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Evaluation of some biochemical variables in women with polycystic ovary syndrome

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Abstract

Background: Polycystic Ovary Syndrome (PCOS) is a heterogeneous, complex disorder of the endocrine system affecting 6–15% of women of reproductive age worldwide; consequently, PCOS is one of the most common causes of infertility and metabolic dysregulation in women. It includes hyperandrogenism, ovulatory dysfunction, and the polycystic ovarian morphology within a spectrum of clinical manifestations.

Methods: The study included (60) samples of women with polycystic ovary syndrome and compared them with (30) samples of the control group. The pathological conditions of women with polycystic ovary syndrome were confirmed after performing an ultrasound examination. Blood was drawn and samples were taken on the second or third day of the menstrual cycle.

Results and Conclusion: The levels of Vitamin D, Calcium, Vitamin C, Vitamin B12, and Vitamin E were measured and the results showed a significant decrease in patients with polycystic ovary syndrome compared to control group. There was a significant increase in the levels of estrogen, insulin hormone, glucose and HOMA-IR in patients with polycystic ovary syndrome compared to the control group at the probability level of $P \leq 0.05$.

تقييم بعض المتغيرات البيوكيميائية لدى النساء المصابات بمتلازمة تكيس المبايض

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الخلاصة :متلازمة تكيس المبايض (PCOS) اضطراب معقد ومتغاير في الجهاز الهرموني، يصيب ما بين 6 و15% من النساء في سن الإنجاب حول العالم؛ ولذلك، تُعدّ متلازمة تكيس المبايض من أكثر أسباب العقم واضطرابات التمثيل الغذائي شيوعًا لدى النساء. تشمل أعراضها فرط الأندروجينية، واضطرابات التبويض، وتكيس المبايض، ضمن طيف واسع من المظاهر السريرية. شملت الدراسة 60 عينة من النساء المصابات بمتلازمة تكيس المبايض، وقورنت بـ 30 عينة من المجموعة الضابطة. تم تأكيد التشخيص المرضي للنساء المصابات بمتلازمة تكيس المبايض بعد إجراء فحص بالموجات فوق الصوتية. سُحبت عينات الدم في اليوم الثاني أو الثالث من الدورة الشهرية. قُيست مستويات فيتامين د، والكالسيوم، وفيتامين ج، وفيتامين ب 12، وفيتامين هـ، وأظهرت النتائج انخفاضًا ملحوظًا لدى المصابات بمتلازمة تكيس المبايض مقارنةً بالمجموعة الضابطة. لوحظت زيادة كبيرة في مستويات هرمون الاستروجين، وهرمون الأنسولين، والجلوكوز، ومؤشر مقاومة الأنسولين (HOMA-IR) لدى المرضى المصابين بمتلازمة تكيس المبايض مقارنة بالمجموعة الضابطة عند مستوى الاحتمالية P≤0.05.

الكلمات المفتاحية: فيتامين د، الأنسولين، الأستروجين، الجلوكوز، مؤشر مقاومة الأنسولين (HOMA-IR)

