RESEARCH ARTICLE

A Study of Hepcidin Levels and Other Biochemical Parameters in Women with Osteoarthritis: As a Pharmaceutical Control

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ABSTRACT

Background: Hepcidin, one of the peptide hormones generated from the liver, can be defined as an important regulator regarding systemic iron homeostasis, while its lopsided production plays a role in the pathogenesis of a variety of iron diseases. Osteoarthritis (OA) frequently coexists with a number of diseases, including hemochromatosis, ß-thalassemia, cell disease, sickle cell disease, and hemosiderosis caused by iron overload. Evidence suggests that iron deficiency and iron overload negatively impact the bone, which acts immediately on the bone's cells. The present work aims to determine whether a direct correlation exists between female patient's OA and levels of hepcidin, DHVD3, and other indicators.

Methods: A total of 60 participants were split into two groups for this work: 30 healthy controls and 30 female OA patients. This work was carried out from Nov. 2021 to the end of June. 2021. The ages ranged from (40 to 55). In Al-Mahmudiya Hospital and in Al-Yarmok Teaching Hospital, an endocrinologist assessed each one of the patients. We measured Hb, BMI, ferritin, hepcidin, DHVD3, iron, Ca, and PO4.

Results: Hepcidin levels significantly increased, whereas ferritin, BMI, iron, vitamin D, calcium, and PO4 levels significantly decreased compared with the control group. However, alterations in lipid profile and glycemic profile were not statistically significant.

Conclusion: By connecting low levels of iron, ferritin, calcium, vitamin D3, and PO4 with high levels of hepcidin in women with OA, it may be concluded that vitamin D3 insufficiency is associated with hepcidin malfunction as a factor affecting women with OA.

Keywords: BMI, Hepcidin, Osteoarthritis, Vitamin, Lipid profile.

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INTRODUCTION

Hepcidin, one of the peptide hormones generated from the liver, can be defined as an important regulator regarding systemic iron homeostasis, and its lopsided production plays a role in the pathogenesis of various iron diseases. Hepcidin performs the next activities by preventing iron from flowing into plasma: stored iron mobilization, absorption of the nutrients from the hepatocytes, and release of old RBCs from the macrophages.[1] The levels of hepcidin could reflect the integration regarding a number of key signals involved in the iron regulation process. Hepcidin is responsible for the direct regulation of iron absorption; therefore, measuring bio-availability must be one of the valuable clinical tools for treating iron problems.[2,3] Osteoporosis (OP) frequently coexists with a number of illnesses, including hemochromatosis, ß-thalassemia, cell disease, sickle cell anemia, and hemosiderosis caused by iron overload.[4,5] Evidence suggests that iron deficiency as well as iron overload have a deleterious impact on bone, operating immediately on the bone's cells. Iron deficiency appears to lower BMD (i.e., bone mineral density), which appears to cause a significant alteration in the bone's structural makeup. Iron overload encourages bone resorption and causes osteopenia and OP, which prevents the formation of the bone.[6] Iron is a crucial element for almost all living things and cells. Iron, however, may become one of the potential biohazards when present in excess due to its redox reactivity, promoting oxidative stress. Balanced iron metabolism and its dysregulation are essential for health because they prevent sickness.[7] In mammals, hemoglobin contains the vast bulk (more than 70%) of the body's iron. It facilitates oxygen transport and has been divided in the RBCs. Active 1,25-dihydroxyvitamin D (Vit D) supports phosphorous absorption in kidneys, intestinal calcium absorption, and calcium and phosphate release in bones.[8-10] The enzyme driving this process is CYP27B1,

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which is a hydroxylase of 25-hydroxyvitamin D. Both the severe syndrome of rickets as well as the rare autosomal recessive disorders with an early onset could be brought on by inactivating mutations in (CYP27B1).[11]

MATERIALS AND METHOD

In the presented work, a total of 60 women between the ages of 40 and 55 were included. These were divided into the following two groups: the OA group (G2) had 30 patients, while the control group (G1) included 30 healthy individuals. Following a period between 12 and 14 hrs of fasting, blood samples were taken from both groups. Endocrinologists assessed patients at Al- Al-Mahmudiya Hospital and Al-Yarmok Teaching Hospital from November 2021 until the end of June 2021. The serum was acquired. Fasting blood triglycerides,[12] HDL,[13] LDL,[14] and lipid profiles, in addition to total cholesterol,[15] have been assessed in the control and patient groups. Measurements of the blood pressure, waist circumference, and BMI were taken. BMI was determined with the use of the formula weight/height squared.[16]

Determination of Ferritin[17]

VIDAS Ferritin (FER) assay is ELFA, or enzyme-linked fluoresce immuno-assay, carried out on an automated instrument. The instrument manages the assay temperature and all assay procedures.

