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Measuring the Levels of AMH, FSH, LH, TSH, Progesterone, Estrogen, Vitamin D, Calcium, and Magnesium in Women with Premature Ovarian Insufficiency

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Abstract This research was designed to study some hormonal and physiological aspects of women with Premature Ovarian Insufficiency (POI). Blood samples were collected from the Infertility and Gynecology Unit at Al-Batoul Teaching Hospital in Baqubah and private women's clinics, including ages between 20 and 40. The study took place from 12/1/2022 until 5/1/2023, and informed consent was obtained from all patients. The study included 45 women with premature ovarian insufficiency. It was compared to a control group that consisted of 45 healthy women after confirming their fertility, at ages ranging from 20 to 40 years.
The study investigated the effects of specific physiological and biochemical parameters on the disease. This was done by examining hormone levels and various biochemical levels in women with premature ovarian insufficiency. Some of the hormones examined were anti-Mullerian hormone (AMH), follicle-stimulating hormone (FSH), luteinizing hormone (LH), thyroid-stimulating hormone (TSH), progesterone, and thyroid hormone. The levels of vitamin D, calcium, and magnesium were also measured. The results of the current investigation showed that the age groups had a significant difference ($P<0.05$) with the study groups. (patients and healthy people). The age groups 31-35 and >35 years recorded the highest percentages (33.3% and 31.1%) in female patients compared to the groups <20-25 and 26-30 years, which recorded the lowest percentages (15.6% and 20.0%), respectively.
The outcomes of the current investigation regarding the concentration of reproductive hormones showed a significant difference. ($P<0.05$) between the AMH, FSH, and LH hormone levels in the two study groups (patients and healthy individuals). The levels of FSH and LH were higher in patients (6.780 ± 15.54 and 5.44 ± 12.40) compared to healthy individuals (4.75 ± 6.51 and 4.04 ± 0.94), respectively. On the other hand, AMH levels were lower in patients (0.18 ± 0.42) compared to healthy individuals (0.92 ± 3.15). In terms of TSH, E2, and Progesterone hormone levels, there were no significant differences ($P>0.05$) between the two study groups.
About the physiological investigation that included measuring the concentration of magnesium, calcium, and vitamin D, the current study demonstrated a significant difference ($P<0.05$) in the concentration of magnesium between the two groups. (patients and healthy people). The level of magnesium was low in patients (0.29 ± 0.75) compared to healthy people (0.30 ± 2.07). There was also a statistically significant difference ($P<0.05$) in the level of calcium concentration between the two study groups (patients and healthy people). The calcium level was high in patients (0.45 ± 8.33) compared to healthy people (0.58 ± 8.02). Also, significant differences ($P<0.05$) in the levels of vitamin D were observed between the two groups of study (patients and healthy individuals). The levels of vitamin D were low in the patients (8.62 ± 18.88) compared to the healthy people (11.53 ± 44.15).

Key Words Premature ovarian insufficiency, POI , Vitamin D, Hormones, Calcium, Magnesium, POI

1. Introduction

Also known by other terms (which are sometimes called premature/early menopause, primary insufficientness, and premature failure of the ovaries), there is still limited agreement on the proper term [1]. As a result, premature ovarian failure (POF) is a condition that is derived from the dysfunction or early depletion of the group of ovarian follicles, accompanied by a loss of fertility that is greater than usual in young women (under the age of 40 years). Premature ovarian failure or menopause can be natural or man-made. If the illness occurs, it is possible that it was caused by medical procedures like chemotherapy or surgical procedures like surgery that removed both ovaries [2].

Hormones are the most significant component of the body due to their influence on human behavior, particularly regarding sex hormones. They are accountable for the reproduction procedure and its subsequent development, thus having a significant role in the continued life and production of new individuals. The hypothalamic-pituitary-ovarian axis is responsible for the development and completion of the Follicle. The process of follicular development is complete in the ovaries, which produce the egg and promote ovulation [3]. The hypothalamic gland has a significant role in controlling the menstrual cycle via its release of Gonadotropin-releasing hormones (GnRH), which causes the pituitary to release Follicle stimulating hormones (FSH), Luteinizing hormones (LH), and Prolactin hormones.

