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ORIGINAL PAPER

Exploring the role of B complex vitamins in reproductive health – valuable insights and unresolved issues regarding premature ovarian failure

Dena A. Hashim [1], Alaa Sh. Abdulbari [2], Noor M. Ali [1], Alaa Hussein J. Al-Qaisi [1], Ahmed Rahmah Abu-Raghif [3], Hayder Ridha-Salman [4]

[1] Department of Chemistry, College of Sciences, Al-Nahrain University, AL-Jadriya, Baghdad, Iraq
[2] Department of Applied Chemistry, College of Applied Sciences, University of Technology-Iraq, Baghdad, Iraq
[3] Department of Pharmacology, College of Medicine, Al-Nahrain University, Baghdad, Iraq
[4] Al-Mustaqbal University, College of Pharmacy, Hillah, Babylon, Iraq

ABSTRACT

Introduction and aim. Premature ovarian failure (POF) is a condition distinguished by a decline in ovarian follicles in women under 40 leading to a disruption in the normal menstrual cycle and fertility. Vitamin B_6 (pyridoxine) and vitamin B_{12} (cyanocobalamin) play an important role in ovarian health. The aim was to investigate the effects of vitamins B_6 and B_{12}, for predicting POF via the association of these vitamins with other biochemical parameters.

Material and methods. A total of 80 participants with age ≤ 40 years, 40 POF women, and 40 fertile control women were recruited in this study from November 2023 to March 2024. Serum levels of prolactin (PRO), thyroid hormones, estradiol (E-2), progesterone, follicle stimulating hormone (FSH), luteinizing hormone (LH), 17-alpha hydroxylase, and inhibin alpha (INH-α) were measured using the ELISA technique while vitamins B_6 and B_{12} were measured by HPLC.

Results. Serum levels of reproductive hormones (LH, E2, progesterone, and testosterone), vitamin B_6, B_{12}, T_3, T_4, 17-alpha hydroxylase, and INH-α were markedly reduced in POF patients, while levels of FSH, TSH, and PRO were significantly elevated compared to healthy controls.

Conclusion. This study highlights the critical role of vitamins B_6 and B_{12} in the pathophysiology of POF, suggesting their potential as biomarkers for diagnosis and prognosis.

Keywords. 17-alpha-hydroxylase, inhibin-alpha, premature ovarian failure, vitamin B_6, vitamin B_{12}

Introduction

Premature ovarian failure (POF) is a condition that affects women's reproductive health, with its occurrence becoming more prevalent in recent years.[1] POF, often referred to as premature menopause, occurs when a woman experiences cessation of ovarian activity and amenorrhea before turning 40, along with the serum FSH level that rise to the menopausal range. This condition can result in infertility and can cause both physical and emotional challenges.[2-4] Women with POF face a higher risk of psychological disorders, cardiovascular problems, osteoporosis, autoimmune diseases, cognitive impairment, and infection of the urinary and reproductive systems compared to those without the condition.[5-7]

Corresponding author: Hayder Ridha-Salman, e-mail: hayder80.ridha@gmail.com

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Ovarian follicles are composed of three types of cells: granulosa cells, oocytes, and theca cell. Granulosa cells and oocytes contain follicle-stimulating hormone (FSH) and luteinizing hormone (LH) receptors, which play a critical role in follicular growth and development.[8-10] Folliculogenesis is a structured process involving the maturation of follicles in preparation for ovulation.[11-13] However, this process is disrupted in cases of POF.[1,14,15] Biochemically, POF is defined by reduced levels of gonadal hormones, such as estrogens and inhibins, accompanied by elevated gonadotropins (LH and FSH), a state referred to as hypergonadotropic amenorrhea. This increase in FSH is typically more pronounced than that of LH, with FSH levels exceeding 30 IU/L serving as a diagnostic indicator of ovarian failure.[16-19]

