EVALUATION OF THE LEVELS AND ETHIOLOGİC ROLES OF HEPCIDIN AND IRON METABOLISM IN PRE-DIABETIC PATIENTS

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ABSTRACT

Objective*: Iron can increase the development of type 2 Diabetes Mellitus (DM) through the formation of reactive oxygen radicals and subsequent oxidative distress. Hepcidin is a hormone that regulates iron absorption and distribution to tissues. Both hepcidin and ferritin are acute phase reactants and their serum levels may increase with all kinds of inflammations. Proinflammatory cytokines are known to increase in type 2 DM. For these reasons, we aimed to investigate the role of serum hepcidin and iron markers in the etiology of diabetes in pre-diabetic patients.*

Method: *It is a single-center, prospective, cohort study. 60 prediabetic and 30 healthy women was included according to the Oral Glucose Tolerance Test (OGTT) results. None of them had a systemic disease or use any drugs and cigarettes that could affect glucose concentration. The Study was approved by the Ethics committee of Kocaeli University. Informed consent was obtained from all individuals participating in the study.*

Result: *In our study, although ferritin and hepcidin values were found significiantly high in the prediabetic patient group, no significant difference was found between the two groups in terms of iron, total iron binding capacity and saturation of the transfer.*

Conclusion: *These findings suggest that the levels of ferritin and hepcidin may increase even in the prediabetic period due to chronic inflammatory process.*

Keywords: *Hepcidin, Ferritin, iron metabolism, diabetes mellitus.*

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Introduction

Iron is a powerful pro-oxidant which catalyzes many reactions that cause the formation of reactive oxygen radicals. As a result of these reactions, it may increase oxidative stress and thus type 2 Diabetes Mellitus (DM) formation[1]. Increased iron levels have been found in many cases which can be seen with insulin resistance such as obesity, metabolic syndrome, and increased body mass index[2]. In addition increased serum ferritin levels are thought to be a component of insulin resistance syndrome. Indeed, in many studies, there is an inverse relationship between serum ferritin levels and insulin sensitivity indices[3]. Hepcidin which is a very important hormon in iron metabolism consists of 25 amino acids that regulate iron absorption and distribution to tissues, synthesized mainly by hepatocytes and excreted by circulation[4]. When iron stores are sufficient or high, the liver increases the production of hepcidin that inhibits the absorption of iron from enterosides. In addition, it prevents iron release from broken red cells and captured it into macrophages until it is used in erythropoiesis again. Thus, in the presence of hepcidin, iron is trapped in macrophages.

When iron stores are low, production of hepcidin decreases, whereas absorption of iron from enterocytes and release of iron stored in macrophages into circulation increases[5]. In addition to its role in iron homeostasis, hepcidin is also known as an inflammatory-induced acute phase reactant in mice and humans[6].Epidemiological studies have shown

the relationship between iron overload and peripheral insulin resistance[7].

The relationship between ferritin levels (indicator of iron stores) and serum iron levels, and the risk of developing diabetes were mentioned. Ferritin is an acute phase reactant which is stimulated with all kinds of infections and inflammation[8].

Type 2 DM is closely related to chronic inflammation and it is known that proinflammatory cytokines increase in type 2 DM[9]. Therefore, serum ferritin and hepcidin levels may have increased in type 2 DM patients. These findings suggest that there may be a relationship between hepcidin and diabetes and iron metabolism. In this study, we tried to find answers to these questions by comparing the level of serum ferritin, which is a direct indicator of iron metabolism and hepcidin in pre-diabetic women and healthy volunteers.

Matherials and methods

This study was conducted between December 2010 and December 2011 in which a total of 60 prediabetic female patients and 30 healthy female control groups who applied to Kocaeli University Hospital Diabetes Policlinic were included.

Ethics committee approval was given by Kocaeli University Ethics Committee and informed consent was obtained from all individuals participating in the study. Our primary endpoint was to measure serum hepcidin level measured during Oral Glucose Tolerance Test (OGTT) test.