Hepcidin Principle of Assay Determination[18]

According to the manufacturer's manual instructions, HEPC ELISA kit uses the quantitative sandwich enzyme immunoassay method.

Iron Determination[19]

This Beckman Coulter technique makes use of a modified version of these techniques. This Beckman Coulter approach uses a variant of those techniques and TPTZ [2,4,6Tri-(2pyridyl)-5-triazine] as a chromogen.[20] The acidic medium separates iron coupled to transferrin to apo-transferrin and free ferric ions. Ferric ions are lowered by sodium ascorbate and hydrochloric acid to the ferrous state. Then, TPTZ interacts with ferrous ions to produce a blue-hue complex that might be detected bi-chromatically at 600/800 nm.

Determination of DHVD3 Levels in Blood Serum

The quantities of plasma 25(OH)D were measured with use of the enzyme-linked fluorescence assay (ELFA) method on a small VIDAS Biomerieux automated immunoanalyser (Biomerieux, Marcy-Ietoile, France).

Calcium Determination[21,22]

This process of calcium relies on the reaction of (Ca2+) Arsenazo III (2,2'-[1,8-Di-hydroxy-3,6-disulphonaphthylene-2,7-bisazo]- bisbenzenear-sonic acid) with calcium ions to produce a vivid purple colored complex.

Determination of Inorganic Phosphorus

Use the technique created by Ertingshausen and Daly.[23]

Statistical Analysis

Data has been transformed into electronic database. In Excel 2010, the student's F-test was used to do statistical analysis from a one-way ANOVA to compare parameters measured in controls and patients. Measurement contains the means and the standard error of the means. Statistical significance was defined as a level of significance that is less than 0.05.

RESULT AND DISCUSSION

Table 1 lists anthropometric data of OA patients for this investigation. In OA patient group, there was a substantial increase in SBP and DBP ($p \leq 0.001$), as well as a non-significant increase in age, height, and weight, compared with controls. BMI and weight clearly increased in OA group when compared to control groups, as seen in Table 1. The findings were consistent with Fawzy *et al.*[24]. Reduced body mass is related to reduced BMD and a greater OA risk. WHO defines OA as a progressive systemic skeletal illness that is defined by low bone mass as well as micro-architectural degradation regarding the bone tissue, increasing the fragility of bones and fracture risks as a result. Dual-energy X-ray absorptiometry (DXA) that measures the BMD, is the gold standard for OA diagnosis.

Dyslipidemia does not manifest in the OA group, which in the presented work had non-significant changes in serum level of the TAG, TC, LDL-C and VLDL.

Due to the fact that iron is a separate risk factor for OA, iron reduction treatment approaches could aid in OA prevention. Hepcidin that is produced in the liver and is an iron-reducing parahormone, is a crucial iron homeostasis regulator *in-vivo*. In the case where levels of iron are high, iron absorption is blocked and iron that is absorbed is stored in the organs; however, in the case where the levels of iron are low, the iron that has been absorbed by gut is released. Hepcidin is a good pharmacological target for postmenopausal OP as it is an endogenous iron-reducing hormone, and targeting hepcidin will typically have fewer adverse impacts compared to chelating medicines, which are concerned with hyperpigmentation (Table 2).

