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A Study of Hepcidin Levels and other Biochemical Parameters in Woman with Osteoporosis with Type 2 Diabetes Mellitus

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Abstract:

Background:

Diabetes mellitus (DM) is regarded as a set of chronic metabolic disorders which have a common aspect of hyperglycemia. The resistance in the peripheral actions of insulin or impaired insulin secretion can be the reason for hepcidin, a peptide hormone derived from the liver. In systemic iron, homeostasis is an essential regulator, and its lopsided production participates in the pathogenesis of iron disorders in the spectrum**.** Osteoporosis often accompanies diseases such as ß-thalassemia, hemochromatosis, sickle liver diseases, cell disease, and hemosiderosis featured to iron overload. Iron overload and iron deficiency are suggested by evidence that they affect bone negatively, acting straightway on the cells of the bone. This study aims to find the direct relationship between the levels of hepcidin, DHVD3, and other parameters in osteoporosis with type 2 DM, in a female patient. This study involved 60 subjects divided into two groups 30 female osteoporosis with type 2 DM patients and 30 healthy controls. From November 2021 until the end of June 2021, this study was conducted. The ranges of age were 40 and 55 years. All diabetic patients were examined by an endocrinologist in the Al-Yarmouk Teaching Hospital and Al-Mahmudiya Hospital. The control subjects were referred to Laboratories Yarmouk Teaching Hospital, where all measurements were made. Control subjects were collected with glycemic control inclusion criteria when fasting serum glucose (FSG) < 100 mg/dL and HbA1c < 5.7%. BMI, Hb, hepcidin, ferritin, iron, DHVD3, Ca, and PO4 were determined.

The results showed a significant increase in glycemic and lipid profiles compared to the control group. A significant decrease compared with the control group in Hb, ferritin, iron, Vit D, Ca, and PO4. It also showed a considerable increase in hepcidin. By linking ferritin, iron vitamin D3, Ca, PO4 deficiency, and hepcidin overload to women, diagnosed osteoporosis with T2DM, which indicated vit D3 deficiency related to hepcidin dysfunction. as an influencing factor in osteoarthritis women.

Keywords: Osteoporosis, Hepcidin ,Vit D, Type 2 Diabetes Mellitus

1.Introduction

A rise in bone mineral density (BMD) can cause osteoporosis because of multiple factors [1]. Diabetes mellitus (DM) could be regarded as a set of chronic metabolic disorders with a common aspect of hyperglycemia. The resistance in the peripheral actions of insulin or impaired insulin secretion might be the reason for this [2]. Hepcidin, a peptide hormone derived from the liver, in systemic iron homeostasis is an essential regulator, and its lopsided production participates in the pathogenesis of iron disorders in the spectrum. By closing the iron flows into plasma, hepcidin functions as the following: the absorption of duodenal from hepatocytes and mobilization of stored iron recycling old red blood cells released from macrophages [3].

In regulating iron, hepcidin levels can reflect the integration of many significant signals, and iron absorption is directly controlled by hepcidin. Bioavailability should be a useful clinical tool in circulation and measurement for iron disorders management [4,5]. Osteoporosis (OP) often accompanies many diseases such as ß-thalassemia, hemochromatosis, sickle liver diseases, cell disease, and hemosiderosis featured by iron overload [6,7]. Iron overload and iron deficiency are suggested evidence that negatively affects bone, acting straightway on the cells of the bone. Bone mineral density (BMD) reduced by iron deficiency resulting in an intense change in the structure of the bone. Bone resorption is promoted by iron overload, inducing osteopenia and OP. It inhibits the formation of bone [8].

For nearly all organisms and living cells, iron are a key element. Nevertheless, iron might become a possible biohazard when present in excess because of its redox reactivity, which promotes oxidative stress. Thus, leading to disease, balanced iron metabolism and its deregulation are fundamental for wellbeing [9]. The immense majority of the iron in the body (>70%) in mammals is within haemoglobin. It mediates oxygen transport and is divided into red blood cells.

Active 1,25-dihydroxy vitamin D (Vit D) reinforces phosphate reabsorption in the kidney, phosphorous absorption, and intestinal calcium, releasing phosphate and calcium in the bone [10-12]. The 25-hydroxyvitamin D 1α-hydroxylase (CYP27B1) is the enzyme responsible for this reaction. Inactivating mutations in (CYP27B1) can cause a severe syndrome of rickets and an uncommon autosomal recessive disorder featured by the early onset [13].

