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Hormonal Changes in a Sample of Iraqi Women and their Relationship to Liver Dysfunction

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Abstract

There are numerous bidirectional interactions between the reproductive system and the liver. Sex steroids regulate metabolic health through signaling effects in both peripheral and central metabolic tissues, including adipose tissue, liver, skeletal muscle, and brain, and have a role in the etiology of structural and functional liver diseases. Blood samples were obtained from 90 healthy women (control group) and 90 women that have hormonal changes (patients' group). The levels of reproductive hormones (follicle stimulation hormone/FSH, luteinizing hormone/LH, estradiol/E2, progesterone/P4) were measured by using fully automated Cobas E411, whereas those of liver enzymes (alanine transaminase /ALT, aspartate aminotransferase/AST, alkaline phosphatase/ALP) were measured by using fully automated Cobas C111. Levels of copper and ceruloplasmin were measured as well. The results demonstrate a strongly significant positive correlation between the levels of FSH and ALT ($r = 0.45$, $p = 0.0001$), FSH and AST ($r = 0.48$, $p = 0.0001$), and FSH and ALP ($r = 0.303$, $p = 0.005$), LH and ALT ($r = 0.301$, $p = 0.005$), LH and AST ($r = 0.34$, $p = 0.001$), and LH and ALP ($r = 0.307$, $p = 0.004$). The reproductive hormones and liver enzymes had a strong positive correlation. The results demonstrate a correlation between estrogen and copper ($r = 0.38$, $p = 0.0001$), as well as a highly significant positive correlation between estrogen and ceruloplasmin ($r = 0.43$, $p = 0.0001$) and a positive significant correlation between progesterone and copper ($r = 0.26$, $p = 0.01$). These findings are consistent with earlier research that has found a clear link between low estrogen levels in the blood and liver disease in women. These findings imply that an abnormally low level of estrogen may render women more susceptible to developing liver damage.
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Keywords: Liver, Hormones, Female, Ceruloplasmin, ALT, AST

التغيرات الهرمونية في عينة من ألنساء العراقيات وعلاقتها بضعف الكبد

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الخلاصة

هناك العديد من التفاعلات ثنائية الاتجاه بين الجهاز التناسلي والكبد. الستيرويدات الجنسية تنظم الصحة الأيضية من خلال تأثيرات الإشارات في كل من الأنسجة الأيضية المحيطية والمركزية ، بما في ذلك الأنسجة الدهنية والكبد والعضلات الهيكلية والدماغ ولها دور في مسببات أمراض الكبد التركيبية والوظيفية. تم الحصول على عينات دم من 90 امرأة طبيعية (مجموعة السيطرة) و 90 امرأة لديهن تغير هرموني (مجموعة

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المرضى). تم قياس الهرمون التناسلي (الهرمون المنشط للحوصلة ، الهرمون اللوتيني ، الاستروجين)، و ، تم قياس اختبار البروجسترون باستخدام Cobas E411 ، تم قياس إنزيمات الكبد (ناقلة امين الالانين ، ناقلة امين الاسبارتات ، فوسفاتاز قلوي) باستخدام Cobas C111 ، وتم قياس النحاس والسيرولوبلازمين أيضًا أظهرت النتيجة وجود علاقة ارتباط موجبة عالية بين الهرمون المنشط للحوصلة و ناقلة امين الالانين = r) (0.45، p = 0.0001)) ، علاقة ارتباط موجبة عالية بين الهرمون المنشط للحوصلة و ناقلة امين الاسبارتات (r = 0.48) ، p = 0.0001)) ، وعلاقة موجبة عالية. ارتباط كبير بين الهرمون المنشط للحوصلة و فوسفاتاز قلوي (r = 0.303) ، p = 0.005)). أيضا ، كان هناك ارتباط معنوي إيجابي مرتفع بين الهرمون اللوتيني و ناقلة امين الالانين (r = 0.301) ، (p = 0.005) ، هناك علاقة ارتباط موجبة بين هرمون LH و هرمون AST حيث بلغت قيمتها (p = 0.001) r = 0.34 ، وارتفاع إيجابي. وهناك علاقة ارتباط كبيرة بين LH و ALP بلغت قيمتها (r = 0.307)، (p = 0.004). وكان هناك ارتباط إيجابي كبير بين الهرمون التناسلي وإنزيمات الكبد. أظهرت النتيجة وجود علاقة ارتباط بين هرمون الاستروجين والنحاس (ص = 0.38) ، (ع = 0.0001) وارتباط معنوي إيجابي مرتفع مع سيرولوبلازمين (ص = 0.43) ، (ع = 0.0001) وأظهرت ارتباطًا إيجابيًا معنويًا بين البروجسترون والنحاس (r = 0.26)، (p = 0.01). تتوافق هذه النتائج مع الدراسات السابقة التي أظهرت ارتباطًا قويًا بين المستويات المنخفضة من هرمون الاستروجين وزيادة خطر الإصابة بأمراض الكبد لدى النساء. تشير هذه النتائج إلى أن مستويات هرمون الاستروجين قد تجعل النساء أكثر عرضة للإصابة بتلف الكبد.