Whereas secondary endpoints of this study are listed as follows:

• Serum iron, total iron binding capacity, serum transferrin receptor level, ferritin level in blood taken during OGTT test;

• The relationship between parameters related to iron metabolism and serum hepcidin level;

• Relationship between iron metabolism markers (including ferritin) and hepcidin and age, height, weight, waist circumference, body mass index, serum hemoglobin, platelet, leukocyte, cholesterol and triglyceride in the patient and control group.

Inclusion criteria

OGTT was applied to each of these women, and those with glucose levels less than 140 mg/dl at the 2nd hour were included into healthy controls, and those between 140-200 mg/dl were included into prediabetes groups.

Exclusion criteria

None of the women included in the study had a systemic disease such as hypothyroidism, hyperthyroidism, chronic kidney failure, chronic hepatobilier failure, chronic obstructive pulmoner disease, chronic rheumatological disease, malignancy, anemia and etc. The prediabetic and healthy control group did not use any drugs and/or cigarettes that could affect glucose concentration.

Observation indicators

Weight measurements of the participants were made by the doctor using a sensitive scale during the clinical examination. Their height was again measured by the doctor, and then their body mass indexes were calculated by dividing their weight by the square of their height in meters. Waist circumference measurements were also performed at the same time.

Taking samples

Volunteers who applied to endocrinology, internal medicine outpatient clinics but had no significant features and additional diseases other than pre-diabetes and who had hemoglobin A1c (HbA1c) , triglyceride, low density lipoprotein (LDL), total cholesterol, creatinine, hemoglobin, mean corpuscular volume (MCV), white blood cells (WBC), neutrophils, platelets (PLT), measurements was included. OGTT test was performed after 12 hours of fasting for hematological and biochemical examinations of patients and control group. Before the test, 2 cc blood samples were taken into 2 biochemistry tubes. Blood samples were centrifuged for 10 minutes at 3500 cycles for the analysis of iron, total iron binding capacity, ferritin and hepcidin, and stored in the seperated serum ependorphs at -80ºC in Kocaeli University Clinical Research Department.

Determination of samples

Serum fasting glucose was determined using the ARCHITECT C 16000 ABBOTT Laboratories (Illinois U.S.A.) autoanalysis device. Serum HbA1c level was studied by HPLC method with Shimadzu device. Triglyceride, LDL, total cholesterol, serum iron, total iron binding capacity, creatinine levels were studied with the Abbott Architect c16000 device by spectrophotometric method. Ferritin level was studied by chemiluminescence method with Advia centaur XP device. Hemoglobin values were measured with CELL-DYN 3700 ABBOTT Laboratories (Illinois U.S.A) blood count device. Hepcidin kit, serum hepcidin measurements in the study were

made using the elisa method with a detection range of 62.5 to 4000 pg/mL. Uscn Hepcidin hormone ELISA (E91979Hu) (Enzyme-Linked Immunosorbent Assay Kit for Hepcidin) kit was used to evaluate hepcidin. Hepcidin is a solid phase enzyme-linked immunoassay ELISA kit based on the principle of hormone-competitive binding. Microtiter wells are covered with a monoclonal antibody directed against the antigenic region of the hepcidin hormone molecule. The hepcidin hormone-coated antibody in the patient serum competes with the biotin conjugate of hepcidin for binding.

After incubation, the unbound conjugate was removed by washing. It was incubated with streptavidin-peroxidase enzyme complex and washed again. The color intensity formed after adding the substrate solution is inversely proportional to the amount of hepcidin in patient samples. The intensity of the formed color is at 450 nm. DYNEX -DSX ™ was read on the Four-Plate Automated ELISA Processing System (North Carolina U.S.A). Normal values are between 13.3-54.4 ng/ml.

Statistical analysis

SPSS 13.0 (Statistical Package for the Social Sciences) package program was used in the statistical analysis of the research. Conformity tests of all data to normal distribution were performed.

Continuous variables that are suitable for normal distribution (parametric) were compared with Student's t test and non-parametric variables were compared with Mann-Whitney U test. Pearson was used for parametric correlation analysis and Spearman tests were used for non-parametric correlation analysis. In each analysis, $p<0.05$ was considered statistically significant.

