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# Determination and data analysis for Pb, Ni, Fe, Mn and Co elements in serum of PCOS patients

**Salam A.H. AL-AMERI[1], Amer Hasan ABDULLAH[1], Muntadher H. CHALLOOB[2*]**

[1] Chemistry department, College of Science, Mustansiriyah University, Baghdad- IRAQ
[2] College of Medicine, Mustansiriyah University, Baghdad- IRAQ

* Corresponding Author. E-mail: muntadther.hussein@uomustansiriyah.edu.iq (M.H. C.); Tel. +964 772 217 5013

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**ABSTRACT**: Number of women with polycystic ovary syndrome (PCOS) who have an insulin resistance at the same time was increased to large numbers in Iraq, with a lack of statistics regarding the number of PCOS patients and the role of some of biochemical variables that may interfere and causing this disease. The research aimed to study the levels of some trace elements namely; lead, nickel, iron, manganese and cobalt in the serum of Iraqi PCOS patient in a case control study in which the serum Pb, Ni, Fe, Mn and Co concentrations were determined in sixty women with PCOS with an age ranged from 20-40 years old and compared with those levels in 60 age, gender, and BMI-matched healthy volunteers selected as a control group which showed that the levels of Lead and nickel were significantly higher ($p<0.05$) in the levels of Fe, Mn and Co in patients comparing with controls. The analysis of the ROC curve analysis revealed that all studied trace elements provide significant results with highest values of AUC, sensitivity and specificity obtained with Ni which is 0.954, 85% and 98.3% respectively and in Mn of 0.908, 86.7 % and 88.3 % respectively which indicate that these elements can be considered as good predictive markers for PCOS

**KEYWORDS**: PCOS; Lead; Nickel; Manganese; Iron; Cobalt.

## 1. INTRODUCTION

Trace elements are important and necessary in avoiding diseases and human health and at the same time their presence and function require specific levels. Trace elements have several main roles as stabilizers, structural elements, and important elements for hormonal function and as enzymes cofactors [1]. Manganese is essential micronutrients trace element that incorporated in the defence mechanisms that protect the body from the dangers of oxidative stress damage through their role as a cofactor for the enzyme Mn-superoxide dismutase (Mn-SOD) which is considered as a contributing factor in the activity of this enzyme and also contributed to an imbalance in glucose metabolism. It was demonstrated that its levels may play secondary roles in the occurrence of abnormal glucose levels as a significant variable that indicates suffering from polycystic ovary syndrome (PCOS) [2- 4]. It is well known that SOD enzyme plays crucial role in the scavenging of reactive oxygen species (ROS) such as superoxide which converted by SOD into hydrogen peroxide which is reduced subsequently in the mitochondria by catalase into water [5].

Cobalt is a heavy metal that indirectly causes the free radicals formation and affects the genetic material as it may cause DNA strand destruction; also, Co works to reduce the production of glucose in the blood, which is a useful feature for PCOS women which is caused by it is role in reducing the gluconeogenesis which result in reduces the level of sugar in the blood, in addition to reducing the oxidation of fat, and these variables are associated with the occurrence of PCOS [6- 8].

Lead is a systemic toxin that is found in large quantities in the environment, damages many organs, and has to be closely monitored. The health of women who are at a reproductive age should be concerned about lead exposure since it can lead to hormonal changes that impact a woman's menstrual cycle, ovulation, and fertility [9]. Lead incorporates to the ROS production by reduces glutathione (GSH) and a protein-bound sulfhydryl group and the excessive ROS generation is showed in patients with PCOS which may provide a link between Pb and PCOS [10].

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The effects of iron excess on endocrine functions vary, as evidenced by variations in the severity of illnesses and patient hormonal profiles [11, 12]. Research confirms high iron levels in PCOS patients, and the iron levels also considered as one of the causes of congenital heart disease that is caused by iron accumulation in the cardiac tissue. The possible role of iron in PCOS is owned to an elevated levels that might be caused by the irregular menstrual cycle or for more evident the hyperinsulinemia that may be considered as the main cause of iron accumulation in patients [13].

In PCOS patients, nickel may contribute to folliculogenesis, which is detrimental to ovulation, and it can also increase free radical oxidation in cell membranes which also can be considered as another link to the incidence of PCOS [4].

In order to confirm the disease's causes and use some elements like lead, nickel, iron, manganese and cobalt as indicators to forecast the likelihood of contracting the disease, its stages, and the potential for treatment with particular dietary supplements, current research has focused on studying these elements by assessing their levels in the sera of patients in comparison with healthy controls.

