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Serum Ferritin in Healthy Subjects and Type 2 Diabetic Patients

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Abstract

In order to study the relationship between the serum ferritin level and the components of the insulin resistance syndrome in type 2 diabetic patients, we evaluated fifty type 2 diabetic patients who were selected according to NDDG/WHO criteria from those patients attending Korea University Hospital from 1997 to 1998. Twenty-five healthy non-diabetic subjects of comparable age and sex distribution acted as a control group. The results showed that the value of log ferritin was higher in the type 2 diabetes patients than the control subjects, but not at a statistically significant level ($p=0.09$). Log ferritin was correlated with fasting blood sugar level ($r=0.235$, $p=0.048$) and body mass index (BMI) ($r=0.285$, $p=0.05$). In the type 2 diabetic patients, log ferritin was correlated with fasting C-peptide ($r=0.478$, $p=0.009$). In the control subjects, log ferritin was correlated only with BMI ($r=0.477$, $p=0.012$). In a stepwise multiple regression analysis, the diabetic group showed a significant correlation between fasting C-peptide and log ferritin ($p=0.001$). In the control group, the fasting sugar level was significantly correlated with log ferritin ($p=0.034$). These results suggest that serum ferritin can be employed as a marker of not only glucose homeostasis but also insulin resistance both in type 2 diabetic and control subjects.

Key Words: Type 2 diabetes mellitus, insulin resistance, ferritin

INTRODUCTION

Excessive iron accumulation can induce organic damage that leads to diabetes. For example, 50% of transfusion-treated thalassemia patients have an abnormal glucose tolerance[1] and up to 65% of hereditary hemochromatosis patients develop diabetes mellitus.[2] But an even smaller accumulation of iron can alter the glucose and insulin homeostasis of the body. This suggestion is based on the observations that increased serum ferritin level was associated with poor glycemic control in type 1 diabetes mellitus patients[3] and that therapy with an iron-chelating agent led to an improvement in the control of diabetes in a group of patients with poorly controlled type 2 diabetes mellitus.[4]

In addition, serum ferritin, which is a good measure of body iron stores,[5] has been proposed as a component of insulin resistance syndrome. In a study by Salonen et al.,[6] serum ferritin concentration had a significant positive correlation with blood glucose, serum triglyceride, and serum apolipoprotein B concentration, and was inversely correlated with serum HDL_2 cholesterol, all of which are components of what has been termed the insulin resistance syndrome. In another epidemiological study, men with the higher intake of heme iron had increased levels of serum ferritin and an increased risk of coronary heart disease.[7]

Insulin resistance syndrome is one of the major causes of type 2 diabetes mellitus, and a number of clinical and biochemical features of insulin resistance occur in subjects with type 2 diabetes mellitus: especially, hypertension; obesity (particularly central obesity); accelerated artherosclerosis; and dyslipidemia characterized by elevated levels of serum total and very low-density lipoprotein (VLDL) triglyceride and reduced high-density lipoprotein (HDL) cholesterol concentration.[8]

Thus, in order to investigate the relationship between serum ferritin and the components of the insulin resistance syndrome in type 2 diabetes mellitus, we

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evaluated the serum ferritin concentration in the type 2 diabetic patients and the control subjects.

MATERIALS AND METHODS

Subjects

Fifty type 2 diabetic patients were selected according to NDDG/WHO criteria from those patients attending Korea University Hospital from 1997 to 1998. Age, sex, diabetes duration, BMI, diabetic medication, and laboratory examinations (see below) were recorded. Each subject had his/her body weight (to the nearest 0.1 kg) and height (to the nearest 0.1 cm) recorded while wearing only light indoor clothes and no shoes. BMI was calculated as the weight divided by height squared (kilograms per square meter).

After the subjects had been resting in a sitting position for at least 5 mins, their blood pressure was measured with an appropriate-sized cuff on the right upper arm. Blood pressure was measured two times, and the average of the two measurements was used throughout the study. The subjects were considered to have hypertension if they had a blood pressure of greater than 140 mmHg systolic, 90 mmHg diastolic, or if they were receiving antihypertensive medication. These hypertensive patients were treated by an angiotensin enzyme inhibitor, a calcium channel blocker, or an alpha blocker. Cardiovascular disease was proven by electrocardiogram, echocardiogram or coronary angiogram.

Twenty-five healthy non-diabetic subjects of comparable age and sex distribution acted as a control group. Patients with acute or chronic liver disease, malignant process, uremia, inflammatory disease, hyperthyroidism, hemolytic anemia, pregnancy, recent blood donation or transfusion, or recent iron therapy were excluded on the basis of their clinical history and biochemical data.