FSH and LH are hormones secreted by the anterior portion of the pituitary in response to the release of GnRH from the hypothalamus [4]. FSH is dedicated to the growth and development of the ovarian follicles. LH is responsible for ovulation from the ovary after the follicles have matured; this is sometimes referred to as the ovulation hormone, Luteinizing hormone [5]. Regarding the anti-Müllerian substance (AMH), it reduces the sensitivity of the ovarian Follicle to the hormone FSH and is responsible for controlling sex determination in the fetal stage. In males, it activates the formation of the male tract and inhibits the formation of the female tract, and vice versa. As for the hormone estrogen, it works on the growth of the reproductive organs. Primary and secondary sexual characteristics usually develop. The hormone progesterone works on the growth and development of endometrial cells and reduces the occurrence of uterine contractions during pregnancy. This also facilitates the preparation of the uterus for the baby's arrival [4]. The estrogen hormone is involved in the reproduction of both females and males and other biological systems, including the neuroendocrine, vascular, skeletal, and immune systems. As a result, it is also involved in multiple diseases, including infertility, obesity, osteoporosis, and cancer [6].

Vitamin D is considered a steroidal hormone primarily produced by the skin in response to sunlight exposure, with less than 20% derived from dietary sources. Vitamin D is a precursor to several hormones that are soluble in fat. Thus, it may have a role in regulating the female reproductive system. Fat-soluble vitamins participate in multiple physiological processes. These vitamins have multiple functions, including being an antioxidant, a cofactor, and a precursor of steroid hormones. As a result, they have no adverse effects on the recipients. The ovary is considered one of these organs, and a deficiency of fat-soluble vitamins results in the premature failure of the ovary. At least some of the fat-soluble or protein-bound vitamins may be associated with POI [7].

Calcium is a vital nutrient that is necessary for the majority of human health functions. Calcium is the most common mineral in the body, with 99% of it found in teeth and bones. Many recent studies have reported that calcium is closely related to reproductive function. Calcium also helps activate signaling pathways related to reproductive function through its role as a secondary messenger. Calcium stimulates oocyte maturation, either spontaneously or via LH. This is considered to be due to calcium modulating intracytoplasmic cAMP concentrations [8]. Calcium is a mineral that has various reproductive benefits and may prevent POI [9].

Magnesium is an essential dietary mineral necessary for a healthy and functioning body. Estrogen deficiency may cause tissue loss of magnesium. During menopause, estrogen levels begin to decrease, which means magnesium levels decrease as well [10].

2. Materials and Methods

A. Sample Collection

Blood samples were taken from 90 Iraqi women, distributed as follows: 45 samples from patients diagnosed with premature ovarian insufficiency (POI) and 45 samples from women who do not suffer from POI. The samples were collected from the infertility and gynecology unit at Al-Batoul Teaching Hospital in Baqubah and private women's clinics. The ages ranged between 20 and 40 from 12/1/2022 until 5/1/2023, and informed consent was obtained from all patients.

B. Preparation of Blood Serum

(5 ml) of blood was drawn from the antecubital vein using a medical syringe at the Infertility and Fertility Department of Al-Batoul Teaching Hospital and private women's clinics. The blood was then placed in a special gel tube and left for a period ranging between (10-15) minutes. A minute later, the samples were centrifuged at a rate of 4000 revolutions per minute (r.p.m) for 15 minutes to obtain the serum. The serum was then distributed into small Eppendorf tubes, and several replicates were saved for each sample to prevent repeated dissolution. Lastly, the samples were stored at a temperature of -20°C until tests were conducted.