## 2. RESULTS and DISCUSSION

Certain elements are necessary for specific biochemical reactions, particularly those involving enzyme cofactors that are connected to glucose metabolism, homeostasis, and hormone regulation, particularly insulin. However, the activities of several elements in PCOS remain completely unknown [3]. The findings of the present study demonstrated that, in comparison to the healthy groups, PCOS patients had significantly higher Pb and Ni levels ($p < 0.05$) and the results also showed significant decrease in the serum concentrations of iron, manganese and cobalt in these PCOS patients comparing to controls.as illustrated in Table 1.

**Table 1.** Elements data results for patients and control groups

| Trace elements | Patient group, N = 60 | | Control group, N = 60 | | P-value |
|---|---|---|---|---|---|
| | Mean | Std. Deviation | Mean | Std. Deviation | |
| Pb, µg/dl | **0.0925** | 0.0237 | 0.074 | 0.00985 | 0.048* |
| Ni, µg/dl | **0.06557** | 0.0236 | 0.034 | 0.006829 | 0.016* |
| Fe, µg/dl | 129.12 | 7.662 | **138.88** | 5. 73 | 0.025* |
| Mn, µg/dl | 0.16 | 0.007 | **0.217** | 0.03 | 0.037* |
| Co, µg/dl | 0.07557 | 0.0112 | **0.097** | 0.0117 | 0.04* |

* Sample t-test, $p \leq 0.05$, Non-significant: NS

Comparing Pb levels across the two groups, it was found that PCOS patients had higher Pb levels than the control. This finding suggests that the rise in Pb levels may be a growth risk factor or contribute to the pathophysiology of PCOS. These findings were in consistency with those of previous investigations findings that confirm the raising the Pb limits in PCOS patients comparing to controls [9, 14], whereas other results showed that PCOS patients' blood Pb levels were lower than those of the controls [3]. According to previous reports, women who have lead levels more than 25 µg/L are three times more likely to have infertility than women who have lower levels. The core pathophysiology of PCOS is dependent on oxidative stress, which can be produced by heavy metal poisoning [9]. In PCOS-affected individuals, blood lead levels were strongly correlated with both insulin and insulin resistance. Exposure to hazardous metals, such as lead, has been linked to PCOS in a beneficial manner. As are the primary contributors, which were connected to many clinical PCOS phenotypic features [15].

Results illustrated in Table 1 showed that the serum Mn concentrations in PCOS patients were significantly lower ($p< 0.05$) than the concentrations determined in the control group. The explanation of the reduction in Mn levels in PCOS patient may originate from the fact that Mn-SOD was participate extensively in the protection against the increased oxidative stress that occur in PCOS which cause a consumption of Mn that leads to a decrease in its serum levels, given that the patients received an adequate amount of Mn [16]. The levels of Mn that demonstrated in the current study showed to be consistent with earlier studies which found a decreases in Mn level in patients with PCOS compared to control which indicate an imbalance in Mn levels that may causes an imbalance in glucose metabolism in women with PCOS [3, 16], whereas other researchers noticed an increase in manganese levels in women with PCOS, indicated that the increases in Mn concentration is the result of women developing PCOS and other contributing factors [4], whereas the others demonstrate no difference in Mn levels between the groups [17].

From the data obtained, significant difference in Ni levels was evident in the samples of PCOS patients comparing to controls as shown in Table 1. The levels of Ni in the sera of PCOS patients were significantly higher than those in healthy group, ($p < 0.05$). These results are in agreement with the data presented previously and reported that the patients' serum Ni concentration was significantly higher than that in healthy controls [18]. But the result of the current research was dissimilar with other previous results in which patients with PCOS showed non-significant lower Ni levels comparing with controls [4]. In consistency with the present results, previous study demonstrated that the exposure Ni may be the cause of PCOS. It was also reported that the PCOS patients showed an increase in Ni levels with an increase in the LDL levels which is observed specially in in women with insulin resistance, while in patients without insulin resistance, the HDL level decreased which confirms the observation that nickel levels may be have a risky lipid profile effect in patients with PCOS [19].

The results obtained in the present work showed that the levels of iron in sera of PCOS patients were significantly lower ($p<0.05$) than those in controls as postulated in Table 1. The results consistent with the previous studies which notify those high levels of Fe was related to the risk increases of PCOS [4, 13, 20], whilst former studies illustrate non-significant variation in the levels of Fe among the studied groups [17, 21, 22]. Abnormal levels of iron which is termed as "iron overload" showed to affect the menstrual cycle of women with PCOS and reduce the pregnancy probability; it may lead to insulin resistance, diabetes, and an increase in ferritin which is the iron storage protein, thus increasing the possibility of developing PCOS [12]. Increased ferritin levels are a sign of increased iron stores in the patient's body, which results from high insulin and a state of secondary hyperinsulinism, which increasing the ability to absorb iron in the digestive system. This can be explained that obesity leading to increased iron absorption, while the results may show a lower iron level than in the control group, but in reality, the excess iron will accumulate in various tissues, including adipose tissue, especially for women with obesity and PCOS [11].

