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# The Impact of Vitamin B12 Deficiency on Reproductive and Metabolic Outcomes in Polycystic Ovary Syndrome

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## Abstract

Polycystic Ovary Syndrome (PCOS) is an endocrine disorder that has a wide range of clinical heterogeneity with a global prevalence from 5 to 13% in women of reproductive age. It has been progressively proposed that shortages of micronutrients like vitamin B12 are related to both metabolic and reproductive disorders in PCOS females Vitamin B12 plays a critical role in the metabolism of homocysteine, the methylation pathways and oocyte quality. On the contrary in B12 deficiency, the influence of deficiency can be seen on clinical features such as insulin resistance, gangrene, menstrual abnormalities, anovulation and dermal symptoms such as acne and hirsutism. Ninety-three women were assessed for the structured questionnaire and biochemical parameters such as serum B12, HOMA-IR, BMI and reproductive history from clinical and outpatient gynecology centers in Wasit Province, Iraq. In addition to greater menstrual cycle irregularities and lower parity, the low B12 group also had higher HOMA-IR and BMI as identified using ng-H. Nevertheless, an association was reported between metformin and menstrual irregularity, but it was relatively weak (OR = 2.17, 95% C. I: 0.60-7.91). Results contribute to the rising evidence that deficiency of vitamin B12 may exacerbate the clinical features of PCOS. Also, emphasize the importance of adding micronutrient screening, namely B12, to routine examination and management of PCOS, especially in long term Metformin users. Additional interventional studies on the impact of B12 supplementation on metabolic and reproductive outcomes among women with the condition.

**Keywords**: PCOS, Vitamin B12, Insulin Resistance, and Reproduction.

## 1. Introduction

PCOS (Polycystic Ovary Syndrome) is a multisystem disorder of heterogeneous etiology involving both reproductive and metabolic dysfunction and affects 5-13% of reproductive-age women worldwide [1, 2]. Three features are hyperandrogenism, ovulatory dysfunction, and polycystic ovarian morphology. Reproductive features of PCOS include infertility, menstrual irregularities, and impaired folliculogenesis. However, the syndrome is also linked intimately to many metabolic complications, including insulin resistance, obesity, dyslipidemia and an increased risk of type two diabetes mellitus [3, 4].

Human research investigating micronutrient status as a putative factor involved in PCOS pathogenesis and clinical expression is limited and largely unpublished. The mechanistic role of vitamin B12, particularly in DNA synthesis and methylation, energy metabolism, and in the regulation of homocysteine, has been emphasized as especially important of these [5-7]. In this context, B12 deficiency can disturb metabolic homeostasis which may contribute to the development of insulin resistance, a hallmark feature of the pathophysiology of PCOS [8, 9].

In addition, Vitamin B12 deficiency has been found to be associated with prolonged usage of metformin, used extensively as a first line treatment of insulin resistance in PCOS [10-12]. Thereby creating a feedback loop of energy metabolism either improving with a drug targeting insulin resistance or driving the drugs increased micronutrient depletion and therefore worsening the metabolic profiles over time. It has also been found that B12 deficiency might have an adverse effect on ovulation, oocyte quality and reproductive outcomes [13, 14].

Based on the findings, the relationship between Vitamin B12 and metabolic and reproductive dysfunctions associated with PCOS needs to be further investigated. For minimize the frequency of these disorders, the present study conducted to investigate the relationship among a clinical population of women diagnosed as PCOS in Wasit Province.

This work adds to the international evidence base on micronutrients and polycystic ovary syndrome (PCOS), providing region specific information by examining the relationship between serum B12 concentration and a range of biochemical and reproductive outcomes.

## 2. Methodology

Ninety-three women with Polycystic Ovary Syndrome (PCOS) aged between 18 and 40 years old were recruited from clinical and outpatient gynecology centers in different parts of Wasit Province, Iraq. Diagnosis of PCOS was established according to the Rotterdam criteria with a minimum of two features required which included oligo/anovulation, clinical and/or biochemical evidence of hyperandrogenism, and polycystic ovarian morphology on ultrasound. For avoid confounding effects, subjects with pregnancy, obstetric complications, known thyroid or adrenal disorders or receiving vitamin supplementation were excluded.

The questionnaire form was structured for each participant for assessment of sociodemographic background, menstrual history, fertility status, use of Metformin and clinical symptoms (i.e., acne and hirsutism). Body mass index (BMI) was calculated based on anthropometric measures (height and weight). Venous blood samples were obtained after an overnight fast to measure serum vitamin B12 levels (pg/mL) and to assess insulin resistance using the Homeostasis Model Assessment of Insulin (HOMA-IR).