C. Measurement Hormone Levels in the Blood

The following hormones were measured: AMH, FSH, LH, TSH, Progesterone, and Estrogen at Al-Batoul Teaching Hospital - Infertility Unit. The Cobas E-411 chemical, hormonal, and tumor marker analysis device was used, which works according to the principle of electrochemiluminescence immunoassay. The blood sample was well separated. To obtain pure blood serum, it is then placed in tubes and inserted into the device. The required tests are determined, and the hormone results are compared with normal values.

D. Measurement of Vitamin D Concentration in Blood Serum

Vitamin D was measured using the Cobas E-411 chemical, hormonal, and tumor marker analysis device, which works

Age			Patients	P value
Age groups (years)	<20-25	n	7	P<0.05*
		%	15.6%	
	26-30	n	9	
		%	20.0%	
	31-35	n	15	
		%	33.3%	
	>35	n	14	
		%	31.1%	
Total		n	45	
		%	100.0%	

Table 1: Comparison of age groups between the two study groups

according to the principle of electrochemiluminescence immunoassay.

E. Measuring the Concentration of Calcium and Magnesium in Blood Serum

The calcium concentration was estimated using a ready-made kit from the Indian company Biotech, using the Semi-Auto chemistry Analyzer model BA- 88A from Mindray. This device is distinguished by its modernity and advancedness, as well as accurate results.

F. Statistical Analysis

Statistical analysis was conducted using SPSS v. 25, GraphPad Prism v.6, and Excel 2013. Ordinal and nominal data were described in number and percentage form, and comparisons between percentages were done using the chi-square test. The quantitative data was described as mean $\pm$ SD, and the arithmetic means were compared using the T and F tests. The least significant difference (LSD) test assessed the differences between the average numbers. Significant differences were considered significant at a significance level of $P < 0.05$.

3. Results and Discussion

A. Distribution Results of Samples According to Age

The results of the current investigation indicated that the age groups and study groups had a significant difference (P<0.05) in regards to their age, (Patients and healthy people). The age groups 31-35 and >35 years have recorded the highest percentages (33.3% and 31.1%) in patients compared to the groups <20-25 and 26-30 years, which have recorded the lowest percentages (15.6% and 20.0%), respectively, as shown in Table 1.

The results of the current study showed a high prevalence of premature ovarian insufficiency in the age groups 31-35 years and >35. The prevalence rates were 33.3% and 31.1% respectively, with a significant difference of P<0.05. This was proven in a study conducted by [11] in Turkey. The study found that the highest prevalence of premature ovarian insufficiency was in the age group <30, where it reached 34%. These findings are consistent with the results of a study conducted by [12] in Libya, which also showed that the highest prevalence of premature ovarian insufficiency is in the age groups 31-35 and 30.

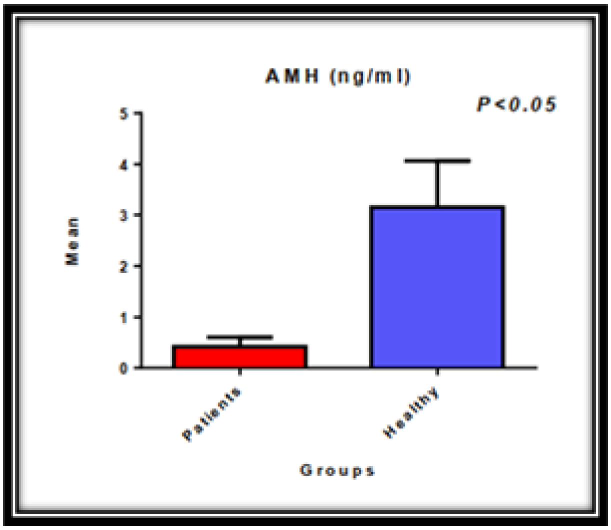

Figure 1: AMH level in the serum of females with premature ovarian insufficiency compared to healthy women

Clinical studies have shown that age is a significant risk factor for POI [13]. Normal ovarian function itself depends on age, as ovarian function decreases with age. Therefore, it is not surprising that older women have a higher incidence of complete infertility and permanent infertility, compared to their younger counterparts [14].