Genetic, immunological, metabolic, viral, and medicinal factors all contribute to the etiology of POF.[2,20-23] B complex, as water-soluble vitamin, is a critical compound for overall cellular metabolism.[24] Pyridoxine ($B\text{-}_6$) plays an essential role in the regulation of thyroid and sex hormones, the promotion of ovarian maturation, and the production of vital female hormones such as estrogen and progesterone. It is believed to regulate the activity of various steroid hormone receptors at the transcriptional level.[25] Cobalamin ($B\text{-}_{12}$) is a corrin-based compound containing a corrin ring and plays a critical role in human somatic cell metabolism.[26] $B\text{-}_{12}$ is essential for nervous and reproductive systems, supporting normal RBC formation, growth, synthesis of DNA, as well as amino acid, fatty acid, and cellular energy metabolism.[27-29] Once inside the cell, $B\text{-}_{12}$ is converted into two active forms: adenosylcobalamin and methylcobalamin. Methylcobalamin functions as a coenzyme for methionine synthase, which plays vital role for metabolism of methionine-homocysteine, commonly referred to as the cycle of methionine. $B\text{-}_{12}$, a key player in one carbon metabolism, is a complex metabolic factor essential for human reproductive health.[30] Vitamins, particularly $B\text{-}_{12}$, are crucial for reproductive health, promoting organ function and mitigating oxidative stress, pro-inflammatory cytokines, and circulating homocysteine levels.[31-33] Homocysteine acts as a metabolic linker that hinders the synthesis of intracellular antioxidants. It inhibits key antioxidant enzymes, such as superoxide dismutase and glutathione peroxidase.[34] A subclinical deficiency of $B\text{-}_{12}$ impairs the conversion of excess homocysteine to methionine, leading to an increase in homocysteine levels. This, in turn, promotes the production of H_2O_2, which contributes to the accumulation of reactive oxygen species (ROS) accumulation.[35-37] Oxidative stress, caused by an imbalance between the generation of free radicals and antioxidant levels, can cause detrimental transformation of mitochondrial DNA and protein damage, inevitably provoking cell death.[38-41] Methionine serves as a key precursor for S-adenosyl-methionine, an avital compound required for the methylation of phospholipid, neurotransmitters, amines, DNA, RNA and myelin basic protein.[42,43] A reduction in S-adenosyl-methionine levels can disrupt DNA methylation, potentially altering fetal metabolic programming and increasing susceptibility to non-communicable diseases later in life.[44] Consequently, $B\text{-}_{12}$ deficiency may lead to homocysteine buildup and reduced circulation methionine, impacting reproduction, as well as protein and DNA methylation.[45,46] The disruptions in redox balance, DNA methylation, and mitochondrial function are particularly detrimental to ovarian tissue, which is highly sensitive to oxidative stress. Granulosa cells, essential for follicle maturation, are especially vulnerable. Experimental studies have shown that elevated homocysteine levels promote apoptosis in granulosa cells, contributing to follicular atresia. Kaplan and Türk showed that B-12 administration reduced histological markers of tissue damage in rat ovaries by reducing oxidative stress.[34] Additionally, Deniz et al. revealed that treatment with $B\text{-}_{12}$ was effective in decreasing inflammation and oxidative stress caused by ovarian ischemia-reperfusion by downregulating the TLR-4/NF-kB signaling pathway, which is associated with inflammation.[47]

The enzyme 17-alpha hydroxylase is expressed in the adrenal glands and gonads, serving as a key branching point in the synthesis of cortisol and sex hormones. This enzyme facilitates two distinct steps in steroidogenesis. Individuals with deficiency in this enzyme commonly present with hypertension, hypokalemia, amenorrhea and absent of secondary sexual characteristics.[48,49] Inhibin-alpha (INH-α)Plays a role in regulating follicle development by responding to increased levels of FSH in gonads, which in turn triggers negative feedback to the anterior pituitary, reducing circulating FSH levels.[50-52] The interaction between vitamins of the B complex, particularly $B\text{-}_6$ and Cobalamin ($B\text{-}_{12}$), and the risk of POF remain intricate and not yet fully understood.