INTRODUCTION

PCOS is a common endocrine-metabolic condition that most often develops during the teenage years or early adulthood, and is characterized by a set of symptoms, which include hyperandrogenism, insulin resistance, irregular menstrual periods, and polycystic ovarian shape. The etiology is complex, as it involves genetic factors, environmental factors and lifestyle factors [1]. The central role is played by insulin resistance, which causes hyperinsulinemia and excessive production of androgens, which additionally contribute to the development of reproductive and metabolic abnormalities. Diagnostic instruments like the Rotterdam Criteria document two of three of the following characteristics, including hyperandrogenism, ovulatory dysfunction and polycystic ovaries to establish diagnosis with the presence of heterogeneous symptoms making clinical diagnosis difficult [2,3]. Treatment of PCOS is multidisciplinary and aims at symptom management, prevention of complications and fertility support. Behavioral change, especially weight control and physical exercise, are considerations even here. Hormonal contraceptives, anti-androgens and insulin sensitizers such as metformin are commonly used. To perform fertility treatment there is ovulation induction or assisted reproductive technologies [4-6]. There is emerging evidence regarding the role of micronutrient status in the case of PCOS pathophysiology. The problem of vitamin D deficit is common and is associated with the aggravation of insulin resistance and reproductive failure. Oral administration can increase insulin sensitivity and menstrual regularity [7]. Calcium, usually combined with vitamin D, is considered useful with bone health and in insulin metabolism, and there are possible effects in the treatment of metabolic imbalances. Vitamin C is a strong antioxidant that could prevent oxidative stress and inflammation caused by PCOS and thus facilitate better insulin functionality. In a similar manner, Vitamin E could serve to reduce oxidative damage and enhance the hormonal status, which might lead to the improved results in fertility [8-10]. Vitamin B12 deserves special consideration in patients with long term Metformin intake because it might interfere with its absorption causing deficiency and typical neuro- or hematologic manifestation. It is recommended to be monitored regularly and supplemented [11,12]. Insulin is a key metabolic component of PCOS. This hyperinsulinemia contributes to hyperandrogenism and an impaired ovulatory function, greatly increasing the likelihood of developing the type 2 diabetes [13]. Continuous glucose monitoring, and following a low-glycemic index diet are essential requirements for effective glycemic control [14]. The Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) is a well-known clinical method to assess the degree of this metabolic dysfunction and is directly related to negative metabolic and reproductive effects [15]. Additionally, non-cycling estrogens in PCOS cause a hormonal imbalance which increases the likelihood of endometrial hyperplasticosis and cancer. Hormone replacement and lifestyle changes are essential to maintaining fertility and reducing the overall risk of long-term conditions; however, these are typically not sufficient to treat the whole of the PCOS disease process. The addition of micronutrient support has become a possibility in addition to lifestyle and pharmaceutical options [16.17]. The precise association between vitamin D status and important metabolic/hormonal factors in PCOS patients is, however, not well understood. With this gap in mind, the aim of this study was to compare serum vitamin D level and to examine the correlation between serum vitamin D level and some biochemical parameters in women with PCOS. This work outlines these metabolic and hormonal associations and serves as novel empirical evidence that shows the significance of vitamin D status in the physiological presentation of PCOS, which will help guide more comprehensive management strategies. This study aims to establish the differences and associations between five micronutrients namely Vitamin D, calcium, Vitamin C, Vitamin B12, Vitamin E as well as metabolic and hormonal marker such as insulin hormone levels, glucose, HOMA-IR and estrogen, in women with polycystic ovary syndrome (PCOS). These biochemical markers, vitamins and hormones were determined using enzyme-linked immunosorbent assay (ELISA) kits. The research aims to establish how deficiencies in vitamins and minerals intake might impact on insulin sensitivity, glucose levels and estrogen levels in order to establish if nutritional changes can be effective in the treatment of insulin resistance and reproductive issues in women diagnosed with PCOS.

Statistical Analysis

The data obtained from the present study was analysed with the help of XLSTAT software and SPSS (Version 23.0). Descriptive statistics were presented as Mean ± SD, as appropriate. For continuous variables, across study group comparisons were made using one way Analysis of Variance (ANOVA). Tukey's post-hoc test was used when making pairwise comparisons between individual group means. For all analyses, a P-value of ≤ 0.05 was regarded as statistically significant [18].

MATERIALS AND METHODS

Data were collected from a sample of 60 patients whose ages ranged between (19-38 years). Additionally, 30 control subjects whose ages ranged between (19-35) were collected. The samples were obtained from Salah Al-Din Laboratories. Both patients and controls had 5 mL of venous blood collected. The blood samples were placed in a gel tube then left to clot for 10 minutes at room temperature. The samples were centrifuged for ten minutes at a speed of 3000 revolutions per minute (rpm) and divided into three aliquots. The specimens were stored at a temperature of -20°C in tubes with a capacity of 1.5 mL until analysis using ELISA kits and a flameless atomic absorption spectrometer. The medical conditions of women with polycystic ovary syndrome were confirmed after an ultrasound examination [19].

Ethical Approval

This study protocol was approved by the Institutional Ethical Committee and written informed consent was obtained, explicitly, from all the subjects involved in the study. This research was approved by the Scientific Research Ethics Committee of the College of Pharmacy, Tikrit University (Ref. No. SREC21/2024-08-01).