B. Hormonal Study Results

The results of the current investigation demonstrate that there are significant differences (P<0.05) in the levels of AMH, FSH, and LH hormones between the two groups of study (patients and healthy individuals). The concentrations of FSH and LH were elevated in patients. (6.780 $\pm$ 15.54 and 5.44 $\pm$ 12.40) compared to healthy people (4.75 $\pm$ 0.651 and 4.04 $\pm$ 0.94), respectively. As for AMH levels, they were lower in patients (0.18 $\pm$ 0.42) compared to healthy people (0.92 $\pm$ 3.15). As for the levels of the hormones TSH, E2, and Progesterone, they were not significantly different (P>0.05) between the two study groups, as shown in Table 2.

The concentration of the AMH-hormone decreased significantly in the patients' group with early ovarian failure. (Mean = 0.42 ng/ml) compared to the control group, and the result was consistent with what was shown by some research studies in this field [11], [15], [16]. As shown in Figure 1.

AMH is considered a beneficial and dependable marker of ovarian reserve, low levels may indicate low ovarian reserve, even if the woman has a regular period and normal FSH and E2 levels. [17] documented that the decrease in serum AMH levels in patients with POI is associated with deficiencies in granulocytes in the sinuses of the follicles, these cells are therefore involved in the production of AMH.

While the concentration of follicle-stimulating hormone (FSH) witnessed a significant increase in the group with

Groups		N	Mean	Std. Deviation	P value
AMH (ng/ml)	Patients	45	0.42	0.18	P<0.001***
	Healthy	45	3.15	0.92	
FSH (IU/ L)	Patients	45	15.54	6.78	P<0.001***
	Healthy	45	4.75	1.65	
TSH (mIU/ ml)	Patients	45	2.46	0.92	P>0.05
	Healthy	45	1.93	0.66	
LH (IU/L)	Patients	45	12.40	5.44	P<0.001***
	Healthy	45	4.04	0.94	
E2 (pg/ml)	Patients	45	49.61	17.57	P>0.05
	Healthy	45	51.17	15.23	
Progesterone (ng/ml)	Patients	45	0.66	0.29	P>0.05
	Healthy	45	0.84	0.33	

Table 2: Comparison of hormones between the two study groups

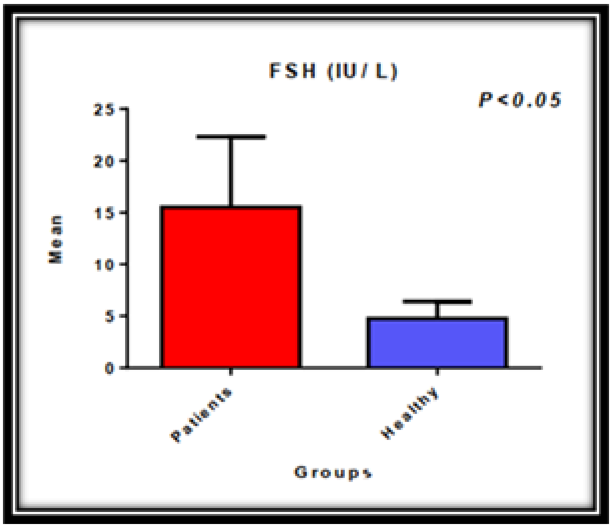

Figure 2: FSH level in the serum of females with premature ovarian insufficiency compared to healthy women