Results

No statistically significant difference was observed in body mass indexes, ages, waist circumference, height, and weight between women in the prediabetes group and the control group (p values were determined as 0.477; 0.213; 0.328; 0.293; 0.318, respectively). Although no statistically significant difference was observed in serum HbA1c and serum LDL levels between prediabetes and women in the control group (p values 0.117 and 0.150, respectively), there was a trend in favor of the patient group.

There was a statistically significant difference in serum triglyceride, OGTT 0 and 2 hours serum glucose levels between both groups (p values 0.001, 0.00 and 0.00, respectively). When the results of women in prediabetes group and control group were compared, no statistically significant difference was observed in blood hemoglobin, PLT, WBC levels (p values 0.568, 0.059, 0.993, respectively). Again, no statistically significant difference was observed in serum total iron levels, total iron binding capacity and saturation of transfer between women in the prediabetes group and the control group (p values 0.415, 0.716 and 0.394, respectively).

A statistically significant difference was found in serum ferritin and hepcidin values (p values 0.009 and 0.034, respectively). Clinical and laboratory findings and iron metabolism values of the patient and control groups are shown in Tables 1 and 2.

Variable	Pre-diabetic group (N=60)	Control group (N=30)	P value
Age (year)	47.27±13.56	43.63±11.63	0.213
Waist circumference (cm)	90.00±8.31	88.87±10.88	0.328
Body mass index	29.29±3.63	28.71±3.5	0.477
Height (cm)	158.53±5.5	157.86±4.21	0.293
Weight (kg)	73.82±10.68	71.50±8.60	0.318
HbA1c (%)	5.46±0.41	5.37±0.41	0.117
Triglycerides (mg/dl)	143.65±76.77	93.59±26.98	0.001*
LDL (mg/dl)	130.73±31.66	120.47±30.01	0.15
Total cholesterol (mg/dl)	208.55±39.29	194.87±34.45	0.125
Hemoglobin (gr/dl)	13.41±0.63	13.31±0.62	0.568
WBC x10^3/uL	9.96±15.52	9.71±13.90	0.993
PLT x10^3/uL	298.42±68.2	270.33±59.83	0.059
Fasting blood glucose (mg/dl)	109.17±9.68	93.27±4.58	0.000*
OGTT (2. Hour blood glucose) (mg/dl)	160.33±16.96	106.47±14.74	0.000*

Table 1: Clinical and laboratory findings of prediabetes group and control group.

Variable	Pre-diabetic group (N=60)	Control group (N=30)	P value
Iron (mcg/dl)	62.62±27.51	67.63±27.14	0.415
Total iron binding capacity (mcg/dl)	330.18±45.00	334.03±51.35	0.716
Ferritin (ng/dl)	46.70±54.43	26.21±24.87	0.009*
Hepcidin (pg/ml)	633.43±217.00	582.71±251.4	0.034*
Transferin Saturation	19.29±8.90	21.10±10.32	0.394

Table 2: Iron metabolism variables of prediabetes group and control group.
*Data are given as mean ± standard deviation. *p<0.05 statistically significant.*

In the pre-diabetic patient group, a statistically significant positive relationship was found between serum ferritin level and age and serum transferrin saturation level (p values 0.038 and 0.020, respectively), whereas a statistically significant negative relationship was found between ferritin and total iron binding capacity (p value 0.001).

When we looked at the pre-diabetic group of patients again, a statistically significant positive correlation was found between hepcidin and blood platelet level (p=0.01), a statistically significant negative relationship was found between serum hepcidin level and serum triglyceride and LDL (p values, respectively, 0.034 and 0.05) (Table 3).

When the results of healthy volunteers constituting the control group were examined, a statistically significant positive relationship was found between serum ferritin level and serum triglyceride and transferrin (p values, respectively, 0.032 and 0.029), and statistically significant negative relationship was found between serum ferritin and total iron binding capacity (p=0.001) (Table 4).