Aim

This study aims to investigate the correlation between vitamin $B\text{-}_6$ and $B\text{-}_{12}$ levels and POF. More research is required to elucidate their roles and potential therapeutic applications.

Material and methods

Subjects

In this study, 80 participants aged 40 years or under were split into two groups: group one consisted of 40 women with POF, whereas group two included forty healthy fertile women as a control group. From November 2023 to March 2024, POF patients were collected from the Higher Institute of Infertility Diagnosis and Assisted Reproductive Technologies located in Baghdad, Iraq. Women with an unclear etiology of POF, autoimmune

diseases, and pelvic surgery were excluded. The research was approved by the University of Technology-Iraq Bioethics Committee on Scientific Research, approval no. BCSR11, on March 24, 2024.

Methods

Blood samples were collected from two groups: the first group (healthy control) on the second day of their menstrual cycle and the second group (POF group) under conditions appropriate to their health status. For each participant, 5 mL of peripheral blood was drawn and centrifuged for 5 minutes at room temperature.

ELISA methods technique

The serum obtained was analyzed for hormone levels including FSH, LH, thyroid hormones, prolactin (PRO), progesterone, estradiol (E-2) and testosterone, 17-alpha hydroxylase and INH-α using the ELISA technique (CUSABIO BIOTECH COM), E-2, INH-α, and progesterone measured used competitive inhibition ELAZA kits, the microtiter plate included in the kits is pre-coated with goat anti-rabbit antibody, standards and samples were placed in designated wells along with specific antibody for these hormones and horseradish peroxidase (HRP) conjugated antigen.[53-55] A competitive reaction occurs between HRP labeled to target hormones and the unlabeled form of hormone for antibody binding. A substrate solution is then introduced, leading to color development inversely proportional to hormone concentration in the sample.[56-59] The reaction halted and the color intensity is measured, while the other tests was measured by used quantitative sandwich ELAZA kits, the microplate included in the kits is pre-coated with antibody specific for target hormone, Standards and samples were added to wells along horseradish peroxidase (HRP)-conjugated antibody targeting hormone. After washing to remove unbound reagents, a substrate solution is introduced, resulting in color development inversely proportional to the hormone concentration bound in the initial step.[60-62] The reaction is stopped and the color intensity is measured.

HPLC technique

Vitamin B_6 and B_{12} concentrations were measured using the HPLC technique, the HPLC Autosampler SyKNM from Germany S5200 pump model S1122 Column oven S4011, and a UV detector S3240 set at wavelength 230 nm with a C-18 column., using acetonitrile (A) and NaH2PO4 (B), with a gradient of 100% (B) at 3 minutes, 80% (B) at 6 minutes, and 60% (B) at 15 minutes. The flow rate remained constant at 1.0 mL/min.[40,63,64] Standard vitamins B_6, B_{12} were sourced from Sigma. Creating a standard solution began with dissolving 10 mg of each of vitamins B_6 and B_{12} in methanol to form a 100 parts per million (ppm) stock solution, diluted to 100 mL, then the stock solution was further diluted to prepare other standard solutions by subsequent dilution of the stock solutions.[65-67]

Post hoc power analysis

The results of the post hoc power analysis indicate that the study had a very high ability to detect differences in testosterone levels between the control and POF groups. The mean testosterone level in the control group was 0.47 with a standard deviation of 0.22, while the mean in the POF group was 0.09 with a standard deviation of 0.02. The effect size, calculated using Cohen's d, showed a very large difference between the two groups, reaching 2.55, which reflects a significant variation in testosterone levels. The sample size consisted of 40 individuals in the control group and 40 in the POF group, further strengthening the reliability of the study. The post hoc power reached 1.0 (100%), which means that the study was fully capable of detecting any real effect if it existed, which is a strong indicator of the statistical reliability of the results.