Introduction

The liver is an important organ in the body that helps with digestion, blood pressure regulation, protein production, detoxification, hormone management, combating infections and disease, repairing after injury, and metabolizing cholesterol, glucose, and iron and regulating their levels. Nonetheless, many people develop chronic liver disease as a result of excessive alcohol consumption or consuming unhealthy meals. Obesity, diabetes, and even cancer are all increased in risk by liver dysfunction [1]. Several other hormonal illnesses, such as Graves' disease (GD), are associated with liver impairment [2]. Both excess and deficiency of adrenal activity can cause changes in liver function, and adrenocortical dysfunction can occur in cirrhotic individuals, particularly during periods of decompensation. [3]

Women are more likely to develop acute liver failure, autoimmune hepatitis, benign liver lesions, primary biliary cirrhosis, and toxin-mediated hepatotoxicity [4]. The female preponderance of drug-induced hepatic damage may be attributable in part to gender differences in drug absorption, metabolism, and excretion, which have been seen in rat and mouse models as well as in humans [5]. In healthy women, menopause frequently results in a decrease in estradiol and an increase in follicle-stimulating hormone, both of which are connected to an increased risk of heart disease [6] and bone disease [7]. The current study aimed to investigate the associations between sex hormone and liver enzyme levels in women with hormonal changes (premature ovarian failure/POF) in different age stages.

Material and method

Subjects of the study

This study comprised 180 women, among whom 90 were patients (POF) and 90 were appearing healthy. Both groups were divided into three age groups: the first (15-25 years old), the second (26-35 years old), and the third (36-45 years old). Before collecting blood for laboratory analysis, all of the volunteered women filled out a questionnaire.

Blood collection

Venous blood samples (4 ml) were taken from all participants (patients and control). Serum was collected for each sample after centrifugation at 3500 rpm for 15 minutes and kept in the freezer (-20 C) until used [8]

Biochemical measurements

Serum was used to measure the following vital parameters: The levels of Estradiol (E2), Progesterone (P4), Luteinizing hormone (LH), and Follicle-stimulating hormone (FSH) were determined using Roche Kit (Germany) applied on Cobas E 411 system [9], a fully automated analyzer that uses patented Electrochemiluminescence (ECL) technology for immunoassay analysis. Liver function tests (ALT, AST, and ALP) were determined using Roche Kit (Germany) applied on Cobas C 111 system. The Cobas C 111 analyzer is the smallest member of the Cobas serum work area platform family and the ideal solution for the clinical chemistry testing in laboratories running 10 to 50 samples per day. Copper was measured using the Itanoline kit (Italy) which consisted of Acetate buffer, Reducing agents, Preservatives, 3,5-Di-Br-PAESA, Ion copper, and Preservatives). Ceruloplasmin was measured using the Centronic GmbH kit (Germany) that comprises Buffer reagent (Tris buffer, 20 mmol/L, PEG 8000, pH 8,3. Preservative) and Antibody solution (Goat serum, anti-human Ceruloplasmin, pH 7,5. Preservative) by using a colorimetric spectrophotometer, following the manufacturer's instructions.

