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Evaluation the Role of Hepcidin in Women with Osteoporosis

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Abstract

The research included (93) cases collected from Ibn Sina Teaching Hospital in Mosul city, the cases were divided into two group, the first group included (58) patients with osteoporosis either the second group included (35) healthy cases, both groups were divided into three subgroups:(40-49) (50-59) (60 and over) year, and included estimation of hepcidin hormone, parathyroid hormone, vitamin D_3, iron, calcium, total cholesterol, triglycerides, high density lipoprotein (HDL), very low density lipoprotein (VLDL), paraoxonase, glutathione-S-transferase, body mass index (BMI), creatinine, urea, bilirubin, hemoglobin, albumin, and ceruloplasmin in blood serum of women with osteoporosis and comparing with control group., the results showed a decrease in the levels of (Hepcidin, vitamin D_3, calcium, hemoglobin, body mass index (BMI), HDL-C and albumin) in patient group compared to control group and an increase in the levels of (parathyroid hormone, iron, paraoxonase, glutathione-S-transferase, ceruloplasmin, creatinine, urea, bilirubin, total cholesterol,and triglycerides) in patient group compared to control group. The results also showed appositive correlation between hepcidin hormone and iron, calcium, ceruloplasmin, total cholesterol, VLDL-C, urea, albumin, and bilirubin, while there was negative correlation between hepcidin and parathyroid hormone, vitamin D_3, hemoglobin, paraoxonase glutathione-S-transferase, triglyceride, HDL-C, BMI and creatinine.

Keywords: Osteoporosis; Hepcidin; Parathyroid; Oxidative stress; Lipid profile; Anemia.

1. Introduction

Osteoporosis is a silent disease [1] because it develops without any symptoms until fracture occurs [2] it is a skeletal disease characterized by low bone density and micro-architectural degradation of bone tissue with an increase in osteoporosis in addition to fracture[3].The risk of developing osteoporosis in women is greater than in men [4], in women iron levels in the form of ferritin (iron storage protein) increase significantly after menopause, increased iron concentrations contribute to enhancing bone resorption and suppressing bone formation [5].

Hepcidin a liver-derived peptide hormone, is a major regulator of systemic iron homeostasis [6]. Hepcidin negatively regulates the export of macrophage iron [7], as it binds to the source of iron in target cells, macrophages, and intestinal cells to some extent. In hepatocytes [8], its unbalanced production contributes to a group of iron disorders by blocking the flow of iron into the plasma[6].

Vitamin D3 is an important nutrient in maintaining bone health [9]. The primary role of Vitamin D3 is in bone metabolism and in increasing levels of calcium and phosphorous in the plasma[10], as vitamin D3 enhances calcium and phosphate absorption. Low concentrations of vitamin D3 are associated with impairment. Calcium absorption, negative calcium balance, and compensatory elevation in parathyroid hormone, leading to bone resorption [11].

Calcium has a key role in many physiological processes including skeletal mineralization [12], decreased dietary calcium intake is associated with lower BMD [13].

Parathyroid hormone plays a key role in maintaining calcium homeostasis[14], which is secreted by the parathyroid gland in response to low calcium[15], it works to maintain calcium levels within the normal range through its effects on the

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bones, kidneys and also reduce phosphorous in the blood through renal reabsorption [16].The hormone has multiple effects including increasing bone turnover by stimulating both osteoblasts and osteoclasts [17]

Anemia of Chronic Disease also known as secondary anemia, where there is a permanent increase in the incidence of this type of anemia associated with age and tendency to develop chronic diseases [18] among elderly patients with anemia, about 20% are considered to have anemia due to chronic diseases or inflammatory anemia is commonly seen in chronic kidney disease, acute or chronic infection, malignant tumors, and inflammatory disorders such as rheumatoid arthritis [19]. Bone marrow to produce an adequate number of red blood cells in order to maintain tissue oxidation when anemia is severe, hypoxia stimulates erythropoiesis by increasing renal synthesis and this leads to suppression of hepcidin transcription

[20].

Aim of the work:

Estimate hepcidin level in women with osteoporosis in combaretion with control group and study its relationship with some biochemical parameters.

EXCLUSION CRITERIA: Women without any diseases.