	FERRITIN		HEPCIDIN	
	r	p	r	p
Ferritin (ng/dl)			-0.570	0.665
Hepcidin (pg/ml)	-0.57	-0.665		
Age (year)	0.269	0.038	-0.168	0.200
Height (cm.)	-0.620	0.640	-0.470	0.720
Weight (kg.)	0.184	0.160	-0.080	0.890
Waist Circumference (cm.)	0.154	0.241	0.076	0.563
HBA1c	0.069	0.601	0.202	0.123
Triglyserid (mg/dl)	0.019	0.884	-0.274	0.034
LDL (mg/dl)	0.043	0.748	-0.256	0.050
T.cholesterol (mg/dl)	0.115	0.383	0.235	0.071
BMI	0.207	0.113	0.053	0.686
OGTT (0. HOUR) (mg/dl)	0.165	0.207	-0.143	0.275
OGTT (1.HOUR) (mg/dl)	0.176	0.178	-0.137	0.295
OGTT (2.HOUR) (mg/dl)	-0.08	0.952	0.093	0.478
Platelet x 10^3/uL	-0.300	0.819	0.330	0.010
Iron (mcg/dl)	0.195	0.135	0.023	0.861
Total iron binding Capasity (mcg/dl)	-0.404	0.001	0.111	0.400
Transferrin saturation	0.299	0.020	-0.008	0.955

Table 3: Correlation analysis between serum ferritin, hepcidin and other biochemical parameters in the prediabetic group.

	FERRITIN		HEPCIDIN	
	r	p	r	p
Ferritin (ng/dl)			0.285	0.127
Hepcidin (pg/ml)	-0.285	0.127		
Age (year)	0.056	0.769	-0.130	0.494
Weight (kg.)	-0.144	0.466	0.085	0.665
Waist Circumference (cm.)	0.081	0.682	0.071	0.720
Triglyserid (mg/dl)	0.400	0.032	0.017	0.932
BMI	-0.018	0.928	0.153	0438
OGTT (0. HOUR) (mg/dl)	0.024	0.901	-0.043	0.821
OGTT (1.HOUR) (mg/dl)	0.263	0.160	-0.038	0.841
OGTT (2.HOUR) (mg/dl)	0.321	0.084	0.088	0.645
Platelet x 10^3/uL	-0.235	0.212	-0.280	0.134
Iron (mcg/dl)	0.279	0.135	-0.082	0.666
Total iron binding Capasity (mcg/dl)	-0.568	0.001	-0.150	0.429
Transferrin saturation	0.398	0.029	-0.840	0.658
HbA1c (%)	0.217	0.250	0.075	0.692
LDL (mg/dl)	0.275	0.148	0.047	0.800

Table 4: Correlation analysis between serum hepcidin, ferritin and other biochemical parameters in the control group.

Discussion

Receptor and especially postreceptor level disorders are frequently determined in the development of insulin resistance. Visceral adipose tissue has an important role in the development of insulin resistance compared to subcutaneous adipose tissue. The free fatty acids released here increase depending on the level of insulin resistance in the skeletal muscle and liver. In addition, the contributions of tumor necrosis factor alpha (TNF-alfa) released from visceral adipose tissue to insulin resistance is explained by the fact that insulin receptor substrate 1 (IRS-1) increases serine phosphorylation, decreases IRS-1 expression, inhibits tyrosine kinase activity, and reduces glucose carrier protein-4 (GLUT-4) expression, due to impaired relationship between IRS-1 and phosphatidylinositol 3 kinase (PI3K9[10]. Animal experiments have shown that iron deficiency is associated with increased insulin sensitivity[11]. Epidemiological studies have also shown an association between iron overload and peripheral insulin resistance[7]. Iron stores, expressed as serum ferritin levels, are thought to be a component of insulin resistance syndrome. Indeed, many studies have shown

an inverse relationship between serum ferritin levels and insulin sensitivity indices such as higher serum ferritin level in individuals with impaired fasting glucose compared to the healthy control group[3, 12-13].