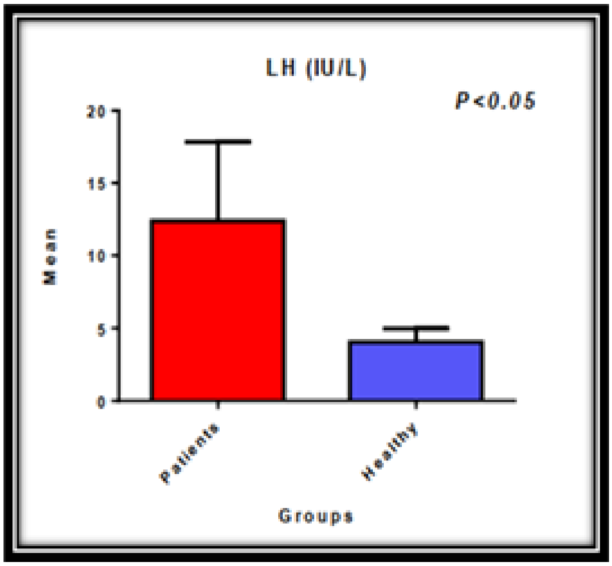

Figure 3: LH hormone level in the serum of females with premature ovarian insufficiency compared to healthy women

premature ovarian insufficiency (Mean = 15.54 IU/L) Compared to the control group, the graphite group demonstrated a higher degree of improvement, as shown in Figure 2. This research concurred with the investigations of [16], [18], [19].

A high concentration of FSH indicates ovarian dysfunction (hypothyroidism or hyperthyroidism). Either low or normal FSH levels indicate a defect in the pituitary gland or hypothalamus [20]. The high concentration of FSH shown by the results of the current study indicates a decrease in the reserve of early ovarian reserve or early impotence, and as a result, the woman becomes progressively less fertile [21]. Moreover, other reasons for high FSH may include autoimmune disorders such as hypothyroidism, adrenal gland disorders, chromosomal defects, intermittent use of oral contraceptive pills, pelvic inflammatory disease, miscarriage, chemotherapy, and treatment with radiology [21].

In this research, the concentration of the ovulation hormone LH in the serum of females with early ovarian failure was recorded to be elevated. The average concentration in the blood serum was 12.40 IU/L, compared to the control group with an average concentration of 4.04 IU/L, as shown in Figure 3. This finding is consistent with the observations made by many researchers in this field [16], [18].

The reason for the increased concentration of LH is due to the increased concentration of the LH hormone in the majority of patients with early ovarian failure. There was a defect in the secretion of the hormones LH and FSH, and this defect made the ovaries unable to produce hormones properly, leading to a disturbance in the menstrual cycle [21]. As for the level of thyroid-stimulating hormone (TSH), it was noted that there is no significant difference in the serum of females with premature ovarian insufficiency, as its average concentration in the blood serum was (Mean = 2.46 mIU/mL) compared with the control group, as shown in Figure 4. This finding is in agreement with what was mentioned by [15] in Turkey and [22] in India. The results of their study showed that TSH levels were normal in the serum.

In this research, a link between low ovarian reserve and elevated TSH levels was not observed. Some of the results of investigations on this topic concur with the findings of this study. One study demonstrated that women with low ovarian reserve had higher levels of TSH than others, however, the

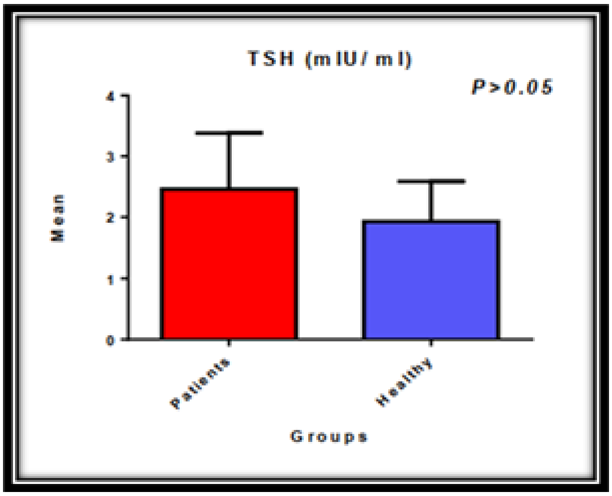

Figure 4: TSH level in the serum of females with premature ovarian insufficiency compared to healthy women