The results obtained regarding Co showed clearly the significant decreases ($P <0.05$) in cobalt levels in patients with PCOS in comparison with healthy subjects as demonstrated in Table 1 which may indicate that cobalt can be considered as one of trace elements that participate in the PCOS pathogenesis, but the present result is disagree with previous results which reported that Co levels were increased in PCOS patient in a comparison with controls [21] rather than decreased as demonstrated in current work. Also, based on the Co levels, other studies have shown no significantly differences between PCOS patients and control [8, 3, 16].

The results of trace elements and heavy metals obtained in this study may help in providing an assumed mechanism for the incidence of PCOS and provide an information regarding the role of these element in the PCOS pathogenesis. The level of these parameters in patients is not clear and requires other assessment, so, more comprehensive studies are needed to clarify their exact role in PCOS progression taking in consideration other trace elements, especially zinc, copper and chromium, to elucidate their role in disease.

The Pearson correlation results showed a direct significant correlation between Pb and Ni at ($r = 0.258$ $p <0.001$) which confirm the strong correlation between these element levels and the possible relationship with PCOS, also these results may prove the possibility of using these elements as a marker in patients with PCOS and also confirm the strong correlation between these element levels and the possible correlation to PCOS, Furthermore, this study revealed other positive and negative non-significant correlations with others as shown in Table 2.

**Table 2.** Elements Pearson correlation in patient women with PCOS.

| Pearson Correlation | Pb | Ni | Fe | Mn | Co |
|---|---|---|---|---|---|
| Pb | - | 0.26 * | - 0.13 | 0.11 | - 0.02 |
| Ni | 0.268 * | - | 0.18 | 0.13 | - 0.14 |
| Fe | - 0.137 | 0.180 | - | 0.14 | - 0.11 |
| Mn | 0.11 | 0.140 | 0.16 | - | - 0.18 |
| Co | - 0.02 | - 0.153 | - 0.11 | - 0.18 | - |

*. Significant correlation at 0.05 levels (2-tailed).

To assess the significance of the of measuring the studied elements as a powerful marker for PCOS, ROC curve analysis was performed [23] and area under the curve (AUC) were assessed as measure of the test accuracy and reliability to choose the appropriate analytical components marker [24]. The results illustrated in Table 3 and Figure 1 showed significant associations between the study's trace elements and PCOS and the results revealed that nickel and Mn provide the best values of AUC, sensitivity and specificity that may indicate that these elements are very good markers that can be used to ass the PCOS progression and pathogenesis. Additionally, other elements subjected to the current study (Pb, Fe and Co) also showed a good AUC value which are also used greatly as predictive marker for the occurrence and development of PCOS.

Nickel ROC curve validate (AUC= 0.954), sensitivity of 85.0%, a specificity of 98.3% with Cut-off value 0.059 µg/dl whilst for the manganese; (AUC= 0.908), sensitivity of 86.7% also a specificity of 88.3% with Cut-off value 0.1695 µg/dl, whereas Pb, Fe and Co element also gives a good results as seen in Table 3 and Figure 1.

**Table 3.** The ROC curve results.

| Elements | AUC | Std. Error [a] | Asymptotic Sig *P*-value | Sensitivity% | Specificity% | Cut-off value |
|---|---|---|---|---|---|---|
| Pb | 0.864 | 0.038 | < 0.001 | 76.7 | 98.3 | 0.0765 |
| Ni | 0.954 | 0.017 | < 0.001 | 85.0 | 98.3 | 0.059 |
| Mn | 0.908 | 0.034 | < 0.001 | 86.7 | 88.3 | 0.1695 |
| Fe | 0.841 | 0.035 | < 0.001 | 66.7 | 86.7 | 145.5 |
| Co | 0.883 | 0.030 | < 0.001 | 78.3 | 85.0 | 0.0865 |