MATERIALS AND METHODS:

This study was conducted during the period of September 2020 to April 2021, which is the period of case - control study. (58) postmenopausal women with osteoporosis out of seventy were diagnosed by specialized doctors at the Ibn Sina Teaching Hospital in Mosul, they were compared with (35) healthy women. Of the same age, the study protocol was approved by the Ethical Research Committee at the Nineveh Health Department in Mosul, about 5 ml of venous blood sample were drawn from the patients and the control group, the sera were separated by centrifugation at a rate of 3000×g for 10 minutes[21]. Each sample was analyzed directly for Hepcidin, vitamin D3. and parathyroid. the serum were separated and used to estimate the following clinical parameters:

1. Hepcidin hormone was measured by competitive-enzyme linked immunosorbent assay (ELISA) technique using Shanghai yehua biological technology, Cat.No.20201103.
2. Vitamin D3 and Parathyroid hormone were determined by VITROS ECIQ from Diagnostics orthoclinical (USA), Cat. No.1300 for parathyroid hormone, Cat. No.1008533280 for vitamin D3.
3. Calcium: was determined by CPC method using BIOLABO kit Cat. No.052010A (France).
4. Iron: was determined by Colorimetric method using BIOLABO kit Cat. No.121915A. (France).
5. Total Cholesterol (TC): was determined by enzymatic colorimetric method using BIOLABO Kit Cat. No.071930A1 (France).
6. Triglycerides (TG): was estimated by enzymatic colorimetric method using BIOLABO Kit Cat. No.032023A1 (France).
7. High density lipoprotein-cholesterol (HDL-C): was estimated by precipitation method using BIOLOABO kit Cat. No.111913 (France),
8. Very low density lipoprotein –cholesterol (VLDL-C): was calculated using the equation: VLDL-C (mmol/L) =TG/ 5 (mmol/L) [22].
9. Body Mass Index (BMI): was calculated as weight in kilogram divided by square height in meters [23].
10. Paraoxonase enzyme: The effectiveness of the paraoxonase enzyme was estimated according to the researchers method Toma´s et al [24].
11. Ceruloplasmin: The concentration of ceruloplasmin was determined according to the method of the researcher Sunderman and Nomoto[25].
12. Glutathion-S-transferase(GST):The activity of the enzyme glutathione-S-transferase was estimated according to the method used by researchers Habig et al [26].
13. Creatinine: was determined according to the method used by researchers Tietz [27].
14. Urea: was determined by urease-modified berthelot reaction using BIOLABO Cat. No.061930A kit (France).
15. Albumin: was determined by bromocresol green method using BIOLABO kit Cat.No.061916A1 (France).
16. Bilirubin: was determined according to the method of the researcher Walters and Gerarde [28].
17. Haemogobin (Hb): was determined by microhaematacrit method used by reserchers Barbara et al [29].

Results and discussion:

Hepcidin hormone

There was a significant decrease in hepcidin hormone in the patients compared to control group in all age groups as shown in table (1), these results were in agreement with sato et al [8]. In severe cases of a disease, Hepcidin production tends to decrease even if inflammation is present [6] the levels of hepcidin in the blood ofpatients with rheumatoid arthritis and iron deficiency are significantly lower compared to patients with rheumatoid arthritis and chronic inflammatory anemia, due to the influence of hepcidin by inflammation and iron metabolism [8].

Parathyroid hormone

There was increase in parathyroid hormone in the patients compared to control group in all age groups as shown in table (1), these results were in agreement with Tisato [30]. The hormone can produce a catabolic or anabolic pathway on bone metabolism depending on the hormone level and the duration of exposure [16].

Vitamin D3

There was a significant decrease in levels of Vitamin D3 in patients compared to control group in all age groups as shown in table (1) these results were in agreement with Thomas,vitamin D3 deficiency in older populations in many countries [31] a risk factor to bone health, osteomalacia hyperthyroidism with muscle weakness and osteoporosis, the amount of sun exposure is a possible factor that contributed to this finding[32].

Iron

There was increase in levels of iron in patients compared to control group in all age groups as shown in table (1), these results were in agreement with Zhang [33]. This may be due to a lack of estrogen due to menopause, which increases bone resorption and accelerates bone loss[34] excess iron in the blood serum disrupts the dynamic balance between bone formation, which ultimately leads to osteoporosis, in addition to excess iron promotes the differentiation of osteoclasts which leads to enhanced bone resorption and causes apoptosis in osteoblasts, thus inhibiting bone formation [33].

Calcium

There was a significant decrease in levels of calcium in patients compared to control group in all age groups as shown in table (1) these results were in agreement with Shukla [35]. The reason for this is due to the effect of testosterone and estrogen on the intestine's absorption of calcium from the bloodstream [36].

Table (1): The levels of hormones and some parameters in serum of patient with osteoporosis in comparison with control group.