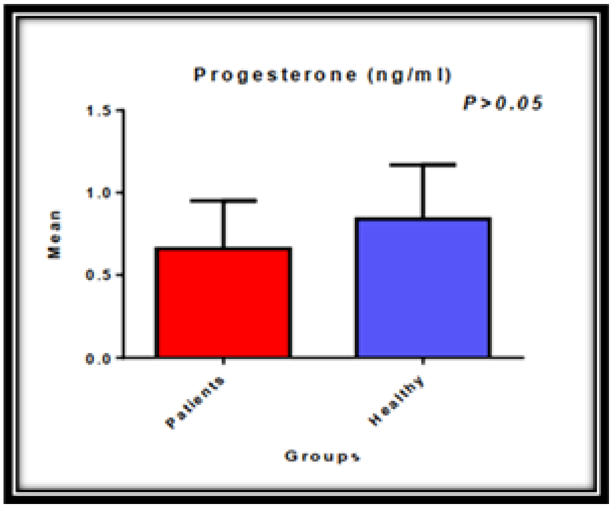

Figure 5: Progesterone level in the serum of females with premature ovarian insufficiency compared to healthy women

presence of antibodies to thyroid hormones in the follicular fluid of women with autoimmune thyroid disease was demonstrated. A positive association has been documented. The mechanism of this specific association is explained by the fact that antibodies against TPO cross the barrier between blood and follicles and cause a toxic environment that involves the destruction of the oocyte as it matures [15].

The results demonstrate that the average concentration of progesterone in the serum of females with premature ovarian failure (Mean = 0.66 ng/ml) was not different than the control group, as seen in Figure 5. This is consistent with the results of other studies that indicate no significant difference in progesterone concentration in POI women [22].

Progesterone is considered one of the most effective progestins from a biological standpoint. It is mainly produced by the corpus luteum in the ovary, as well as the adrenal cortex, placenta, and testicles. It suddenly appears in the urine on the day of ovulation, as a result of its metabolism and conversion, mainly to pyranadiol [23]. Progesterone is biosynthesized from acetate, which converts into cholesterol, and then into the main intermediate compound, pregnenolone. Pregnenolone is then converted into progesterone, which is metabolized in the liver and further converted to pregnendiol before being excreted in the urine.

Finally, no significant difference was recorded in this study for serum estrogen levels in females with premature ovarian insufficiency compared with the control group. The average concentration of the hormone was 49.61 pg/ml (Mean = 49.61 pg/ml), and this result was consistent with the results obtained from [24]–[26]. The findings are presented in Figure 6.

No significant difference in the level of E2 was observed between women with POI in this investigation. Low concentrations of AMH accompanied by high concentrations of FSH lead to a greater recruitment of follicles into the growing batch. Despite the fact that it promotes the rapid depletion

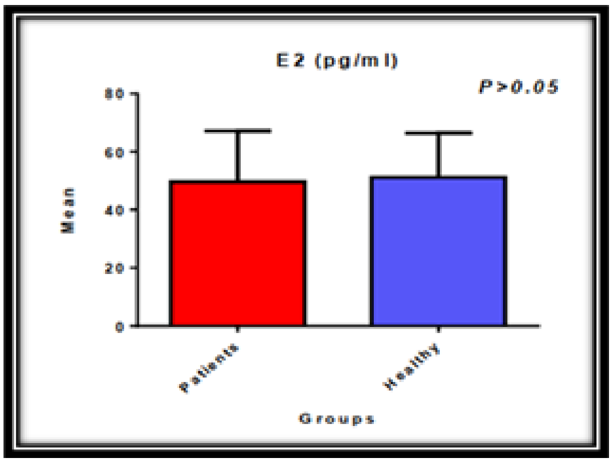

Figure 6: E2 hormone level in the serum of females with premature ovarian insufficiency compared to healthy women

of follicles, increasing the number of growing follicles and maintaining the cycle of estradiol can also have a positive effect on fertility and efficiency in reproduction [24], [27]. Therefore, basal estradiol levels may fluctuate over different time periods and should not be used alone to predict premature ovarian insufficiency.