Statistical analysis

To assess the effect of different age groups on the study parameters, the Statistical Analysis System- SAS (2018) program was utilized. The least significant difference -LSD test (Analysis of Variation-ANOVA) was used to compare between means [10].

Results and Discussion

Hormonal analysis

The level of LH in patient group in comparison to the control group

Table 1 shows that LH level was significantly higher ($p \leq 0.01$) in the patient group compared to its level in the control group for all samples. It was also observed that within the group of patients, the (15-25 years old) group had a lower level of the hormone compared to the (26-35 years old) and (36-45 years old) groups, as shown in Table 1.

Table 1: The level and p-value of LH in patient and control groups.

Age groups	LH mean ± SE (mIU/ml)		Probability (p-value)
	Control	Patients	
15 – 25 years	7.60 ± 0.25	22.17 ± 0.90	9.59×10^{-11}
26 – 35 years	8.55 ± 0.25	32.12 ± 1.65	1.91×10^{-22}
36 – 45 years	12.28 ± 0.28	42.28 ± 2.60	3.82×10^{-29}

This result was consistent with [11], who showed that POF is biochemically defined by low levels of gonadal hormones (estrogens and inhibin) and high levels of gonadotropins (LH and FSH). FSH and LH levels in the blood are quite high during menopause because they are attempting to stimulate the ovaries to perform their functions. POF is therefore diagnosed by at least two blood tests that revealed high FSH and LH levels. This result also agrees with [12] that noted that LH is a hormone secreted by the pituitary gland that plays a crucial role in the regulation of the menstrual cycle and ovulation. In healthy women, the level of LH is tightly controlled by a negative feedback loop involving the hypothalamus, the pituitary

gland, and the ovaries. However, in women with POF, the depletion of ovarian follicles leads to a decrease in the production of estrogen, which disrupts the negative feedback loop and results in an increase in LH secretion [13]. As women with POF age, the level of LH tends to increase further due to several factors. One of the main reasons is the gradual decline in the number of follicles in the ovaries, leading to decreased production of estrogen. This decline in estrogen production causes an increase in LH secretion, as there is less negative feedback to inhibit its production. Additionally, as women with POF age, their remaining ovarian follicles become less responsive to FSH and LH, which are important hormones for follicular development and ovulation. This reduced responsiveness of the ovaries to FSH and LH causes an increase in their levels in the blood, including LH [14]. In addition, [15] stated that women with PCOS had higher LH levels and that LH and FSH levels increased in overweight PCOS patients.

The level of FSH in patient group in comparison to the control group

In this study, the level of FSH was significantly higher in the patient group as compared to its level in the control group for all groups. It was observed that the levels of the hormone in the (26-35 years old) and (36-45 years old) groups of patients were higher than that in the (15-25 years old) group, as shown in Table (2).

Table 2: The level and p-value of FSH in patient and control groups.

Age groups	FSH means ± SE (mIU/ml)		Probability (p-value)
	Control	Patients	
15 – 25 years	8.95 ± 0.45	41.39 ± 1.72	6.53×10^{-27}
26 – 35 years	11.24 ± 0.36	49.92 ± 1.94	2.73×10^{-34}
36 – 45 years	14.70 ± 0.34	49.72 ± 3.15	1.39×10^{-27}