RESULTS AND DISCUSSION

Vitamin D plays a crucial role in metabolism of calcium, immune systems and normal glucose homeostasis. It also contributes substantially to insulin sensitivity that is of principal importance to the management of PCOS, where insulin resistance is one of its main characteristics. The group of patients shows low Vitamin D levels (32.45 ± 5.00 ng/ml) in comparison with the control group (61.90 ± 6.9 ng/ml). The results agree with the study done by *Wu et al.* which he showed that the difference is statistically significant at the probability level of P≤0.05 and the results presented above indicate that the extent of observed micronutrient deficiencies in PCOS, there is a strong need for further interventional studies to investigate whether micronutrient supplementation is an effective supplement to therapeutic interventions to restore glycemic control and insulin sensitivity [20] as shown in the figure (1).

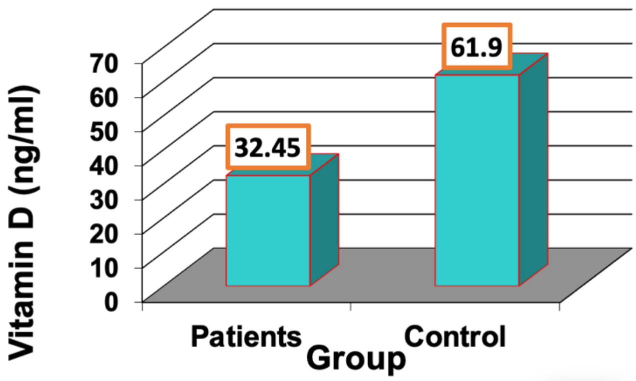

Figure (1): Comparison between patients and control in Vitamin D. Descriptive statistics were presented as Mean ± SD. P-value of ≤ 0.05 was regarded as statistically significant.

Calcium is an essential nutrient in bone health, muscular, and nerve transmission. Irregular menstrual cycles and sex hormonal imbalances in women especially with lower estrogen levels put women with PCOS at a higher risk of losing bone mineral density [21]. The calcium levels in control group are significantly greater (375.21 ± 22.85 mmol/l) than that of the patient group (130.182 ± 6.44 mmol/l) at the probability level of P≤0.05. This study is similar to that by *Ahmed et al.* showed that reduced calcium levels may therefore further increase the vulnerability toward osteopenia and osteoporosis especially in the long run laying emphasis on the need to pay close attention to calcium intake as far as management of PCOS and bone health is concerned [22] as shown in the figure (2).

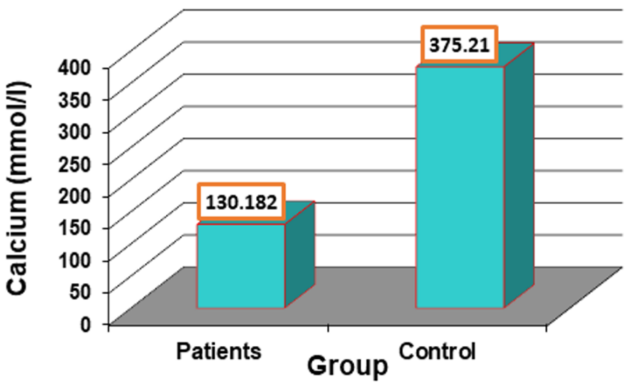

Figure (2): Comparison between patients and control in Calcium. Descriptive statistics were presented as Mean ± SD. P-value of ≤ 0.05 was regarded as statistically significant.

Water soluble vitamin C is an antioxidant that plays a role in collagen synthesis, immune response and facilitates cell protection against damage by reactive oxygen species. Greater oxidative stress leading to insulin resistance and inflammation occurs in women with PCOS that can be ameliorated by the addition of Vitamin C [23]. The values of Vitamin C in the former control group are considerably greater (73.305 ± 1.801 7 µmol/l) than those observed in the patient group (58.832 ± 0.459 8 µmol/l), suggesting that PCOS patients might possess decreased antioxidant reserves at the probability level of P≤0.05. This result is similar to the study made by *Zeber-Lubecka and Agwu et al.* where they showed that this decline in Vitamin C may increase inflammation and insulin resistance which are involved in the metabolic and the reproductive aspects of PCOS [24,25] as shown in the figure (3).