The serum hepcidin level is high in OA patients compared to healthy subjects in this study, which might result from

Table 1: Anthropometric and clinical features of control and OA groups

	Mean ± SD	*Mean ± SD*	*p-value*
	OA (n= 30)	*Control (n= 30)*	
Age (year)	45.76 ± 9.20	42.130 ± 8.960	0.13
Weight (kg)	70.56 ± 7.14	80.88 ± 8.32	0.0001
Height (cm)	166.21 ± 9.63	165.15 ± 12.07	0.711
SBP (mmHg)	135.76 ± 8.25	119.60 ± 2.44	0.0001
BMI (kg/m^2)	26.8 ± 4.45	29.8 ± 4.08	0.007
DBP (mmHg)	80.83 ± 2.29	78.67 ± 2.34	0.0001
Duration of DM (Years)	-	-	0.030
Duration of OA (Years)	3.23 ± 1.85	-	0.932

Table 2: Glycemic profile of control and OA groups

Parameter	*Mean ± SD*	*Mean ± SD*	*p-value*
	OA (n= 30)	*Control(n= 30)*	
FSG (mg/dL)	84.26 ± 15.2	88.23 ± 6.73	0. 2
HbA1c (%)	5.41 ± 0.55	5.19 ± 0.45	0.1
(µU/mL)	15.02 ± 4.44	13.30 ± 3.98	0. 1

inflammation, a necessary factor affecting serum hepcidin. In the current study, iron deficiency and OA patients have clearly decreased levels of serum hepcidin**.** These results agree with study containing patients with iron deficiency and RA had clearly lowered levels of serum hepcidin compared to RA with chronic inflammation and anemia.[25] Results vary on the effect on disease activity and Hb[26,27] as hepcidin is affected by iron metabolism and inflammation. In the current study, Hb was connected with the level of serum hepcidin, whereas strong relationships have been spotted for iron metabolism (ferritin and iron) (Table 3).

In the research that was presented, OA patients had lower serum iron levels and higher levels of serum hepcidin than the healthy control group. Humans with thalassemia and hereditary hemochromatosis have iron excess, and OA is a major side effect.[28,29] Iron excess, which raises ferritin levels, has been linked to progressive bone loss in middle-aged males and healthy postmenopausal women as well as radiographic vertebral fracture in postmenopausal women.[30] The levels of serum hepcidin of 40 OA patients and 40 healthy control individuals were compared in these findings, which contradict with Liu *et al.*[31] In the case when put to comparison with healthy controls, they found that OA patients had low levels of serum hepcidin and high amounts of iron, and that there was a negative correlation between the two.[31] The amount of serum hepcidin and the metabolism of bone markers were not shown to be directly correlated. In individuals who were in remission,

Table 3: Lipid profile of control and patient groups

Parameter	*Mean ± SD*		*p-value*
	OA (n= 30)	*Control (n= 30)*	
TC (mg/dL)	160.3 ± 30.4	155.9 ± 29.74	0.58
TAG (mg/dL)	96.13 ± 56.73	93 ± 9.09	0.05
LDL-C (mg/dL)	93.54 ± 33.7	84.43 ± 30.54	0.28
VLDL (mg/dL)	18.06 ± 0.85	18.6 ± 1.81	0.15
HDL-C (mg/dL)	52. 3 ± 8.5	52.87 ± 5.40	0.76

Table 4: Principal bio-chemical iron parameters with the indications of the iron status of the control and OA groups

Parameter	*Mean ± SD*		*p-value*
	OA (n= 30)	*Control (n= 30)*	
Hb (g/dl)	10.78 ± 1.0	12.93 ± 0.6	0.001
Hepcidin (ng/ml)	9.84 ± 5.06	5.40 ± 1.49	0.05
Ferritin (ng/ml)	15.99 ± 18.67	35.9 ± 7.07	0.05
Iron (mmol/L)	31.5 ± 11. 6	111.53 ± 18.32	0.001

$p < 0.05$: Significant.

Table 5: Levels of Vit D, PO4, Ca of the OA and control groups

Parameters	*Means ± SD*		*p-value*
	OA (n= 30)	*control (n= 30)*	
Vit D(ng/ml)	8.32 ± 0.93	40.9 ± 7.3	0.001
PO_4(mg/dl)	2.54 ± 0.65	3.71 ± 0.44	0.001
Ca(mg/dl)	3.73 ± 0.42	7.2 ± 0.45	0.001

Table 6: Coefficient of correlation of the serum hepcidin, Vit D and some study parameters in OA groups