In this study, it was observed the level of FSH is affected by aging; the results revealed a significant increase of FSH with the increase of age [16]. In women with POF, the loss of ovarian function leads to a decrease in estrogen production. This reduction in estrogen levels removes the negative feedback on FSH secretion by the pituitary gland, leading to an increase in FSH levels in the blood. The increased FSH levels are an attempt by the pituitary gland to stimulate the remaining ovarian follicles to develop and mature. However, as the number of follicles declines in POF, FSH levels continue to rise. This finding is consistent with [2], which states that low E2 levels are caused by ovarian disorders when the feedback system encourages the pituitary gland to release a gonadotropic hormone (high FSH levels). The greater the FSH levels, the more severe ovarian failure. Insufficient ovarian hormone production contributes to a higher increase in FSH over LH via negative feedback due to reduced follicle number or quality. FSH level elevated earlier and more rapidly than LH in the premature ovarian failure stage, increasing the FSH/LH ratio substantially [14]. As women with POF age, the number of ovarian follicles continues to decline, leading to a further decrease in estrogen production and an increase in FSH secretion. This increase in FSH levels can be measured through blood tests and is a diagnostic criterion for POF [12].

Estradiol level in the patient group in comparison to the control group

It was found that the level of estradiol hormone was lower significantly in the patient group as compared to its level in the control group for all age groups. Within the patient's group, it was found that the age group (36-45) occupies the lowest level of the hormone compared to the rest of the age groups and there were significant differences ($p \leq 0.05$), as shown in Table (3).

Table 3: The level and p-value of estradiol in patient and control groups

Age groups	E2 means ± SE (pg/dl)		Probability (p-value)
	Patients	Control	
15 – 25 years	17.0 ± 0.64	31.78 ± 0.86	1.79×10^{-19}
26 – 35 years	15.0 ± 0.68	35.92 ± 0.98	5.59×10^{-32}
36 – 45 years	12.69 ± 0.48	56.24 ± 1.89	1.74×10^{-70}

Estrogen is primarily produced by the ovaries, and its production is regulated by a complex interplay of hormones and signaling pathways. In POF, the ovaries stop producing estrogen due to a loss of follicles, which are the structures that contain eggs and produce estrogen. This loss of follicles can be caused by a variety of factors, including genetic mutations, autoimmune disorders, and chemotherapy or radiation therapy [17]. These findings are consistent with [18], which stated that low E2 levels are caused by ovarian problems when the feedback system encourages the pituitary gland to generate a gonadotropic hormone (high FSH levels). The decrease in estrogen production with age in women with POF is due to the accelerated depletion of ovarian follicles, which can be caused by a variety of factors, including autoimmune disorders, genetic mutations, and certain medical treatments.

Progesterone (P4) level in the patient group in comparison to the control groups

The level of P4 in the patient group was lower significantly than in the control group, while within the patient group, the level of P4 at the (36-45 years old) group was lower than that in the (15-25 years old) and (26-45 years old) groups. There was also a highly significant difference between the patient and control groups, as shown in Table (4).

Table 4: The level and p-value of progesterone in patient and control groups.

Age groups	P4 means ± SE (ng/dl)		Probability (p-value)
	Patients	Control	
15 – 25 years	0.19 ± 0.009	0.38 ± 0.016	2.79×10^{-16}
26 – 35 years	0.14 ± 0.010	0.38 ± 0.018	5.65×10^{-24}
36 – 45 years	0.12 ± 0.011	0.64 ± 0.022	1.02×10^{-59}

As women age, the number of follicles in the ovaries decreases, which leads to a decline in ovarian function and a reduction in the production of progesterone. In women with POF, the decline in ovarian function occurs much earlier than in women without this condition. This is because the ovaries in women with POF have a reduced number of follicles or they do not function properly, leading to a decrease in the production of estrogen and progesterone. Additionally, the decrease in progesterone levels in POF patients may be further exacerbated by the use of certain medications or treatments, such as chemotherapy or radiation therapy [19] These treatments can damage the ovaries and further reduce their ability to produce hormones, including progesterone. This conclusion is consistent with [20]. Women with POI had lower mean blood levels of estradiol and progesterone than controls. When POI patients were compared to healthy controls, the mean value of serum progesterone indicated a smaller difference. The present results are in agreement with [21] and [22]. Low serum progesterone and estradiol levels may be attributed to the ovary's inability to generate them.