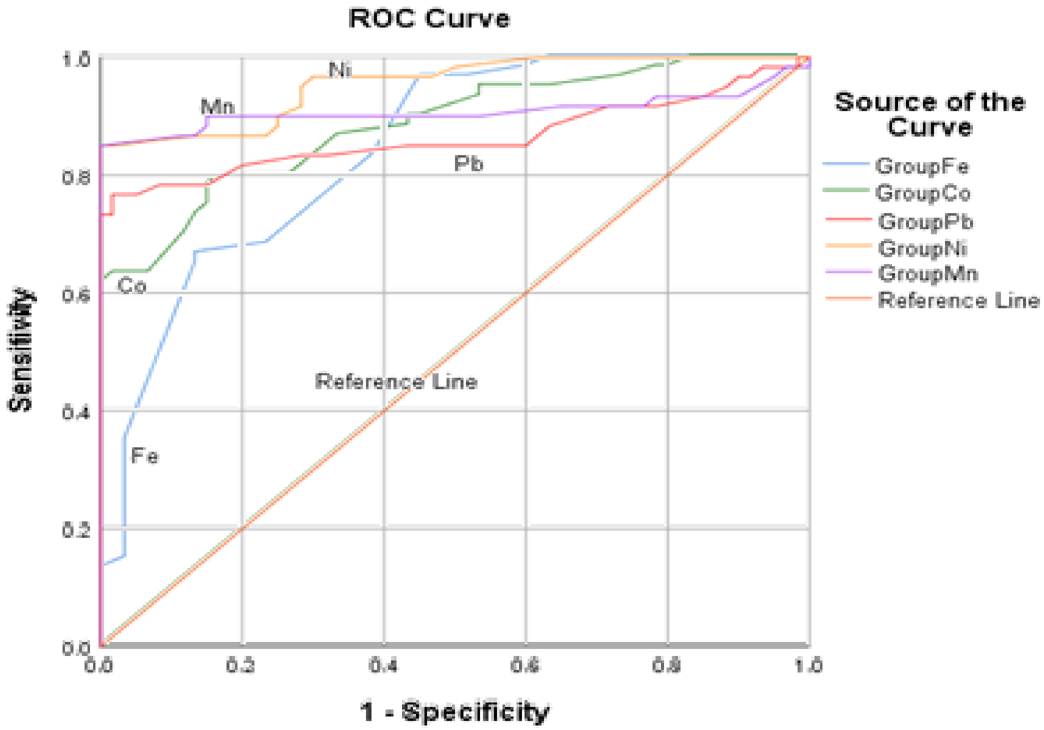


**Figure 1.** Elements ROC curve analysis in women with PCOS serum

## 3. CONCLUSION

It was concluded that the studied elements showed a possible role in the diagnosis and prognosis of PCOS as there are a significant increase in the levels of Lead and Nikel with a significant decrease in the serum levels of Manganese, Iron and Cobalt. Additionally, the difference in the levels of these elements in PCOS patients in comparison with healthy subjects may provide an information regarding the role of these elements in PCOS pathogenesis and my elucidate the mechanism of PCOS development and progression that allow for an optimum treatment in future.

## 4. MATERIALS AND METHODS

### 4.1. Samples

A case control study was conducted on sixty patients diagnosed by medical professionals with primary or secondary PCOS, aged 20–40, were selected from Kamal Al-Samarrai Hospital for Women Infertility and Obstetrics- Baghdad- Iraq between July 1 and November 31, 2022, along with an equal number of age, gender and BMI-matched healthy women. A sample of 10 ml of blood were obtained from PCOS patients and controls, transferred to a plain tube, and allowed to clot for 30 minutes at room temperature before being centrifuged for 10 minutes at 402 x g. The obtained serum was kept at -20ºC until the examination for the identification of trace elements that subjected to the current study (Pb, Ni, Fe, Mn, Co). The levels of the measured trace elements in patients were compared with those in control subjects who appeared healthy.

### 4.2. Analysis

The trace element & heavy metals levels in the PCOS patient and healthy sera samples were determined using the totally control flame atomic absorption (FAAS) and flameless atomic absorption spectrophotometer (FLAAS) at argon gas flow of 0.3L/min at the best conditions at wavelength; 216.9, 232, 248.3, 279 and 240.7 nm for Pb, Ni, Fe, Mn and Co respectively, Slit width; 0.2 - 0.5nm, lamp current 5 mA. Each serum sample was treated according to instrumental specification and the concentration was directly calculated via standard calibration graphs.

### 4.3. Statistical analysis

The obtained data were analysed using statistical package for the social science for Windows software IBM SPSS Statistics, Version 26. The results are illustrated as mean ± SD. A Students independent t test was performed to estimate the differences between the two groups mean. Pearson correlation coefficient was used to determine the relationships among all elements at $p < 0.05$ were considering to be statistically significant, N.S ($p > 0.05$), S ($p < 0.05$), HS ($p < 0.01$), SD: Standard deviation, C.L: Confidence limits (Lower and Upper). The analysis of ROC curve was applied to suppose the suitable analytical elements biomarker by measuring AUC, sensitivity and specificity which provide an information about the accuracy and reliability of the tests used [25].

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