup, the hormone competes with the conjugate for binding to anti-estradiol antibodies that are immobilized on the solid phase receptacle (SPR). The fluorescent product, 4-methyl umbelliferone, produced at the conclusion of the assay demonstrated an inverse correlation with estradiol concentration and was quantified at a wavelength of 450nm. Total testosterone and insulin levels were measured through Electrochemiluminescence Immunoassay (ECLIA), while aromatase concentrations were assessed using a Solid Phase Sandwich Enzyme-Linked Immunosorbent Assay (ELISA).

The analysis of heavy metals, specifically Al, Cd, Pb, As and Ni was conducted utilizing ICP-MS. This procedure involved the digestion of 1 mL of serum with an equal volume of hydrogen peroxide and nitric acid, followed by microwave digestion for 25 minutes in the M3 microwave digestion system, with temperatures varying from 25 to 200^0C. After the cooling phase, the extracts were filtered and the final volume was adjusted to 10 mL using ultra-pure water (Millipore). The concentrations of aluminium, cadmium, lead, arsenic and nickel were then detected and quantified through Inductively Coupled Plasma Mass Spectrometry. Quality control was maintained by analyzing Certified Reference Materials (CRM). Additionally, oxidative stress markers, including MDA and PON-1, were evaluated using standard spectrophotometric techniques.

Biostatistics: The data are presented as mean ± standard deviation (SD). The differences between groups were assessed using the Student's t-test. Additionally, chi-square tests, Pearson's correlation coefficient and multiple regression models were utilized to examine associations and correlations. A p-value of less than 0.05 was deemed statistically significant. Data analysis was conducted using SPSS version 19 software.

Results

Baseline characteristics: This case-control study included participants with an average age of 25 ± 6.4 years in the control group and 24.9 ± 4.9 years in the case group. The body mass index (BMI) was recorded as 26.43 ± 5.03 kg/m² for the case group and 22.7 ± 5.72 kg/m² for the control group. Individuals diagnosed with Polycystic ovary syndrome (PCOS) exhibited a notably higher prevalence of obesity compared to those in the control group. Aromatase levels in the case group were found to be 0.4 ± 0.1 ng/mL which was significantly lower than the control group's measurement of 0.9 ± 0.3 ng/mL. This downregulation resulted in a considerable increase in testosterone levels and a decrease in estradiol (E2) levels within the PCOS group. Additionally, the PCOS group showed significant insulin resistance, with a measurement of 66 ± 22 µIU/mL, compared to the control group's level of 35 ± 11 µIU/mL [Table 1].

Oxidative stress and Heavy metals: Oxidative stress was evaluated by measuring the levels of MDA and PON-1. The subjects with PCOS exhibited markedly higher MDA levels, averaging 1.53 ± 0.71 µmol/L, in comparison to the control group, which had an average of 0.88 ± 0.20 µmol/L.

Additionally, paraoxonase-1 levels were found to be lower in patients with PCOS, indicating a heightened state of oxidative stress within this population. Regarding heavy metal exposure, the PCOS group showed significantly increased aluminium (Al) levels, with an average concentration of 74 ± 26 ppb, as opposed to 62 ± 20 ppb in the control group. No significant differences were observed for other toxic metals such as arsenic As, Ni and Pb between the two groups. Cd was not detected in any of the samples. [Table 2].

It is important to highlight that aluminum concentrations exceeding 100 ppb are regarded as toxic. In this study, 13 (22.8%) of the patients with PCOS surpassed this threshold, in contrast to only 3 (5.6%) individuals in the control group. This difference was found to be statistically significant. While the other metals did not show statistically significant variations, it is alarming that 80% of all participants,

encompassing both cases and controls, had nickel levels classified as toxic.

Additionally, nearly half of the PCOS population exhibited lead levels that were similarly considered toxic [Table 3]. As previously mentioned, PCOS patients exhibited significant oxidative stress, indicated by increased levels of MDA. Among the various toxic metals evaluated, lead was identified as a major factor contributing to oxidative stress, showing a significant positive correlation with MDA. The relationships between other metals and oxidative stress markers did not achieve statistical significance [Table 4].

Impact of oxidative stress and heavy metals on endocrine balance: In individuals diagnosed with PCOS, malondialdehyde (MDA) showed a notable negative correlation with aromatase and a positive correlation with testosterone. This observation strongly suggests that oxidative stress hampers steroidogenesis by diminishing aromatase activity [Table 5]. Additionally, an analysis of the direct impacts of heavy metals on hormonal levels indicated that aluminum (Al) exhibited a significant positive correlation with insulin, implying its possible involvement in causing hormonal imbalance. Conversely, other heavy metals did not reveal any significant direct effects on hormone levels [Table 6].

Table 1
Demographic Characteristics and Clinical Overview.