C. Results of the Physiological Study

1) Vitamin D Concentration Level

The results of the current study showed statistically significant differences ($P<0.05$) in vitamin D levels between the two study groups (patients and healthy people). Vitamin D levels were lower in patients (8.62 ± 18.88) compared to healthy people (11.53 ± 44.15), as shown in Table 3 and Figure 7. These results were similar to the findings of a study by [11] in Iran. The study demonstrated low vitamin D levels in women with premature ovarian insufficiency, with vitamin D reaching a percentage of 12.1. Additionally, these results

	Groups	N	Mean	Std. Deviation	P value
VD mg\dL	Patients	45	18.88	8.62	P<0.001
	Control	45	44.15	11.53	

Table 3: Comparison of vitamin D between the two study groups

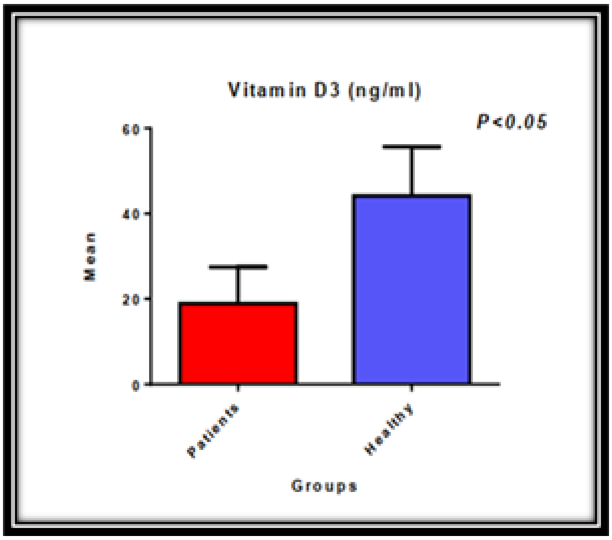

Figure 7: Comparison of vitamin D between the two study groups

were consistent with the findings of the studies by [28], [29].

Premature ovarian insufficiency, defined as the onset of menopause before the 40th year of life, can also be affected by vitamin D levels. In addition to age, the anti-Mullerian hormone (AMH) is well known as a biochemical marker of this syndrome [30]. Vitamin D (VD), a fat-soluble vitamin, is the most important steroid in the human body. Interestingly, studies have documented that VD can prevent the development of NETs in tissues and promote the growth and maturation of follicles by altering the stress-induced production of steroids in the ovary's granulosa cells. It is hypothesized that vitamin D affects the ovaries of women and may enhance the quality of their oocytes [31]. Most women with premature ovarian insufficiency suffer from vitamin D deficiency. Zinc, copper, and vitamin D seem to be contingent on the patients' hormonal status. The probability of having primary ovarian failure (POF) increases in conjunction with autoimmune disorders. Adequate consumption of vitamin D and other trace nutrients is vital to the efficient function of the immune system [28].

2) Calcium Concentration Level

The outcomes of the current investigation demonstrated that there was a significant difference ($P<0.05$) in the concentration of calcium between the two groups of study. (patients and healthy people). The calcium level was high in patients (0.45 ± 8.33) compared to healthy people (0.58 ± 8.02). As shown in Table 4 and Figure 8, this was in agreement with

	Groups	N	Mean	Std. Deviation	P value
Ca mg\dL	Patients	45	8.33	0.45	P<0.05
	Control	45	8.02	0.58	