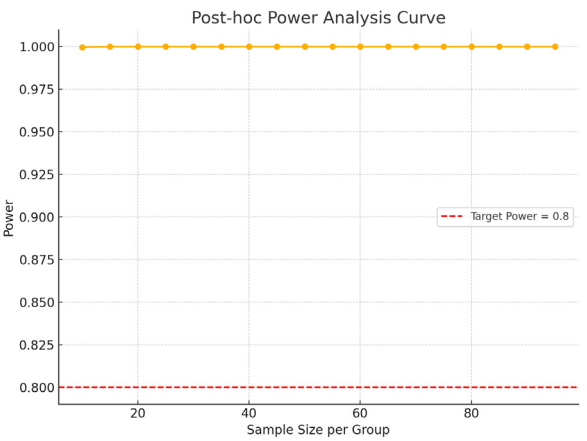

Fig. 1. Post hoc power analysis curve: effect of sample size on statistical power

The post hoc power analysis Figure 1 shows that the study has a very high power to detect differences between the two groups, with the statistical power remaining close to 1.0 (100%) even with small sample sizes. The horizontal axis represents the sample size per group, while the vertical axis reflects the level of statistical power. The orange line indicates how the power remains consistently high across different sample sizes. On the contrary, the red dashed line represents the minimum acceptable power level of 0.8, commonly used as a benchmark for detecting real effects in scientific studies. This result means that the survey can detect any actual differences between the groups, due to the enormous effect size (Cohen's d = 2.55), making the likelihood of a Type II error (failing to detect an actual effect) almost nonexistent.

Statistical analysis

All statistical analyses in this study were conducted using statistical Package for Social Science (SPSS) version 29.0 windows (SPSS, Inc., Chicago, IL USA). Descriptive statistics were expressed as mean±standard deviation (SD). Differences between mean values were evaluated using Student's t test, with significance threshold set at $p< 0.05$, while $p>0.05$ was considered nonsignificant.

Results

Table 1 presents the biochemical profiles of the studied groups, highlighting highly significant differences ($p<0.001$) between the POF group and the control. Serum levels of reproductive hormones LH, E2, progesterone, and testosterone were markedly reduced ($p<0.001$) in POF patients, while levels of FSH and PRO were significantly elevated compared to healthy controls. Furthermore, thyroid hormones (T3 and T4) showed a significant decrease ($p<0.001$) in the POF group, accompanied by an increase in TSH levels compared to the control group. Table 2 and Figure 1 highlight significant differences in levels of $B\text{-}_{12}$, $B\text{-}_{6}$, and INH-α between the two studied groups: POF and healthy controls. These levels were markedly lower in POF patients compared to the control group.

Table 1. The mean (±SD) of age and hormones for the control and POF groups

Characteristic	Mean±SD		95% CI	p
	Control group (n=40)	POF group (n=40)		
Age (year)	32.07±5.370	32.92±4.26	-3.00–1.30	0.43
FSH (mIU/mL)	3.40±1.10	38.64±11.59	-38.95– -31.51	<0.001
LH (mIU/mL)	3.67±1.64	0.83±0.40	2.30–3.38	<0.001
E-2 (pg/mL)	160.31±38.76	10.93±2.31	136.9–161.76	<0.001
Progesterone (ng/mL)	2.27 ±2.03	0.06±0.062	1.56–2.86	<0.001
PRO (ng/mL)	14.62±4.09	38.25±7.25	-26.26– -20.99	<0.001
Testosterone (ng/mL)	0.47±0.22	0.09 ±0.022	0.31–0.45	<0.001
TSH (mIU/L)	2.75±1.11	8.18±1.87	0.34–6.1	<0.001
T_3 (nmol/L)	1.46±0.36	0.68±0.09	0.05–0.66	<0.001
T_4 (nmol/L)	122.99±23.82	40.49±15.62	4.50–73.50	<0.001