Age group (Mean± Standard Deviation)						
Hormones and some parameters	Age group(40-49)year		Age group(50-59)year		Age group(60)year and over	
	Control group (n=15)	Patients group (n=23)	Control group (n=11)	Patients group (n=17)	Control group (n=9)	Patients group (n=18)
Hepcidin hormone (pg/mL)	324±23.1 a	204±12.1 c	441±46.7 b	206±17.3 c	439±16.7 b	220±14.0 c
Vitamin D_3 (ng/ml)	26.3±0.98 a	14.5±0.76 b,c	24.6±0.68 a,d	16.1±0.46 c	22.8±2.16 d	11.2±1.03 b
Parathyroid hormone (pg/mL)	24.1±2.93 a	39.1±2.92 b	27.2±0.99 a,b	56.9±5.34 c	26.1±2.96 a,b	63.7±7.54 c
Calcium (mg/dL)	7.26±0.40 a	3.80±0.28 c	7.26±0.22 a	2.80±0.13 b	5.56±0.22 d	2.20±0.13 b
Iron (µg/dl)	79.6±3.31 a	114±6.85 c	93.6±5.93 a,b	112±12.8 b,c	85.6±3.20 a,d	122±2.70 c
Hb (g/dl)	11.6±1.11 a,b	9.75±0.50 a,c	12.3±1.47 b	8.08±0.23 c	11.3±0.53 a,b	8.32±0.21 c

Hb

There was a significant decrease in level of hemoglobin in patients compared to control group as shown in table (1), these results were in agreement with Batún-Garrido [37].

Paraoxonase

There was a significant decrease in paraoxonase activity in the patients compared to control group in all age groups as shown in table (2). These results were in agreement with those found by Tisato [30]. Reduced enzyme activity leads to decreased functionality of HDL, which in turn increases the risk of inflammatory disease[30].

Glutathione-S-transferase

There was increase in GST enzyme in patients compared to control group in all age groups as shown in table (2). This may be due to increased oxidative stress, which leads to increased levels of Glutathione-S-transferase enzyme [38].

Ceruloplasmin

There was increase in levels of ceruloplasmin in patients compared to control group in all age groups shown in table (2). These results were in agreement with karakas [39]. High levels of ceruloplasmin are predictors of osteoporosis[39].

Table (2): The activity of some enzymes and ceruloplasmin in serum of patient with osteoporosis in comparison with control group.

Age group (Mean ± Standard Deviation)						
Enzymes and ceruloplasmin	**Age group(40-49)year**		**Age group(50-59) year**		**Age group(60)year and over**	
	Control group (n=15)	**Patient group (n=23)**	**Control group (n=11)**	**Patient group (n=17)**	**Control group (n=9)**	**Patient group (n=18)**
Paraoxonase activity (U/L)	**2.06±0.03 a**	**2.75±0.02 d**	**2.27±0.06 b**	**2.81±0.01 d**	**2.47±0.06 c**	**3.03±0.06 c**
Glutathione-S-transferase (U/L)	**198±39.0 a**	**349±21.2 b,c**	**283±24.3 a,b**	**469±59.1 d**	**254±27.7 a,b**	**436.±34.5 c,d**
Ceruloplasmin (mg/dL)	**0.24±0.23 a**	**0.53±0.23 c**	**0.43±0.01 b**	**0.58±0.01 c**	**0.53±0.04 c**	**0.76±0.40 d**

Triglyceride (TG), Total Cholesterol, VLDL-C, and HDL concentration

The results in table (3) showed a significant increase in TC, TG, VLDL-C.and showed a decrease in the level of HDL-C concentration in patients compared to control group. These results were in agreement with those found by Bijelic, Şahin Ersoy [40][41].The accumulation of fats in the blood leads to accumulation in the sub-endothelial matrix of the bone vessels and may inhibit the differentiation and d mineralization of bone cells[42].

BMI

There was a significant decrease in levels of BMI in patients compared to control group as shown in table (3), these results were in agreement with Fawzy et al [43].Low body mass is associated with lower BMD and increased risk of osteoporosis [44].

Table (3): The levels of lipid profile and BMI in serum of patient with osteoporosis comparison with control group.