Table 4: Calcium comparison between the two study groups

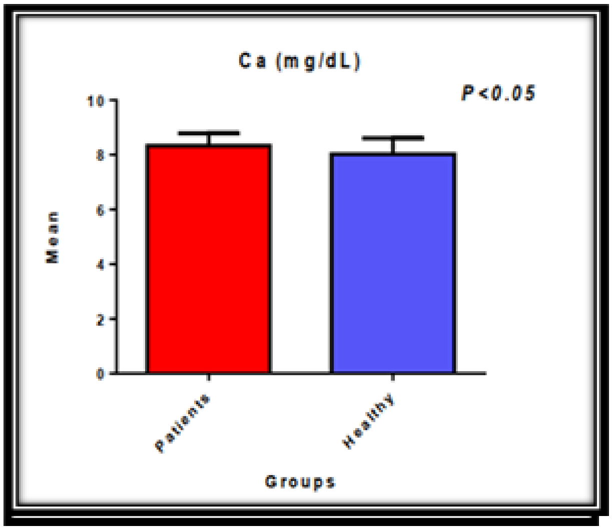

Figure 8: Calcium comparison between the two study groups

what [32] mentioned in Tikrit.

In humans, calcium has been shown to help stimulate egg maturation, either spontaneously or via LH. This is considered due to calcium modulating intracytoplasmic cAMP concentrations [33]. Calcium is also involved in egg activation resulting from sperm-derived factors. Thus, the egg can develop into an embryo. In addition, reproduction also occurs in the presence of calcium [8]. Calcium is a mineral normally found in the body, especially in the bones where its levels are highest. Many recent studies have reported that calcium has a close relationship with reproductive function [33]. In humans, calcium has been shown to help stimulate egg maturation, either spontaneously or via LH. This is considered to be due to calcium modulating intracytoplasmic cAMP concentrations [8].

3) Magnesium Concentration Level

The results of the current study show that there is a statistically significant difference ($P<0.05$) in the level of magnesium concentration between the two study groups (patients and healthy people). The level of magnesium was low in patients (0.29 ± 0.75), compared to healthy people (0.30 ± 2.07). As shown in Table 5 and Figure 9, this is consistent with what was mentioned by [10] in Tikrit.

An often overlooked factor that may contribute to pre-

	Groups	N	Mean	Std. Deviation	P value
Mg mg\dL	Patients	45	0.75	0.29	P<0.001***
	Control	45	2.07	0.30	

Table 5: Comparison of magnesium between the two study groups

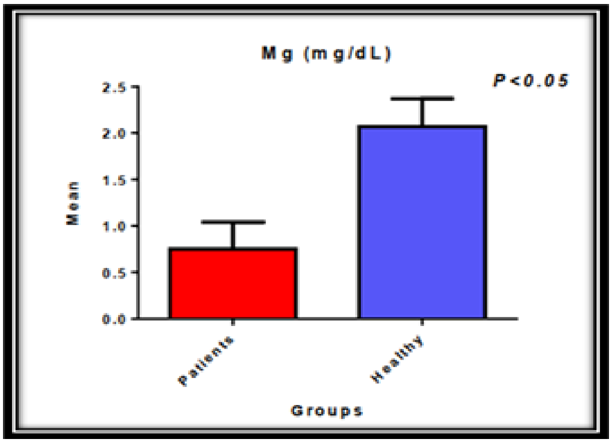

Figure 9: Magnesium comparison between the two study groups

mature ovarian insufficiency is magnesium. Magnesium is responsible for many biochemical processes within bones. Magnesium is necessary for the conversion of vitamin D into its biologically active form [34]. Estrogen enhances magnesium utilization and uptake by soft tissues and bones, which may explain young women's resistance to heart disease and osteoporosis. Estrogen increases the rate at which body tissues and bones absorb magnesium from the blood [35].

4. Conclusions

In this study, it was noted that there was an increase in the concentration level of FSH and LH compared to healthy people, while there was a decrease in the level of AMH in the patient group. As for the levels of TSH, E2, and Progesterone, the percentages were normal and were not different between the two study groups. As for the physiological study of premature ovarian insufficiency, there was an increase in the level of calcium concentration for the patients compared to the healthy group, while the concentration of magnesium and vitamin D levels were lower in the patients compared to the healthy group.

Conflict of interest

The authors declare no conflict of interests. All authors read and approved final version of the paper.

Authors Contribution

All authors contributed equally in this paper.

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