Table 2. The mean (±SD) of vitamin $B\text{-}_{6}$, vitamin $B\text{-}_{12}$, 17-alpha hydroxylase, and inhibin-α for the control and POF groups

Characteristic	(Mean±SD)		95 % CI	p
	Control group (n=40)	POF group (n=40)		
Vitamin $B\text{-}_{6}$ (ng/mL)	40.68±5.75	15.17±3.81	23.32–27.68	<0.001
Vitamin $B\text{-}_{12}$ (ng/mL)	30.68±5.75	5.45±3.42	23.11–27.37	<0.001
17-alpha hydroxylase (ng/dL)	50.68±47.17	0.032±0.018	35.56–65.73	<0.001
INH-α (ng/mL)	29.12±9.11	3.26±1.28	22.91–28.80	<0.001

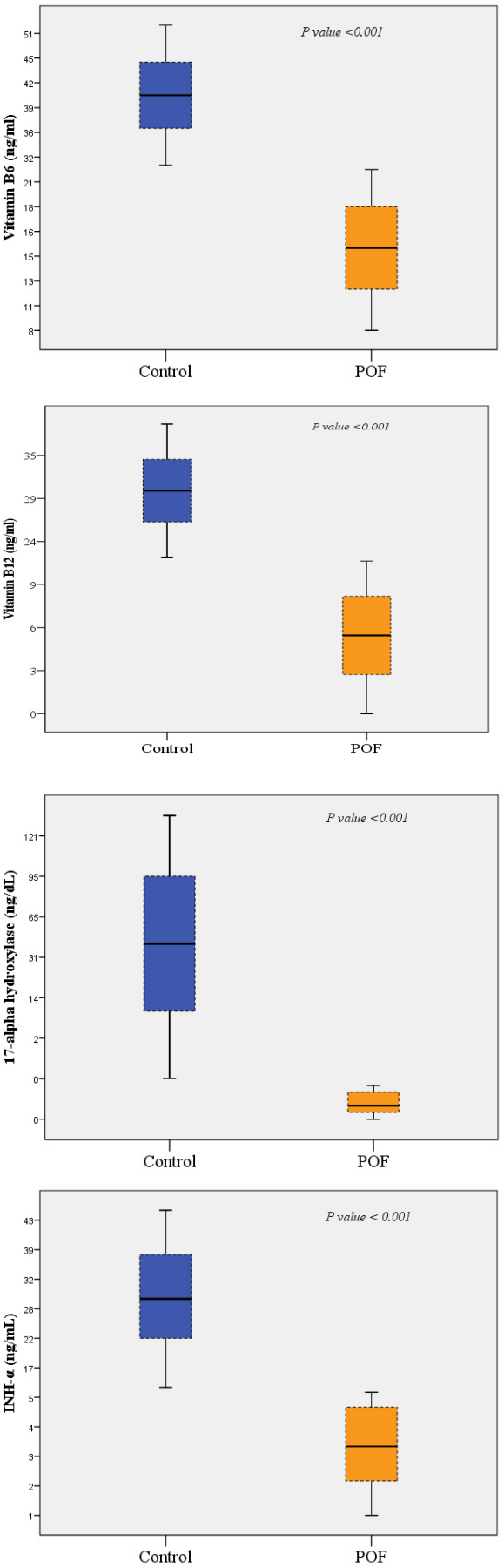

Fig. 2. Mean distribution of biochemical parameters in POF patients group and control group

Discussion

The eight water-soluble vitamins that make up the B-vitamin complex cooperate as coenzymes in an array of anabolic and catabolic enzyme activities to play vital roles in cellular activity.[24,32] Vitamin B_6 is necessary for the synthesis and metabolism of various hormones, including estrogen and progesterone, which is crucial for menstrual regularity, fertility, and overall reproductive health.[68] Several studies evaluated B-vitamin circulating levels in the context of neuroimmune diseases.[69-71] The present study revealed the evaluation of the effect of B_6 and B_{12} vitamins in POF patients and suggests that they are related to POF as well as hormonal dysregulation that occurs with ovarian disorders.