In some tissues, increased iron levels may increase the risk of diabetes through other mechanisms. For example, iron storage in the liver can cause insulin resistance at the hepatic level and hepatic glucose production cannot be suppressed[14]. Similarly, iron may affect glucose uptake of adipocytes by disrupting the effect of insulin in adipose tissue[15]. Storage of iron in the muscles increases free fatty acid oxidation and increases glucose formation[16]. Increased iron levels can cause iron storage in pancreatic beta cells and a decrease in insulin release[17].

As iron has an effect on insulin, insulin can affect iron metabolism. Studies have shown that insulin can alter the transferrin receptor distribution on the cell surface and increase cellular iron uptake in adipose tissue and liver[18]. Increased systemic inflammation can also affect iron metabolism. It has been shown as laboratory data that inflammatory cytokines increase the synthesis of ferritin[19]. An increase in insulin sensitivity due to a decrease in iron stores is another finding that strengthens the connection. Decreased ferritin levels after blood donation and increased insulin sensitivity in parallel have been shown in some studies[20].

Hepsidin is a small, cysteine-rich cationic peptide recently purified from human urine and plasma ultrafiltrate[21]. It interacts with ferroportin to regulate cellular iron release[6]. When iron stores are sufficient or high, the liver increases the production of hepcidin. Hepcidin traps ferroportin in the small intestine inside the cell, blocking the only way that transports iron from enterocytes to plasma. When iron stores are low, hepcidin production decreases, ferroportin molecules take place in basolateral membranes of enterocytes and transfer iron from enteroside cytoplasm to plasma transferritis. In the presence of hepcidin, ferroportin is trapped inside the cell and therefore iron remains in macrophages[5]. In addition to its role in iron homeostasis, hepcidin is also known as an inflammatory-induced acute-phase reactant in mice and humans[6]. Hepcidin is thought to inhibit iron homeostasis in tissue macrophages and regulate cellular iron release. Increased hepcidin levels are associated with hypoferritinemia and anemia. In the absence of hepcidin, ferroportin concentrations increase on the enterocyte surface, as a result of which absorption of dietary iron increases. Ferroportin increases in the macrophage cell membrane and thus iron output increases[22]. In our study, the ferritin and hepcidin concentrations seen in prediabetic women was found to be statistically significantly higher than the control subjects (p values are 0.009 and 0.034 respectively). In addition, a significant relationship, but interestingly statistically significant negative relationship, was found between serum hepcidin and triglyceride and LDL levels in the patient group (p values, respectively, 0.034 and 0.05). However when we looked at the pre-diabetic group of patients again, a statistically significant positive correlation was found between hepcidin and blood platelet level (p=0.01). on the other hand, When the results of healthy volunteers constituting the control group were examined, a statistically significant positive relationship was found between serum ferritin level and serum triglyceride and transferrin (p values, respectively, 0.032 and 0.029), and statistically significant negative relationship was found between serum ferritin and total iron binding capacity (p=0.001).

The relationship between iron metabolism and diabetes has attracted the attention of researchers for years. As the accumulation of knowledge about iron metabolism increases, more data about this relationship emerges. In this study, serum ferritin and hepcidin levels were found to be higher compared to healthy controls even in the early stages of diabetes. Numerous randomized studies are needed to further elaborate possible relationships between iron metabolism, serum hepcidin and diabetes, reveal other factors that may alter the level of hepcidin and the potential beneficial effects of molecules that reduce serum hepcidin levels on diabetes formation.

Conclusion

While ferritin and hepcidin values were found high in the prediabetic patient group, no significant difference was found between the two groups in terms of iron, total iron binding capacity and saturation of the transfer. These findings suggest that ferritin and hepcidin levels may increase even in the prediabetic period due to chronic inflammatory process; however, no statistically significant correlation was found between ferritin and hepcidin in the prediabetic patient group. Given the multifactorial nature of diabetes, there may be other parameters affecting all of them in the chronic inflammatory process. This situation can be elucidated in studies to be carried out by taking more patients and taking into account interleukin-1, interleukin-6, TNF-alpha and C-reactive protein values.

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