2. Materials and Method

This study enrolled 60 female individuals in the age range of (40-55) years. These were classified into two sets as follows: the control group (G1) contained 30 healthy subjects, and the group of osteoarthritis (OS) with T2DM (G2) included 30 patients. Blood samples were collected

from the two groups after 12-14 hours of fasting. The current study was made between November 2021 and the end of June 2021, and an endocrinologist examined all the patients in the Al-Mahmudiya Hospital and Al-Yarmouk Teaching Hospital. Serum was obtained

The sample was poured into a plain tube for 15 minutes for clotting, it was left at room temperature, at 3000 rpm, centrifuged for 10 min, and until the time of assay, the serum was aliquot and stored in the freezer at -20ºC. For the HbA1c assay, two milliliters of blood were poured into an EDTA tube, fasting serum glucose determination [14]. Glycated hemoglobin determination [15] , serum insulin determination [16], fasting blood triglyceride [17], LDL [18]. LDL, HDL [19], and lipid profiles, besides the total levels of cholesterol [20] of the patient and control groups, were measured. Waist circumference, body mass index **(BMI**), and blood pressure measurements were done. Using the formula weight/height squared, body mass index was calculated [21].

2.1. Determination of Ferritin: [22]

Performed in an automated instrument, the VIDAS ferritin (FER) assay is an enzyme-linked fluoresce immunoassay (ELFA). By the instrument, assay temperature and all assay steps were controlled.

2.2. Hepcidin Principle of Assay determination [23]

The quantitative sandwich enzyme immunoassay technique was applied by the HEPC ELISA kit, and as the manufacturer's manual stated

2.3. Iron Determination [24]

This method of Beckman Coulter utilizes a variation of these methods using TPTZ [2, 4, 6-Tri-(2-pyridyl)-5-triazine] as the chromogen, this method of Beckman Coulter utilizes a variation of these methods.[25] Transferrin-bound iron dissociates into apo-transferrin and free ferric ions in an acidic medium. To the ferrous state, sodium ascorbate and hydrochloric acid reduce the ferric ions. TPTZ. After that, it reacts with the ferrous ions to form a complex blue color that could be bichromatically measured at 600/800 nm

2.4. Determination of DHVD3 Levels in Blood Serum [26]

A mini VIDAS biomerieux automated immune analyzer was used to measure the concentrations of the plasma 25(OH)D using the technique of enzyme-linked fluorescent assay (ELFA) (Biomerieux, Marcy-I'Etoile, France).

2.5. Determination of Calcium [27,28]

To form an intense purple coloured complex, this procedure of calcium is based on (Ca2+) arsenio III (2,2'-[1,8-Dihydroxy-3,6-disulphonaphthylene-2,7-bisazo]- bisbenzenear-sonic acid) reacting with calcium ions.

2.6. Determination of Inorganic Phosphorus: Based on Ertingshausen and Daly's developed method [29].

2.7. Statistical Analysis

Data has translated into electronic database structure. Students' F-test did statistical analysis from one-way ANOVA in excel 2010 to compare between the parameters measured in the control groups and patients. The measurement also included standard error mean and the means. A level of significant of less than 0.05 was taken as statistically significant.

Parameters	Means ± SD		*p*-value
	with DM (n= 30)	Control(n= 30)	
Age (years)	45.4 ±5.7	42.13±8.96	0.1
Height (cm)	163.88±8.29	165.15±12.07	0.64
Weight (kg)	80.8 ± 8.6	80.88±8.32	0.97
BMI (kg/m^2)	30.41 ± 3.9	29.8 ± 4.08	0.62
SBP (mmHg)	155.79 ± 8.57	119.60 ± 2.44	0.001
DBP (mmHg)	86.17 ± 3.0	78.67 ± 2.34	0.001

Table 1. Anthropometric and clinical features of the OS with DM and control groups.

3. Result and Discussion

This study shows clinical features and anthropometric for OS with DM patients and control groups as shown in **Table (1).** There was a highly significant increase ($p \leq 0.001$) in SBP, DBP, and non-significant increase in BMI, and a non-significant decrease in high and weight in OS with DM patients compared to the control.

The prevalence of T2DM has increased quickly worldwide, especially in low-and middle-income countries over the past decade. Both comorbid conditions and diabetes complications were recognized to define life quality in patients diabetic with type 2, and poor quality of life causes a high mortality risk [30].The age of patients who have diabetes was realized to be higher than 40 years assured by Hinkel R. et al. [31], who are the earliest researchers in this field; previous studies showed that the age plays a significant part in developing T2DM risk after 40 years.