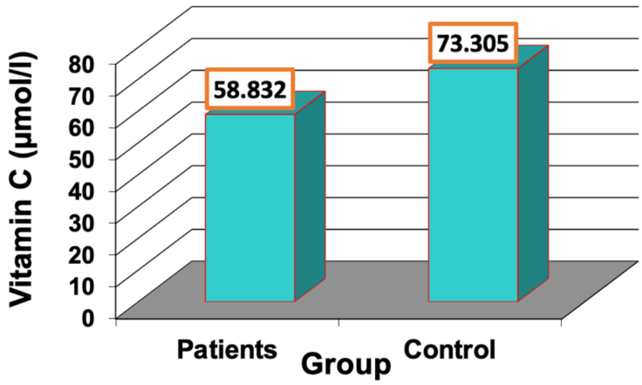

Figure (3): Comparison between patients and control in Vitamin C. Descriptive statistics were presented as Mean ± SD. P-value of ≤ 0.05 was regarded as statistically significant.

Vitamin B12 plays a significant part in neurologic activity, production of red blood cells and DNA synthesis. It also contributes towards homocysteine metabolism which is a factor related to heart health [26]. In the patient group, there are lower percentages of Vitamin B12 (10.423 ± 2.970 ng/ml) when compared to the control group (12.823 ± 2.360 ng/ml) at the probability level of P≤0.05. Study of *Chen and Petrillo et al.* believed that this decrease can be explained by the metabolic abnormalities in PCOS such as insulin resistance and concerns about the ability of certain pharmaceutical treatment such as metformin, which is widely used to treat insulin sensitivity, to interfere with this process. Reduced Vitamin B12 levels in PCOS patients may be associated with metabolic disturbances or metformin use, which may in turn exacerbate fatigue, cognitive and neurological problems, hence the need to monitor and supplement Vitamin B12 among patients with PCOS [27,28] as shown in the figure (4).

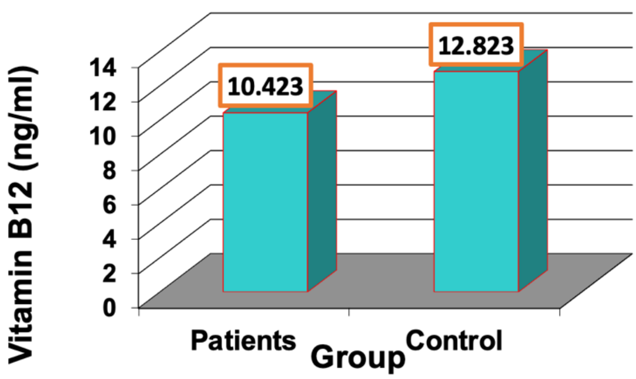

Figure (4): Comparison between patients and control in Vitamin B12. Descriptive statistics were presented as Mean ± SD. P-value of ≤ 0.05 was regarded as statistically significant.

Vitamin E is a fat-soluble antioxidant that has a major role in the protection of cellular damage against oxidative stress through the countering of free radicals. Oxidative stress is a major factor of insulin resistance and other metabolic disorders in PCOS (29). The Vitamin E level in the control group is high (147.8 ± 18.7 mg/100 ml) in comparison to the patients (33.6 ± 3.40 mg/100 ml) at the probability level of P≤0.05. The study by *Yang et al.* agrees with these results and that this deficit indicates that PCOS patients might possess decreased antioxidant barrier, which further manifest the hormonal imbalance, insulin resistance and inflammation (30) as shown in the figure (5).

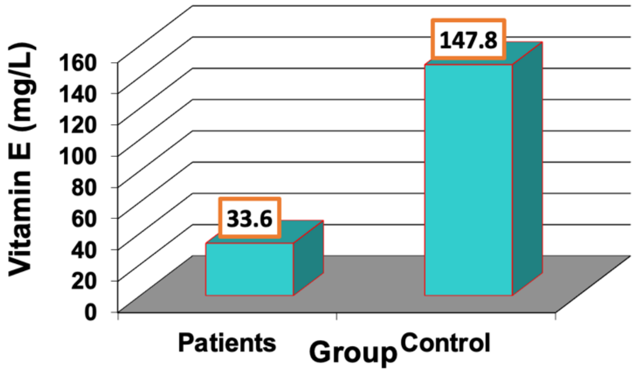

Figure (5): Comparison between patients and control in Vitamin E. Descriptive statistics were presented as Mean ± SD. P-value of ≤ 0.05 was regarded as statistically significant.