Variables	Participants Without PCOS	Participants With PCOS	Significance (p-value)
Age (Years)	25 ± 6.4	25 ± 5	0.9
BMI (Kg/m²)	22.7 ± 5.7	26.4 ± 5	<0.01**
Fasting Blood Glucose (mg/dL)	86 ± 22	95 ± 23	0.07
Insulin Concentration (µIU/mL)	35 ± 11	66 ± 22	<0.01**
Serum Testosterone (ng/dL)	18.64 ± 6	27.76 ± 9	<0.01**
Serum Estradiol (pg/mL)	110.9 ± 36	94.6 ± 31	0.3
Aromatase Enzyme Level (ng/mL)	0.9 ± 0.3	0.4 ± 0.1	0.01*

Table 2
Oxidative Stress Indicators and Metal Concentrations in Study Participants
Markers of Oxidative Stress

Variables	Reference Group	Affected Group	p-value
Lipid Peroxidation Marker (MDA) (µM/L)	0.88 ± 0.20	1.53 ± 0.71	<0.01**
PON-1 Enzymatic Activity (U/L)	89.9 ± 29.99	83.91 ± 27	0.33

Toxic Metal Concentrations

Variables	Reference Group	Affected Group	p-value
Aluminum (Al) (µg/L)	62.34 ± 20.11	74.72 ± 26	0.04*
Arsenic (As) (µg/L)	2.96 ± 0.98	3 ± 1.3	0.84
Lead (Pb) (µg/L)	22.92 ± 8.32	21.63 ± 7.86	0.40
Nickel (Ni) (µg/L)	17.98 ± 5.6	17.37 ± 5.6	0.76

Table 3
Elevated Toxic Metal Levels

Toxic Metal Concentrations	Control (n=53) Prevalence (%)	Case (n=57) Prevalence (%)	p-value
Al (>100 µg/L)	3 (5.6%)	13 (22.8%)	<0.01**
Pb (>25 µg/L)	21 (39.6%)	26 (45.6%)	0.32
As (>50 µg/L)	3 (5.6%)	1 (1.7%)	0.28
Ni (>10 µg/L)	43 (81.1%)	45 (79%)	0.48

Table 4
Correlation of Heavy Metals with Markers of Oxidative Stress in PCOS Patients

Toxic Metal Concentrations	PON-1 (r, p-value)	MDA (r, p-value)
Al	0.29 (0.05)	0.05 (0.85)
As	0.09 (0.50)	0.18 (0.13)
Pb	0.13 (0.30)	0.28 (0.03)*
Ni	0.13 (0.90)	0.09 (0.94)

Table 5
Effects of Oxidative Stress on Hormonal Regulation

Markers of Oxidative Stress	Endocrine Markers	Correlation (r)	p-value
MDA	Testosterone	0.23	0.01*
	Estradiol	-0.10	0.2
	Aromatase	-0.20	0.03*
	Insulin	0.12	0.18
PON-1	Testosterone	-0.08	0.38
	Estradiol	-0.12	0.21
	Aromatase	0.04	0.62
	Insulin	0.12	0.19

Table 6
Impact of Toxic Metal Concentrations on Endocrine Balance

Toxic Metal Concentrations	Endocrine Markers	Correlation (r)	p-value
Al	Testosterone	0.087	0.364
	Estradiol	-0.112	0.242
	Aromatase	0.033	0.729
	Insulin	0.195	0.041*
As	Testosterone	0.079	0.415
	Estradiol	0.003	0.975
	Aromatase	-0.070	0.465
	Insulin	0.023	0.813
Pb	Testosterone	0.041	0.670
	Estradiol	0.075	0.435
	Aromatase	0.149	0.121
	Insulin	0.034	0.726
Ni	Testosterone	-0.067	0.487
	Estradiol	0.081	0.398
	Aromatase	0.001	0.990
	Insulin	-0.089	0.355

Discussion

Our research findings are consistent with the observations reported by Abudawood et al[2] who demonstrated a significant relationship between Pb levels and markers of oxidative stress. Additionally, we have identified the harmful effects of oxidative stress on steroidogenesis, particularly through the inhibition of aromatase activity. Further investigations, including those conducted by Zhang et al[23] have underscored the positive linear correlation between Pb and sex hormone-binding globulin (SHBG), as well as its links to long-term amenorrhea and infertility[11,23]. Research concerning the harmful effects of Al, As and Ni is still limited. This study marks one of the preliminary attempts to explore the influence of Al toxicity on female reproductive health in humans.

We found significantly higher levels of Al in patients diagnosed with polycystic ovary syndrome (PCOS), along with a tendency to promote insulin resistance. Existing animal studies have suggested that exposure to Al in mice results in decreased steroidogenesis, lower estradiol levels, reduced ovarian ATPase activity, altered expression of androgen receptors for follicle-stimulating hormone (FSH) and luteinizing hormone (LH), as well as ovarian damage and infertility[5,8]. Rodriguez Diaz et al[15] studied Al concentrations in human follicular fluid. Their findings indicated no statistically significant difference in Al levels between individuals with PCOS and those without. Notably, a negative correlation was observed between Al levels and the quantity of mature oocytes[15].

As plays a crucial role in this condition, as it may influence the expression of the CYP11A1 and CYP17A1 genes, which are responsible for encoding enzymes vital for steroidogenesis[20]. Our examination of the influence of As and Ni on the CYP19A1 enzyme in blood did not reveal any significant effects. Previous animal studies have suggested a reduction in estradiol levels following exposure to Ni[12]. It is important to note that our research concentrated exclusively on these four metals; however, numerous other toxic metals and environmental contaminants such as phthalates, microplastics, synthetic dyes and fertilizers, may also significantly impact endocrine function and steroidogenesis. Further investigation is necessary to elucidate the potential effects of environmental exposure on the etiology of PCOS.

Conclusion

We assessed the effects of toxic metals namely Al, Cd, Pb, As and Ni on endocrine function. Our findings indicated that heavy metals can trigger pathophysiological changes and

disrupt steroidogenesis, primarily by inducing oxidative stress. Pb was identified as a key contributor to the oxidative stress. The oxidative stress marker MDA was found to be a potent inhibitor of aromatase leading to the accumulation of androgens. Al concentrations were significantly higher in patients diagnosed with PCOS and exhibited a notable positive correlation with insulin levels.

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