Liver function tests

Levels of ALT enzyme in patient groups in comparison to the control groups

The mean concentration of ALT (mean ± SD) in the female patient's group was higher compared with the control group. The level of ALT in the (35-45 years old) group was lower than its level in other patient groups. There was also a significant difference (P≤0.05) between patient and control groups, as shown in Table (5).

Table 5 : The level and p-value of alanine transaminase in patient and control groups.

Age groups	ALT means ± SE (mg/dl)		Probability (p-value)
	Patients	Control	
15 – 25 years	45.45 ± 5.84	26.88 ± 1.62	0.004
26 – 35 years	48.07 ± 6.27	25.08 ± 1.85	0.000453
36 – 45 years	33.71 ± 5.86	25.86 ± 1.37	0.238

Elevated ALT levels may be caused by cirrhosis, liver cancer, infection, hepatitis, or other liver disorders. Damage to the liver might result from a lack of blood flow, some drugs, or toxins. This outcome is consistent with [23]. ALT levels frequently increase in individuals with acute viral hepatitis. The greatest ALT levels are typically observed in individuals suffering from acute toxic damage, such as acetaminophen overdose or acute ischemia insult to the liver. As for why ALT levels may decrease with age, there are a few possible explanations. One is that liver function naturally declines with age, which can lead to a decrease in the production and release of ALT into the bloodstream. Additionally, older adults may be more likely to have conditions or take medications that can affect liver function, which could contribute to decreased ALT levels [24]. One study found that elevated levels of liver enzymes, specifically ALT, were associated with a higher risk of POF in women with polycystic ovary syndrome (PCOS). PCOS is a common endocrine disorder that can lead to hormonal imbalances and disrupted ovarian function, and this study agree with my result [25]. Some studies have suggested that women with POF may have higher ALT levels compared to healthy women, but the evidence is not consistent and more research is needed to confirm any potential association [26].

Levels of AST enzyme in patient groups in comparison to the control groups

The concentration of AST in patient groups was significantly higher than its concentration in the control group (mean ± SD), while the level of AST in the (35-45 years old) group was lower significantly than its level in other patient groups. There was also a significant difference (P≤0.05) between patient and control groups, as shown in Table (6).

Table 6 : The level and p-value of Aspartate aminotransferase in patient and control groups

Age groups	AST mean ± SE (mg/dl)		Probability (p-value)
	Patients	Control	
15 – 25 years	45.43 ± 5.30	31.05 ± 1.55	0.028
26 – 35 years	50.58 ± 6.18	29.98 ± 1.85	0.002
36 – 45 years	36.93 ± 6.62	29.05 ± 1.41	0.256

The presence of hepatitis, cirrhosis, mononucleosis, or other liver illnesses may be indicated by high levels of AST in the blood. High AST levels could also indicate hepatitis or cardiac issues. This result agrees with [27] which noted that AST elevations often

predominate in patients with cirrhosis and even in liver diseases that typically have an increased ALT. As for why AST levels may decrease with age, there are a few possible explanations. One is that the amount of muscle tissue in the body generally decreases with age, which can lead to a decrease in the production and release of AST into the bloodstream [28]. AST levels were significantly higher in women with POF compared to healthy controls. The study also found that AST levels were positively correlated with follicle-stimulating hormone (FSH) levels, which are typically elevated in women with POF, and this agrees with my study [29]. Another study found that women with POF had higher levels of AST compared to healthy controls. The study suggested that the increase in AST levels could be related to the hormonal imbalances associated with POF [30].

Levels of ALP enzyme in patient groups in comparison to the control groups

The level of ALP in the female patient group was lower than its level in the control groups, while the level was insignificantly higher in the (36-45 years old) group than in other patient groups, as shown in Table (7).

Table 7: The level and p-value of alkaline phosphatase in patient and control groups.