### 2. 1 Insulin Resistance (HOMA-IR)

Descriptive statistics were used to examine broad trends, while comparative and inferential analyses (t-tests and chi-square tests) were used to assess if Vitamin B12 status was significantly associated with important metabolic (BMI, HOMA-IR) and reproductive outcomes (parity, menstrual irregularity). Statistical libraries in Python to perform data analysis, providing reproducible and accurate results.

## 3. Results

Metabolic and reproductive characteristics of women with PCOS were compared according to vitamin B12 status. Women with vitamin B12 deficiency demonstrated higher HOMA-IR values and lower parity compared with those with normal B12 levels as listed in table 3. Clinical features such as hirsutism, acne, and menstrual irregularity were also more frequent in the deficient group. Additionally, as illustrated in figure 1, BMI and HOMA-IR differed between the two groups, with higher insulin resistance observed among vitamin B12-deficient women.

**Table 1**: Clinical and metabolic characteristics based on Vitamin B12 status.

| Variable | B12 Deficient (n=51) | B12 Normal (n=42) | Interpretation |
|---|---|---|---|
| **Age (mean ± SD)** | 31.36 ± 8.95 | 33.00 ± 7.31 | Comparable age distribution across groups. |
| **BMI (mean ± SD)** | 26.19 ± 4.36 | 25.83 ± 4.87 | Slightly higher BMI in the deficient group. |
| **PCOS Duration (mean ± SD)** | 3.18 ± 2.14 | 3.46 ± 2.51 | Similar PCOS duration. |
| **HOMA-IR (mean ± SD)** | 2.99 ± 1.40 | 2.32 ± 0.97 | Higher insulin resistance in deficient group. |
| **Parity (mean ± SD)** | 1.27 ± 1.12 | 1.73 ± 1.07 | Lower fertility in deficient group. |
| **Menstrual Irregularity** | 34 cases (66.7%) | 27 cases (64.3%) | Slightly higher prevalence in deficient group. |
| **Hirsutism** | 41 cases (80.4%) | 25 cases (59.5%) | More prevalent in B12-deficient women. |
| **Acne** | 35 cases (68.6%) | 20 cases (47.6%) | Increased dermatologic symptoms with deficiency. |

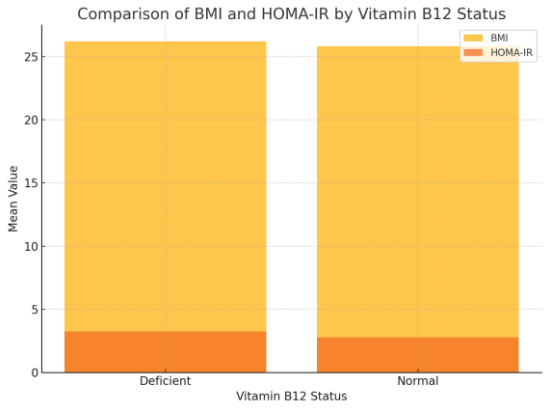


**Figure 1**: Comparison of BMI and HOMA-IR by Vitamin B12 status.

**Table 2**: Results of multiple linear regression analysis evaluating association of vitamin B12 levels and BMI with insulin resistance (HOMA-IR) in PCOS patients.

| Variable | Coefficient | P-Value |
|---|---|---|
| Constant | 4.07 | 0.0003 |
| Vitamine B12 | -0.0038 | 0.3518 |
| BMI | -0.0112 | 0.7003 |

A multiple linear regression analysis was performed to assess the determinants of insulin resistance (HOMA-IR) in women with PCOS as shown in table 2 and figure 2. These included vitamin B12 level and body mass index (BMI) as independent variables. Results showed that BMI was not significantly associated with HOMA-IR (p = 0.7003). HOMA-IR was weak and non-significantly negatively associated with vitamin B12 level (p = 0.3518) as presented in table 2.