There was a highly significant increase (p=0.001) in insulin, HbA1c, and FSG in OS with the DM group as compared to the control group, as shown in **Table (2)**

Table 2. Glycemic profile of OS with the DM and control groups

Parameters	Means ± SD	Means ± SD	*p*-value
	OS with DM (n= 30)	Control(n= 30)	
FSG (mg/dL)	193.1±53.96	88.23±6.73	0.001
HbA1c (%)	8.33±1.35	5.19 ± 0.45	0.001
Insulin (µU/mL)	26.67±5.63	13.30±3.98	0.001

These results showed a significant decrease in FSG in OS with DM patients compared with that of control group. Due to the case that the main characteristic feature of DM was hyperglycaemia, these results are expected. Two key processes strongly organize blood glucose: insulin action on major target organs, i.e., liver, skeletal muscle, adipose tissue, and in response to a nutrient, insulin secretion by pancreatic β-cells. Type 2 DM is often associated with obesity and results from insufficient insulin production/secretion and IR represented by hyperinsulinemia [32].

This study elucidated the (means ±SD) of FSG and HbAlc of OS with DM and control groups as shown in Table (2). Therefore, these findings determined that patients with diabetes were susceptible to higher blood glucose levels connected to lipid peroxidation. For long-term means, glycated Hb is a routinely used marker of the blood glucose level for the development of diabetes complications; HbAlc predicts the risk (33). A rising in HbAlc, as detected in conditions of poor diabetic control, has been connected with increased blood viscosity. Glycosylation of Hb and increased glucose levels may alter membrane lipoprotein interactions in RBCS, altering their internal viscosity, modifying viscoelastic properties of erythrocyte membranes, and impairing RBC deformability [34].
Biochemical results of the tests demonstrated that FSG levels and insulin were higher in OS with the DM groups compared with that of the control; lipotoxicity and hyperglycemia were accepted to play roles in the possible mechanisms underlying impaired β-cell function [35]. If the β-cells were exposed to hyperglycemia, they might promote efflux of the β-cells insulin secretory granules, leading to less insulin being released in response to other hyperglycemia [36].

Table 3. Lipid profile of OS with the DM and control groups

Parameters	**Means ± SD**		***p*-value**
	OS with DM (n= 30)	**Control (n= 30)**	
TC (mg/dL)	230.133±50.91	155.9±29.74	0.05
TAG (mg/dL)	195.1±79.10	93±9.09	0.05
VLDL (mg/dL)	39.02±15.82	18.6±1.81	0.05
HDL-C (mg/dL)	39.6±4.77	52.87±5.40	0.001
LDL-C (mg/dL)	151.51±52.43	84.43±30.54	0.05

In this study, dyslipidemia appeared among OS with DM group which showed a significant increasing in serum levels of TC, TAG, VLDL, LDL-C, and low level of HDL-C as compared to the patients who are non-diabetic which is in agreement with that of previous study [37].
Nearly 40% to 50% of patients who have diabetic present LDL-C values were over 130 mg/dL. In diabetic patients, it has been explained that the cholesterol decreased by more than 70% the death risk and repetition of coronary events [38]. In accordance to standards of the American Diabetes Association and the third report of the National Cholesterol Education Program, the diabetic patients' objectives indicate that the target LDL-C should be 100 mg/dL or lower [39].

Table 4. Principal biochemical iron parameters with indications of iron status in OS with DM and control groups

Parameters	Means ± SD		*p*-value
	OS with DM (n= 30)	Control (n= 30)	
Hb(g/dl)	11,62±1.34	12.93±0.63	0.001
Ferritin(ng/ml)	24.96±5.40	35.9±7.07	0.05
Hepcidin(ng/ml)	15.94±9.28	5.40±1.49	0.05
Iron(mmol/L)	56.23±10.56	111.53±18.32	0.001

In this study, the disease activity of most patients was low; in patients with higher OS activity, the results may have been different. For osteoporosis, the accumulation of iron is a risk factor, and hepcidin is thought to be a beneficial therapeutic target [40]. Hepcidin knockout mice had higher iron and serum ferritin levels in the femur and liver than controls and presented changes in bone and low bone mass microarchitecture in one study [41]. A mouse model overexpression of hepcidin showed higher serum hepcidin levels and lower serum ferritin levels, bone loss, and changes in markers of bone metabolism after ovariectomy were ameliorated [42].

When inflammation occurs, hepcidin production increased because of the expression of inflammatory cytokines, influences lowering iron, and increases ferritin levels. However, in contrast with previous research, opposite impacts of the serum iron level on BMD and the serum 25(OH)D on the serum hepcidin level have been marked. In addition, serum 25 (OH)D and inflammation were widespread factors connected with serum hepcidin levels. More research is necessary to locate these mechanisms.