The current study demonstrated that B_6 and B_{12} vitamins were significantly lower in POF patients compared to healthy control. These results align with studies conducted by Brilliant et al.[72] and Kamrul-Hasan et al.[73], which also reported deficiencies of these vitamins in female reproductive disease, highlighting their potential role in ovarian health.

We did not account for dietary intake, supplementation, or lifestyle factors that could influence these levels. However, it is important to note that none of the participants in our study took vitamin supplements minimizing potential confounding effects for supplementation.

Antioxidant micronutrients, such as vitamin and minerals, are vital for female reproductive health as they help manage oxidative stress and facilitate essential cellular function.[74-76] Recent studies indicate that B vitamins could influence ovarian health and function. Specifically, B_{12} can help mitigate ovarian ischemia-reperfusion injury by reducing inflammation and oxidative stress.[47] Similarly, B_6, which is critical for reproduction, affects sulfur and seleno amino acid metabolism and may influence ovarian health and embryo development under oxidative stress.[77] Although not explicitly related to POF, vitamin B_6 has been shown to lower serum PRO levels in hyperprolactinemic patients, a condition relevant to reproductive health.[78] These findings hint at a relationship between vitamins and ovarian function, but more research is required to confirm these links and explore their therapeutic potential in POF.

17-alpha hydroxylase enzyme was significantly lower in POF group compared to the control group which agrees with certain theory reported that a lower level of 17-alpha hydroxylase enzyme alters ovarian functions and the production of sex hormones, as this enzyme is responsible for converting progesterone into cortisol.[79] Women with 17-alpha hydroxylase deficiency often experience infertility and related complications due to disruptions in the steroidogenic pathway. These abnormalities lead to impaired levels of androstenediones, 17-hydroxyprogesterone, and E-2.[80] Weak estrogen feedback to the pituitary may result in mildly elevated FSH and LH levels, increasing the risk of ovarian cysts, particularly multiple luteinizing ovarian cysts. Furthermore, increasing PRO levels may be due to increased GnRH activity. Another common reproductive endocrine manifestation in women with 17-alpha hydroxylase deficiency is deduced testosterone levels. However, in females, low testosterone has minimal impact on reproductive development and fertility. However, this characteristic can serve as a clinical indicator, emphasizing the need to consider 17-alpha hydroxylase deficiency in cases where pubic hair is absent or absent during gynecological examination. In general, partial 17-alpha hydroxylase deficiency presents with endocrine abnormalities that correspond to the degree of enzymatic dysfunction.[81]

The current study demonstrated a significant decrease in the INH-α ($p<0.001$) in POF group compared to the control group. Ovarian follicle granulosa cells are the main producers of INH-α. Blocks the ability of the pituitary gland to secrete FSH, which is its primary role. INH-α is produced by mature and developing follicles during a normal menstrual cycle. This hormone works with the pituitary gland to decrease FSH levels.[82] In accordance with this theory, the reduced level of INH- α level in patients with POF increases the release of FSH by eliminating the inhibitory feedback of the pituitary gland. Several studies have reported a lower serum inhibin in individuals with POF.[83,84] This deficiency is believed to contribute to disease development, as it leads to increased activation activity. In turn, activation stimulates FSH secretion, accelerating folliculogenesis and speeding up ovarian follicle depletion, which ultimately results in early menopause.[85] Genetic variation in genes responsible for inhibin production has been associated with its reduced serum levels, which may amplify activation effects due to diminished inhibin secretion.[85] Previous study identified INH-α (rs12720062 G / A) and showed genotype GG and allele G as potential risk factors for POF, while genotype GA and allele A appear productive. However, genotype AA does not seem to have a significant influence on disease susceptibility.[86] The reduced levels of 17-alpha hydroxylase and INH-α observed in POF patients' highlights their critical roles in maintaining ovarian function l. While direct evidence linking vitamin B_6 and B_{12} to the synthesis of these proteins is limited l, their fundamental roles in enzymatic reactions and gene expression suggest that deficiency could adversely affect ovarian health. More research is necessary to elucidate these relationships and determine whether supplementation could serve as a therapeutic strategy for POF. Given the observed association between vitamin B_{12} and B_6 levels and POF, it is essential to explore whether correcting such deficiencies by targeted supplementation might influence disease progression, severity of symptoms, or fertility