Besides having myriad effects on the female body, estrogen (E2) is a vital steroid hormone which is predominantly related to the process of regulating the female reproductive system, facilitating the regularity of the menstrual cycle, ovulation, and sustaining pregnancy. It also contributes to bone health, cardiovascular health and condition of skin. Elevated estrogen levels in patients may be associated with anovulatory cycles and is linked to the menstrual disorders including the menstrual irregularities and endometrial hyperplasia. The significant increase in estrogen levels in PCOS patients suggests altered estrogen regulation, which may contribute to menstrual irregularities and endometrial changes. These results validate the study done by *Amiri and Xu et al.* where this constitutional level of elevated estrogens in the PCOS patients is a sign of the hormonal disproportion which is frequently associated with a non-cyclical state of permanent elevated estrogens leading to anovulatory periods and is linked to the menstrual disorders including the menstrual irregularities and endometrial hyperplasia. The drastic difference implies that it is possible that PCOS subjects have alterations in their estrogenic functioning, and it correlates with reproductive and metabolic disorders (31,32) as shown in the figure (6).

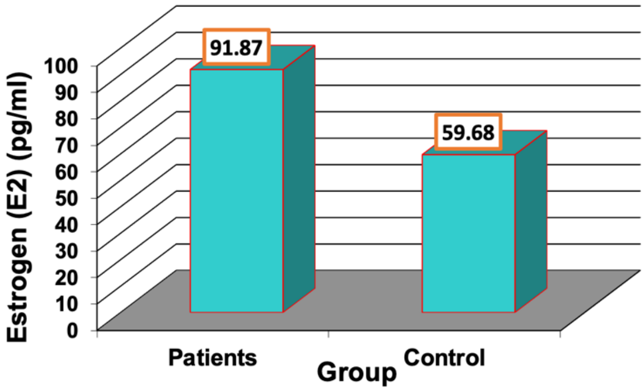

Figure (6): Comparison between patients and control in Estrogen (E2). Descriptive statistics were presented as Mean ± SD. P-value of ≤ 0.05 was regarded as statistically significant.

Additionally, insulin levels in PCOS patients average (25.00 ± 1.33 µIU/ml), which is significantly higher than the (2.3 ± 0.32 µIU/ml) observed in controls. The results agree with a study done by *Houston et al.* that the elevated insulin levels in patients indicate a compensatory response to insulin resistance, as the body produces more insulin to maintain normal glucose levels, (33) as shown in the figure (7).

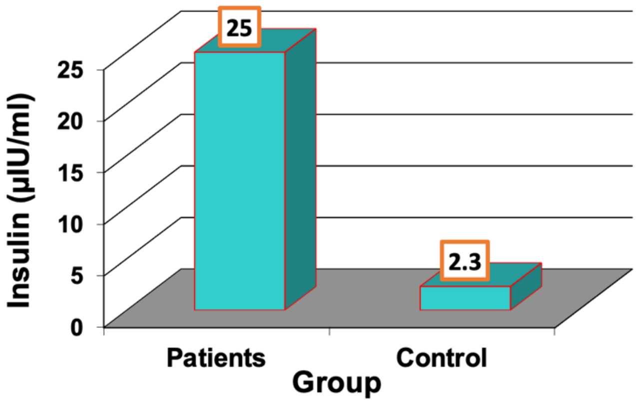

Figure (7): Comparison between patients and control in Insulin. Descriptive statistics were presented as Mean ± SD. P-value of ≤ 0.05 was regarded as statistically significant.

The fasting blood glucose was found to be significantly high in PCOS group (153.30 ± 13.90 mg/dl) when compared with healthy controls (102.88 ± 8.40 mg/dl). This change is consistent with the key metabolic dysfunction associated with PCOS, which is insulin resistance in the tissues (34). Hyperinsulinemia acts mechanically and blocks the production of SHBG in the liver, as well as the uptake of glucose by peripheral tissues, such as skeletal muscle and adipose tissue, resulting in a continuous compensatory hyperinsulinemia and hyperglycaemia. It is important to note, however, that some clinical trials report that young women (or non-obese women) with PCOS have normal fasting glycemic levels, and that the insulin resistance is characterized as post-prandial hyperinsulinemia of an oral glucose tolerance test (OGTT) instead of fasting hyperglycemia. This increase in fasting glucose in our cohort may reflect a more advanced level of metabolic dysfunction partly due to the multiple burden of underlying insulin resistance and, at the same time, systemic oxidative stress and micronutrient deficiencies as shown in the figure (8).