In the cells, systemic hepcidin is made by hepatocytes and is mainly regulated by the iron load. Glucotoxicity inhibits serum hepcidin expression and glucose-stimulated insulin secretion and secretion that, in turn, by inhibiting pancreatic and duodenal homeobox 1 (Pdx-1) expression, reduces β-cell insulin synthesis in T2D, so transitory overexpression in hepcidin was used to flip the down-regulation of Pdx-1 of secretion of insulin induced by glucotoxicity [43].

Med.J et al. [44] found that the hepcidin considerably impaired glucose tolerance in pancreas tissue, as pancreatic beta cells were recognized to have a highest hepcidin expression. Moreover, beta cells can dedicate insulin and hepcidin, reinforcing the connections between iron metabolism and glucose. Those mechanisms must be studied to demonstrate a better description of how insulin resistance impacted levels of hepcidin. But such results contrasted with those of Simcox et al. (2013) [45], who discovered that hepcidin considerably was low in risky type 2 diabetic subjects with (p -value=0.636).

Parameters	Means ± SD		*p*-value
	OS with DM (n= 30)	control (n= 30)	
Vit D (ng/ml)	8.44±0.47	40.9±7.3	0.001
PO_4(mg/dl)	1.98±0.40	3.71±0.44	0.001
Ca (mg/dl)	5.94±1.01	7.2±0.45	0.05

Table 5. Levels of Vit D, inorganic phosphorus, calcium in OS with DM and control groups

Independent of hepcidin level determinants and a wide variety of diabetic risk variables, baseline serum hepcidin levels that were inadequately low in comparison to body reserves of iron significantly predict the risk of T2DM.

3.1. Correlation of serum Hepcidin, Vit D and Study Parameters in OS with the DM Group:

The Vit D, a correlation coefficient of serum hepcidin, and other parameters in OS with DM group are shown in **Table(6).** There were significant negative correlations between age and serum hepcidin. VLDL, HbA1c, TC, TAG, LDL-c, Ca, PO4 levels in OS with DM group. While significant positive correlations were found between FSG, serum hepcidin, BMI, Insulin, HDL-c, and iron levels in OS with the DM group. In addition, there were significant negative correlations between Vit D and BMI, FSG, TAG, VLDL, HDL-c, Hb, ferritin, iron, PO4, and Ca levels in OS with the DM group. Whereas a significant positive correlations were found between Vit D and age, Insulin, TC, and LDL-C levels in OS with the DM group.

Table 6. Correlation coefficient of serum hepcidin, Vit D and some study parameters in OS with the DM group

Parameters	Hepcidin (ng/ml)		Parameters	Vit D (ng/ml)	
	r	*p*		r	*p*
Age (Years)	-0.10	0.001*	**Age (Years)**	0.16	0.001
BMI (kg/m^2)	0.20	0.001	**BMI (kg/m^2)**	-0.05	0.001
FSG (mg/dL)	0.11	0.001	**FSG (mg/dL)**	-0.12	0.001
HbA1c (%)	-0.11	0.05	**HbA1c (%)**	-0.019	0.681
Insulin (µU/mL)	0.09	0.05	**Insulin (µU/mL)**	0.36	0.001
TC (mg/dL)	-0.14	0.001	**TC (mg/dL)**	0.10	0.001
TAG (mg/dL)	-0.16	0.001	**TAG (mg/dL)**	-0.16	0.001
VLDL (mg/dL)	-0.16	0.05	**VLDL (mg/dL)**	-0.16	0.001
HDL-C (mg/dL)	0.25	0.001	**HDL-C (mg/dL)**	-0.13	0.001
LDL-C (mg/dL)	-0.11	0.001	**LDL-C (mg/dL)**	0.16	0.001
Hb (g/dl)	-0.07	0.017	**Hb**	-0.27	0.001
Ferritin (ng/ml)	-0.09	0.001	**Ferritin**	-0.07	0.001
Iron (mmol/L)	0.24	0.05	**Iron**	-0.03	0.001
PO_4 (mg/dl)	-0.17	0.001	**PO4**	-0.15	0.001
Ca (mg/dl)	-0.002	0.05	**Ca**	-0.26	0.001

4. Conclusion

By connecting hepcidin overload, vitamin D3 insufficiency, and iron deficiencies to women with osteoarthritis, it was discovered that vit D3 deficiency associated to hepcidin dysfunction was a contributing cause.

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