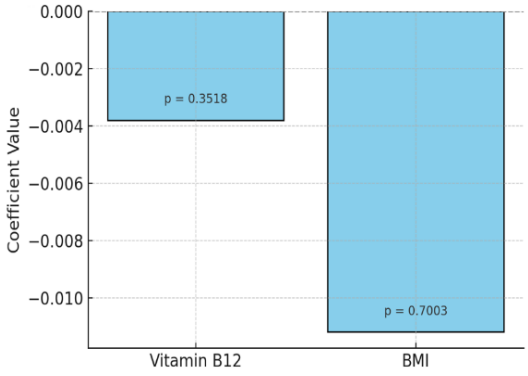


**Figure 2**: Regression coefficients and significance for predictors of HOMA-IR.

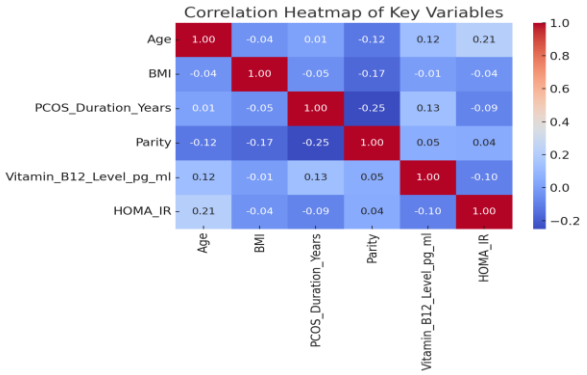


**Figure 3**: Correlation heatmap of key clinical and biochemical variables.

Findings indicate that although the relationship between insulin resistance and BMI is substantial. Vitamin B12 level contributes less to the estimation of insulin resistance in PCOS, more interventional studies may be warranted to investigate causality figure 3.

## 3. 1 Insulin Resistance (HOMA-IR)

Mean HOMA-IR was higher in women with B12 deficiency (2.99 ± 1.40), than in women with normal B12 (2.32 ± 0.97). This is consistent with previous work showing essential roles for Vitamin B12 in glucose homeostasis and insulin signaling. In deficient women, the increased insulin resistance severity may exacerbate PCOS symptoms and risk of metabolic syndrome.

## 3. 2 Body Mass Index (BMI)

The disparity in BMI between the two groups was minimal (26.19 vs 25.83) but the B12 deficient group having a higher mean weight indicates a trend towards weight gain which can worsen the symptoms sporadically seen in PCOS. The coexistence of obesity and B12 deficiency is common, due to faulty dietary diversity and malabsorption, especially in Metformin users [6].

## 3. 3 Reproductive Outcomes: Parity and Menstrual Irregularity

Fewer parities (1.27 vs 1.73) in women with B12 deficiency may relate to reduced fertility. Vitamin B12 is important for DNA synthesis and oocyte quality, and its deficiency can adversely affect conception rates. Rates of menstrual irregularity were high in both groups but slightly more common in the deficient group 66.7% versus 64.3%. Suggesting that other micronutrients, such as vitamin D, vitamin E, and magnesium, may also contribute to hypothalamic pituitary ovarian axis disturbance.

### 3. 4 Androgenic symptoms (Hirsutism and acne)

Compared with the B12-deficient group, hirsutism and acne were more frequent in the B12-deficient group 80.4% vs. 59.5%, and 68.6% vs. 47.6%, respectively. This may correspond to more advanced hyperandrogenic states considering high homocysteine levels along with increased inflammatory stress seen in B12 deficiency.

### 3. 5 Duration of PCOS

The average duration of PCOS was similar between groups 3.18 vs. 3.46 years, p = not significant. Suggesting that the differences were observed are likely due to B12, status rather than chronicity of the disease as shown in figure 4.

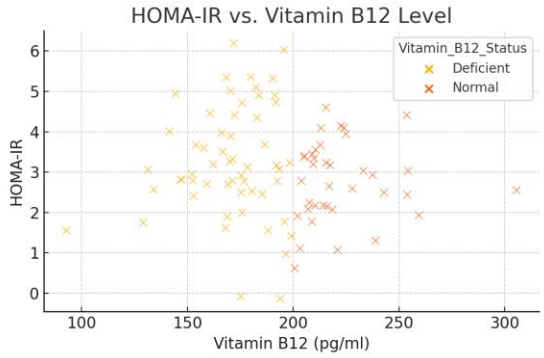


**Figure 4**: Relationship between HOMA-IR and Vitamin B12 levels.

### 3. 6 Clinical Implications

Findings indicate that vitamin B12 deficiency potentiates metabolic and reproductive dysfunctions in PCOS. This can potentially improve clinical outcomes as monitoring and managing B12 levels, particularly in patients on chronic Metformin therapy figure 5. Overall, this population might benefit from dietary interventions or supplementation early in life to mitigate insulin resistance and androgenic symptoms.