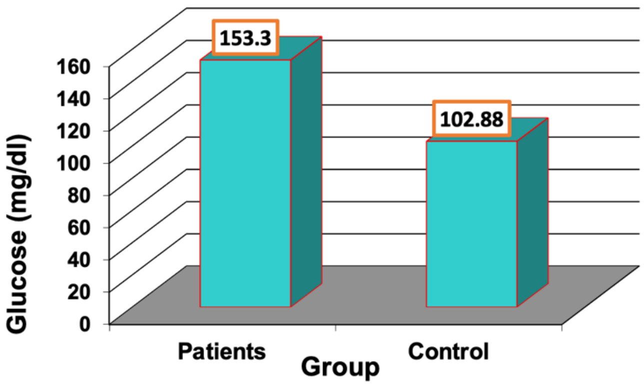

Figure (8): Comparison between patients and control in Glucose. Descriptive statistics were presented as Mean ± SD. P-value of ≤ 0.05 was regarded as statistically significant.

Thus, the insulin resistance determined using the HOMA-IR index was significantly higher in PCOS patients (8.62 ± 0.5) and significantly lower in the women of the control group (1.26 ± 0.08). Increased HOMA-IR in PCOS patients indicates reduced insulin sensitivity, a hallmark of PCOS, in contrast to our findings, *Lewandowski and Li et al*. discovered that these differences which were statistically significant, $P \leq 0.05$ indicate the marked metabolic derangement in PCOS as compared to control participants underscore the need for case-specific approach in the management of insulin resistance and glucose metabolism in women with PCOS [35,36] as shown in the figure (9).

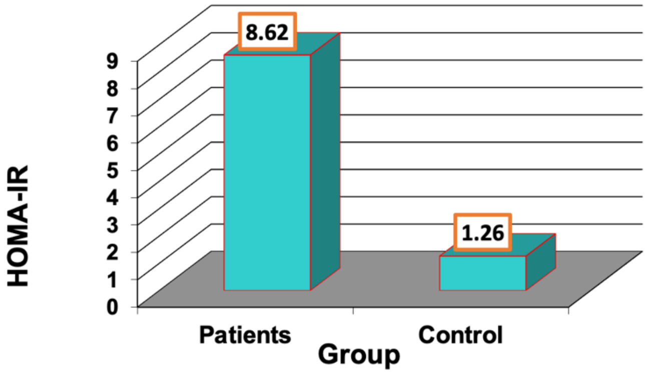

Figure (9): Comparison between patients and control in HOMA-IR. Descriptive statistics were presented as Mean ± SD. P-value of ≤ 0.05 was regarded as statistically significant.

LIMITATIONS

While this study offers valuable information on the different biochemical, hormonal and glycemic profiles of women with polycystic ovary syndrome (PCOS) compared to healthy controls, the following limitations should be taken into consideration. The clinical features in the PCOS group were characteristic of a high prevalence of adiposity and obesity-related metabolic dysfunction. The cross-sectional study design makes it difficult to make inferences about direct cause-and-effect relationships between micronutrient deficiencies and metabolic dysfunction. Second, the concentrations of the micronutrients in the circulation may not have been the only factors affected by unmeasured confounding variables such as detailed dietary intake, physical activity levels, and seasonal variation in sunlight exposure. Last, the findings may be limited to other, demographically more diverse populations because the participants were drawn from a single clinical centre. These observations warrant further studies in multi-center longitudinal designs to confirm and help to elucidate underlying mechanisms.

CONCLUSION

The main conclusion of this study is that women with polycystic ovary syndrome (PCOS) have a considerable decrease in the levels of important micronutrients (Vitamins D, C, B12, E, and Calcium) with a notable increase in fasting glucose, insulin, HOMA-IR, and estrogen levels compared to healthy controls. The results of this study indicate a high potential for concurrent micronutrient deficiencies and dysregulation of important systemic metabolic-hormonal pathways in PCOS, beyond the effects on local reproductive characteristics. But due to the observational nature of the study, a causal relationship cannot be concluded. Although optimization of metabolism and targeted micronutrient supplementation are promising adjunctive treatments, large-scale randomized controlled trials (RCTs) are needed to establish whether therapeutic micronutrient supplementation can have direct effects on glycemic control, insulin sensitivity, and long-term health in women with PCOS.

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CONFLICT OF INTEREST

The author(s) declare no conflict of interest, financial or otherwise.

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