Parameter6	*Hepcidin (ng/mL)*		*Parameter*	*Vit D (ng/mL)*	
	R	*p*		*r*	*p*
Age (Years)	0.06	0.0010	Age (Years)	0.10	0.001
BMI (kg/m^2)	0.09	0.0010	BMI (kg/m^2)	0.11	0.001
DBP (mmHg)	-0.30 NS	0.1180	DBP (mmHg)	0.18 NS	0.386
SBP (mmHg)	-0.26 NS	0.1760	SBP (mmHg)	0.08 NS	0.625
(µU/mL)	0.02	0.050	(µU/mL)	-0.28	0.001
FSG (mg/dL)	-0.18	0.0010	FSG (mg/dL)	0.04	0.001
HbA1c (%)	-0.17	0.050	HbA1c (%)	0.18	0.001
TC (mg/dL)	-0.06	0.0010	TC (mg/dL)	0.33	0.001
TAG (mg/dL)	0.01	0.0010	TAG (mg/dL)	-0.04	0.001
VLDL (mg/dL)	0.07	0.0010	VLDL (mg/dL)	-0.03	0.001
HDL-C (mg/dL)	-0.05	0.0010	HDL-C (mg/dL)	0.31	0.001
LDL-C (mg/dL)	-0.06	0.0010	LDL-C (mg/dL)	-0.08	0.001
Hb(g/dl)	-0.09	0.3410	Hb	-0.30	0.001
Ferritin(ng/ml)	-0.04	0.0920	Ferritin	-0.02	0.031
Iron(mmol/L)	0.07	0.0010	Iron	0.01	0.001
PO_4(mg/dl)	0.11	0.0010	PO4	-0.18	0.001
Ca(mg/dl)	0.22	0.05	Ca	-0.29	0.001

* $p < 0.05$, ** $p \leq 0.01$, NS: Non-Significant.

such findings were a little clearer to see. The intricate interactions between hepcidin levels, iron insufficiency, serum iron and inflammation, and treatment in OA patients may have contributed to the study's unexpected findings regarding OA and iron metabolism. Most of the patients in this work had minimal disease activity; therefore, the outcomes might have been different in patients with higher OA activity. Iron buildup poses a danger for OA, and hepcidin is regarded to be a helpful treatment target (Table 4).[32,33]

Vitamin D influences calcium regulation and mineralization of bones, and 25(OH)D serum level in RA is favorably correlated with BMD.[32] A recent *in-vitro* investigation showed 1,25(OH)2D binding to vit. D receptor immediately reduced transcription of hepcidin gene.[34] On the contrary, BMI, P, and Ca in the sera of OA Iraqi patients indicated no discernible variation between patient groups severe OA, moderate OA] and control group.[35] An earlier investigation found that the extra-cellular iron prevented survival of osteoblast cell and resulted in osteoblast cell death.[36, 37] Also iron also directly negatively affects bone cells and bone remodeling.[38] Kraidith *et al.* have demonstrated that the intestinal iron transport inhibitor hepcidin could increase calcium malabsorption (Table 5).[39]

Correlations amongst Serum Hepcidin, Vit D and Parameters of the Study in the OA Group

Table 6 shows the association coefficient of the serum hepcidin, vitamin D, and other indicators in OA group.

Table 6 shows the correlation coefficient regarding the serum hepcidin, vitamin D, and other indicators in OA group. With regard to OA group, there were strong and highly significant negative correlation between serum hepcidin and FSG, LDL-C, HbA1c, TC, and HDL-C values ($p < 0.05$, $p < 0.001$), whereas in OA group, there were strong and highly significant positive correlations between serum hepcidin and age, , BMI, VLDL, TAG, Ca, Iron, and PO4 ($p < 0.05$, $p < 0.001$). There were significant negative correlations between serum vitamin D and levels of TAG, , LDL-C, VLDL, Hb, PO4, and calcium in OA group ($p < 0.001$). While in the OA group, there have been significant positive correlations between serum vitamin D and age, FSG, BMI, hemoglobin A1c, total cholesterol, HDL-C, and iron.

CONCLUSION

Relating low levels of iron, ferritin, vitamin D3, calcium, and PO4 along with high levels of hepcidin in women with OA showed a vitamin D3 shortage linked to hepcidin malfunction as a factor affecting women with OA.

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