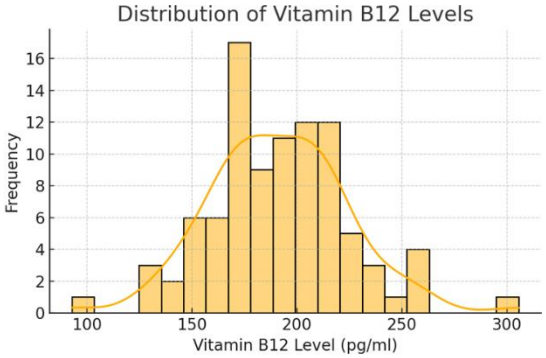


**Figure 5**: Histogram of Vitamin B12 levels among PCOS participants.

## 4. Discussion

No statistically significant independent effects of vitamin B12 level or BMI on HOMA-IR, women with vitamin B12 deficiency had always higher HOMA-IR and worse reproductive outcomes. The finding indicates that the possibility of a clinical use of vitamin B12 status in PCOS's metabolic profile and further studies in larger

prospective cohorts are needed. This result correlates with earlier research which found vitamin B12 to play a crucial role in metabolic pathways involved in insulin signaling, homocysteine homeostasis, and cellular function [15]. Given the clinical importance of insulin resistance as a central feature of PCOS pathophysiology. Vitamin B12 deficiency may further increase the metabolic and reproductive burden associated with the syndrome [16].

Results are also in line with a growing literature suggesting that micronutrient deficiencies, specifically B12, folate, and vitamin D, can impact hormonal dysregulation, follicle maturation, and ovulatory function in PCOS [14]. In this respect vitamin B12 is essential for one-carbon metabolism, a pathway that has effects on oocyte quality and early embryonic development [13]. One of the most concerning clinical implications of the study, the possibility that long-term use of Metformin decreases B12.

For women with PCOS, one of the most common medications given to help regulate insulin sensitivity and menstrual cycles is metformin. However, numerous studies have suggested that chronic Metformin therapy may reduce B12 absorption by inhibiting calcium-dependent mechanisms in the ileum [10-12]. Inadvertently, along with the weight loss, it may lead to an increase in insulin resistance and other metabolic disturbances, particularly if B12 deficiency remains undiagnosed and untreated. In addition, although this cohort did not reveal a statistically significant association between Metformin use and menstrual irregularity, the very high prevalence of reproductive symptoms in our B12-deficient subjects may have masked additional associations that would have emerged with a more granular clinical evaluation.

B12 deficiency may also play a direct and independent role in the pathogenesis of menstrual dysfunction in large part through its impact on hypothalamic-pituitary-gonadal axis regulation [17]. These results are of high relevance to clinical practice and public health. Authors suggest measuring serum levels of Vitamin B12 in every PCOS patient, at least once in a year, and particularly in the patients who need long-term Metformin therapy. Nutritional counseling and tailored supplementation strategies may be necessary for metabolic but also reproductive benefit [18, 19].

In addition, recent findings have shown that there is a link between diet and gut microbiota with metabolic and endocrine changes in women with PCOS, underscoring

the role of nutrition in the pathogenesis of the disease [20]. While the cross-sectional nature of this study limits the ability to infer causality, it provides important baseline data for future longitudinal or interventional studies. It also contributes regional evidence from Iraq, where data on micronutrient status in PCOS is currently limited.

## 5. Statistical Analysis

Statistical analysis was performed using Python (version 3.x) with statistical libraries including Pandas, NumPy, SciPy, and Statsmodels. Descriptive statistics were expressed as mean ± standard deviation (SD) for continuous variables and frequencies with percentages for categorical variables. Comparisons between groups were conducted using the independent samples t-test for continuous variables and the Chi-square test for categorical variables.

A multiple linear regression analysis was performed to evaluate the predictors of insulin resistance (HOMA-IR), including vitamin B12 level and body mass index (BMI) as independent variables. A p-value < 0.05 was considered statistically significant.

## 6. Conclusion

Vitamin B12 is deficient in large number of women with PCOS in the studied population with a significant number having suboptimal levels of vitamin B12. HOMA-IR was found to be higher in women with lower levels of vitamin B12, which may indicate a relationship between vitamin B12 status and metabolic dysfunction. The regression analysis did not show this relationship to be statistically significant. These results suggest vitamin B12 might have an influence on glucose metabolism and insulin sensitivity and are worthy of further study in larger prospective and interventional trials.

Background Women with B12 deficiency had higher Body Mass Index (BMI) and lower parity compared to those with normal B12 levels. Suggesting a connection to metabolic and reproductive dysfunction. Menstrual irregularities again seem to act merely as markers of the related manifestation of PCOS that inherited where no statistically significant association between Metformin and menstrual irregularities is seen giving way to the fact that there are other contributing physiological or environmental weight related factors responsible for the various manifestations resulting due to the polycystic ovarian variant that inherited.

A low and non-significant association between BMI and Vitamin B12 levels suggests that B12 deficiency is not simply

explained by measurement of adiposity alone in this group of individuals. B12-deficient women, compared to non-deficient women, were also significantly more likely to have clinical symptoms such as hirsutism and acne, although this did not reach statistical significance in the present analysis. The analysis of the graphical representation showed a broad distribution of the serum B12 levels, which may reflect individual dietary habits, medication history or absorption problems.

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