outcomes of affected women. Randomized controlled trials are recommended to evaluate this hypothesis.

Reduced PRO, thyroid hormones (T_3, T_4), LH, E2, and progesterone in patients with POF due to ovarian dysfunction and altered hormonal functions can all contribute to the development of POF or exacerbate its symptoms. Support this theory; a study reported that infertility in women is indicated by the significant positive correlation TSH has with PRO, FSH, and LH. When diagnosing infertility, these hormonal assessments enable a standard etiological approach.[87,88]

Our participants were recruited from Baghdad, which may limit the generalizability of the findings. Future studies should consider the possible impact of ethnic and genetic diversity on vitamin metabolism and ovarian health.

Study limitations and recommendations

This study provides a cross-sectional initial snapshot of biochemical parameters in POF patients and does not establish causality. A key limitation of this study is the relatively small sample size (80 participants), which may affect the statistical power and generalizability of the results. Although our findings provide valuable information on the possible association between B-$_6$ and B-$_{12}$ and POF, larger-scale longitudinal studies involving more diverse populations to validate these observations. Further research should incorporate dietary assessment, supplement intake, physical activity, and genetic analysis to provide a more comprehensive understanding of the factors that influence vitamin levels in POF. These unmeasured variables could introduce bias and limit the generalizability of the findings. Another major drawback is the lack of population diversity; more research involving participants from multiple centers is necessary to improve external validity in examining possible environment-gene interactions that can affect POF risk. However, the existing literature suggests that adequate levels of vitamin B are necessary for women's health, especially ovarian function, but also that further investigated studies are required to determine their therapeutic role in POF management.

Conclusion

The study findings revealed a significant deficiency in vitamin B-6, B-12, INH-α, and 17-alpha hydroxylase levels in patients with POF. These results suggest that POF is associated with disturbances in vitamin B-6 and B-12, as well as dysregulation of INH-α and 17-alpha hydroxylase production. These biochemical abnormalities may contribute to the pathogenesis and clinical manifestations of POF. More research is needed to elucidate the underlying mechanisms and explore potential therapeutic interventions that target these deficiencies, aiming to improve the diagnosis and management of POF patients.

Declarations

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Author contributions

Conceptualization, D.A.H. and A.S.A.; Methodology, N.M.A., A.H.J.A. and A.R.A.; Software, H.R.-S.; Validation, H.R.-S., A.R.A. and A.H.J.A.; Formal Analysis, D.A.H.; Investigation, N.M.A. and A.H.J.A.; Resources, D.A.H. and A.S.A.; Data Curation, A.H.J.A.; Writing – Original Draft Preparation, A.S.A.; Writing – Review & Editing, A.R.A. and H.R.-S.; Visualization, N.M.A.; Supervision, A.R.A. and H.R.-S.; Project Administration, N.M.A.; Funding Acquisition, D.A.H. and A.S.A.

Conflicts of interest

The authors declare that they have no conflict of interest.

Data availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Ethics approval

The study protocol was approved by the University of Technology-Iraq Bioethics Committee (approval no.: BCSR11) on 24 March 2024. All procedures were in accordance with internationally recognized, ethical standards, particularly those in the Declaration of Helsinki.

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