Age groups	ALP mean ± SE (mg/dl)		Probability (p-value)
	Patients	Control	
15 – 25 years	92.06 ± 7.85	107.41 ± 6.44	0.365
26 – 35 years	115.51 ± 8.91	103.53 ± 6.78	0.479
36 – 45 years	117.13 ± 13.05	132.70 ± 21.72	0.386

This result agrees with [31] which noted that, in all cases of parenchymal liver diseases (like viral hepatitis or toxic liver diseases), serum ALP activity will be either normal or moderately elevated and seldom rises beyond three folds than normal. As for why ALP levels may increase with age, there are a few possible explanations. The result also agrees with [32]. The researchers measured serum ALP levels in 84 women with POF and 100 healthy women with regular menstrual cycles. They found that the POF group had significantly higher ALP levels compared to the control group ($P < 0.0001$). The authors suggested that elevated ALP levels in women with POF may be related to an imbalance in bone metabolism and increased bone resorption, which can lead to changes in ovarian function. Bone turnover and remodeling naturally decrease with age, which can lead to an accumulation of ALP in the bloodstream as bone cells break down. Additionally, older adults may be more likely to have conditions or take medications that can affect bone metabolism and increase ALP levels.

Biochemical tests

Levels of copper in patient groups in comparison to the control groups

The level of Cu in the patient group was lower than its level in the control group (mean ± SD), while the level of copper in the (36-45 years) age group was insignificantly higher than in other groups, both within the patients and the controls. were Significant differences ($p \leq 0.05$) were only found in the (36-45 years) group, as shown in Table (8).

Table 8 : The level and p-value of copper in patient and control groups.

Age groups	CU means ± SE (µg/dl)		Probability (p-value)
	Patients	Control	
15 – 25 years	110.74 ± 3.43	101.02 ± 2.71	0.953
26 – 35 years	110.40 ± 5.60	116.50 ± 2.41	0.737

36 – 45 years	113.74 ± 3.93	153.86 ± 32.16	0.038

This result agrees with [33]. Ceruloplasmin levels are low in Wilson's illness. A decrease in the rate of synthesis of ceruloplasmin is responsible for copper buildup in the liver due to a copper transport problem in the Golgi apparatus caused by ATP7B deficiency. Elevated copper levels with aging can also be caused by certain health conditions, such as Wilson's disease, a genetic disorder that causes copper to accumulate in the body. Therefore, it is important to consider the context and underlying cause of any changes in copper levels [34]. The result agrees with [35] which noted that copper and zinc levels have been investigated in patients with POF. The study found that women with POF had significantly higher levels of serum copper compared to healthy controls, but there was no significant difference in serum zinc levels between the two groups

Levels of ceruloplasmin in patient groups in comparison to the control groups

The level of ceruloplasmin in the third age group of patients was lower significantly than its level in the control group, while it was higher in the first patient's age group (15-25 years) than in the control group. Significant differences ($p \leq 0.05$) were only found in the (36-45 years) group, as shown in Table (9).

Table 9: The level and p-value of ceruloplasmin in patient and control groups.

Aged groups	Ceruloplasmin mean ± SE (mg/dl)		Probability (p-value)
	Patients	Control	
15 – 25 years	30.82 ± 1.09	27.70 ± 0.90	0.506
26 – 35 years	29.89 ± 1.14	31.18 ± 0.83	0.784
36 – 45 years	31.78 ± 1.19	42.10 ± 8.27	0.039

This result agrees with [36]. Low levels may also be seen in neonates, Menkes disease, kwashiorkor, marasmus, protein-losing enteropathy, copper deficiency, and aceruloplasminemia. This result also agrees with [33]. In Wilson's disease, ceruloplasmin level is depressed. Decreased rate of synthesis of ceruloplasmin is responsible for copper accumulation in the liver because of copper transport defect in the Golgi apparatus, since ATP7B is affected.

Conclusions

The results indicate a relationship between hormonal change and liver dysfunction, as it was found that there is an inverse relationship between estrogen and progesterone hormones and liver enzymes; the decrease in the levels of estrogen and progesterone is accompanied by an increase in the levels of liver enzymes.

Ethical Clearance

This research was ethically approved by the Research Ethical Committees of the Ministry of Environmental and Health and the Ministry of Higher Education and Scientific Research, Iraq, and the approval is numbered CSEC/0122/0051.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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