Age group (Mean± Standard Deviation)						
Biochemical parameters	**Age group(40-49)year**		**Age group(50-59) year**		**Age group(60)year and over**	
	Control group (n=15)	**Patient group (n=23)**	**Control group (n=11)**	**Patient group (n=17)**	**Control group (n=9)**	**Patient group (n=18)**
Total cholesterol (mg/dL)	**77.0±4.93 a**	**150±12.8 c**	**95.0±14.6 a,b**	**158±15.1 c**	**114±2.76 b**	**192±1.87 d**
Triglyceride (mg/dL)	**18.0±2.22 a**	**53.0±2.63 b,c**	**17.0±0.36 a**	**64.6±3.31 c**	**36.6±5.82 a,b**	**84.3±14.2 d**
HDL-C (mg/dL)	**76.0±5.77 b**	**56.5±5.47 a**	**74.5±3.66 b**	**42.8±3.43 d**	**92.1±2.37 c**	**45.8±3.54 a,d**
VLDL-C (mg/dL)	**1.11±0.13 a**	**15.9±1.02 b**	**1.40±0.07 a**	**19.5±1.63 b**	**1.58±0.85 a**	**23.0±7.93 b**
BMI (kg/m^2)	**31.9±1.16 a,b**	**25.3±1.30 c**	**33.1±3.14 b**	**27.5±1.21 a,c**	**30.8±1.24 a,b**	**24.1±0.87 c**

Creatinine

There was a significant increase in levels of creatinine in patients compared to control group in all age groups as shown in table (4), these results were in agreement with Li et al [45]. This may be due to decreased renal function, patients with impaired real function show reduced body mass density and increased risk of fractures [46].

Urea

There was a significant increase in levels of urea in patient compared to control group in all age groups as shown in table (4), these results were in agreement with Park,Jassal [46] [47] this may be due to renal function declines with age due to decreased glomerular filtration rate[47], in addition the acute inflammatory response in bone healing after fracture may lead to further impairment of renal function [46].

Albumin

There was a significant decrease in levels of albumin in patients compared to control group in all age groups as shown in table (4), these results were in agreement with Afshinnia et al [48] this may be to the role of albumin in osteoclast activation through its association with nuclear factor and other inflammatory cytokines that drive osteoporotic activities [49].

Bilirubin

There was a significant increase in levels of bilirubin in patients compared to control group in all age groups as shown in table (4), these results were in agreement with Ruiz-Gaspà et al [50]. High levels of bilirubin and bile acids may contribute to abnormal osteoblast function [50].In addition high levels of bilirubin in the blood serum also indicate abnormal liver function[51].

Table (4): The levels of some biochemical parameters in serum of patient comparison with control group.

Biochemical parameters	Age group (Mean± Standard Deviation)					
	Age group(40-49)year		Age group(50-59)year		Age group(60)year and over	
	Control group (n=15)	Patients group (n=23)	Control group (n=11)	Patients group (n=17)	Control group (n=9)	Patients group (n=18)
Creatinine (mg/dl)	0.07±0.01 a	3.30±0.36 c	0.32±0.11 a	1.78±0.22 b	0.51±0.10 a	1.43±0.31 b
Urea (mg/dl)	33.0±2.19 a	71.8±9.76 c	34.6±5.94 a	79.3±0.80 c	53.5±3.68 b	96.0±2.01 d
Albumin (g/dl)	6.88±0.47 a	b 3.51±0.44	d 5.53±0.15	b,c 4.15±0.30	c,d 5.20±0.19	b,c 4.13±0.51
Bilirubin (mg/dl)	0.14±0.04 a,d	0.45±0.05 b,c	0.08±0.00 a	0.69±0.21 c,d	0.09±0.04 a	0.93±0.14 d

Table (5): The correlation between hepcidin hormones and some parameters.

Hormone and parameters	Positive correlation	Negative correlation
Parathyroid hormone		-0.049
Vitamin D_3		-0.382
Iron	0.325	
Calcium	0.051	
Hemoglobin		-0.030
Paraoxonase		-0.026
Glutathione-S-transferase		-0.174
Ceruloplasmin	0.098	
Total cholesterol	0.111	
Triglyceride		-0.158
HDL-C		-0.186
VLDL-C	0.355	
BMI		-0.125
Creatinine		-0.118
Urea	0.102	
Albumin	0.031	
Bilirubin	0.251	

Conclusion

Through the results of this study, we noticed a significant decrease in the levels of the hepcidin in women with osteoporosis as we noticed a positive correlation was found between hepcidin hormone, ceruloplasmin, total cholesterol, VLDL and biochemical that are an indicator of kidney function such as urea, as well as bilirubin and albumin.

3. Benefits

We learned about the role of hepcidin in women with osteoporosis and its relationship with the rest of the Biochemical parameters, and this gives us an idea about the metabolism of this hormone and its mechanism of action in women with osteoporosis. Next: will women with osteoporosis suffer from anemia? Through the results of the study, it was found that there is a relationship between osteoporosis and anemia in postmenopausal women.

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