Laboratory measurements

Venous blood was taken from all subjects after overnight fasting and the serum was stored at $-20^{\circ}C$.

The level of serum ferritin was determined by immunoradiometric assay (Coat-A-Count kit, Diagnostic Products Corps. DPC USA). The interassay coefficient of variation (CV) was 4.4% at 131 ng/mL and 6.7% at 328 ng/mL. The intraassay CV was 4.6% at 39 ng/ml and 2.7% at 130 ng/ml.

The serum insulin concentration was measured by radioimmunoassay (Coat-A-Count RIA kit, DPC, USA). The interassay CV was 7.1% at 35 μIU/mL and the detection sensitivity was 1.5 μIU/mL using the same day procedure. The C-peptide level was also measured by RIA (Coat-A-Count kit, DPC, USA). The interassay CV was 8.05% at 1.74 ng/mL, and the intraassay CV was 7.41% at 0.74 ng/mL.

Total serum cholesterol concentration was measured through the reaction of cholesterol oxidase-peroxidase, and total serum triglyceride concentration was measured by glycerol-1-phosphate dehydrogenase-diaphorase using CX7 (Beckman instruments, Brea, CA, USA). HDL cholesterol was measured by magnesium precipitation method.

Statistical analysis

Before statistical analysis, normality tests were performed for the variables under study. The variables that are not normally distributed (e.g., serum ferritin) were log-transformed. We used Student's unpaired t-tests for comparison of quantitative variables and used chi-square tests for comparison of proportions. Pearson's correlation coefficients or Spearman's tests were used depending on the normality of the variables. A multiple regression analysis was also performed. A significance level of 5% was chosen for all the tests (p value=0.05). Statistical analyses were performed with the SPSS-win software for IBM computers.

RESULTS

Features of type 2 diabetic patients and control subjects (Table 1)

Table 1 shows the clinical and biochemical characteristics of the type 2 diabetic patients and the control subjects. Typically the diabetic patients were more obese, had higher fasting blood glucose and triglyceride, and had lower HDL cholesterol than the control subjects.

Serum ferritin levels

The log-transformed serum ferritin (log ferritin) level was higher in the type 2 diabetic patients than the controls, but there was no statistical significance ($p=0.09$) (Fig. 1). When the two groups were put together, log ferritin was found to be correlated with fasting C-peptide ($r=0.478$, $p=0.007$), fasting blood sugar ($r=0.235$, $p=0.048$) and BMI ($r=0.285$, $p=0.05$) (Fig. 2) but not with age, systolic or diastolic blood pressure, serum total cholesterol, HDL cholesterol, total triglyceride, serum insulin, or HbA1c.

In the type 2 diabetic patients, log ferritin was correlated with fasting C-peptide ($r=0.478$, $p=0.009$) (Fig. 3) but not with fasting blood sugar or BMI ($p>0.05$). In the control subjects, log ferritin was correlated only with BMI ($r=0.477$, $p=0.012$) (Fig. 4).

A stepwise multiple regression analysis was performed to determine the factors that affect the log ferritin level. In the type 2 diabetic group, the log ferritin level was still significantly correlated with fasting C-peptide ($p=0.001$). In the control group, the fasting sugar level was a significant predictor of the log ferritin level ($p=0.034$).

When the diabetic patients were divided into several groups according to type of antihypertensive

Table 1. Clinical Characteristics of Patients with Type 2 DM and Controls

	Type 2 DM (n=50)	Control (n=25)
Age (years)	58.4±8.3	54.3±7.3
Sex (M/F)	24/26	11/14
Duration of DM (years)	7.6±6.7	–
*BMI (kg/m^2)	24.3±3.2	21.9±2.3
HbA1c (%)	8.2±1.8	–
*FBS (mg/dL)	170.4±48.4	88.6±6.9
Fasting Insulin (IU/mL)	11.3±4.9	–
Fasting C-peptide (ng/mL)	2.55±1.78	–
Ferritin (ng/mL)	91.6±96.2	60.2±94.8
Log ferritin	1.79±0.33	1.64±0.4
*Triglyceride (mg/dL)	222.9±221.7	99.9±36.3
Total cholesterol (mg/dL)	195.6±38.5	177.4±28.7
LDL cholesterol (mg/dL)	114.7±31.8	105.5±24
*HDL cholesterol (mg/dL)	39.6±12.2	51.7±13.3
Diet only (Number, %)	10 (20)	
Oral hypoglycemic agents	31 (62)	
Insulin	9 (18)	

Values are mean±S.D.
* $p<0.05$

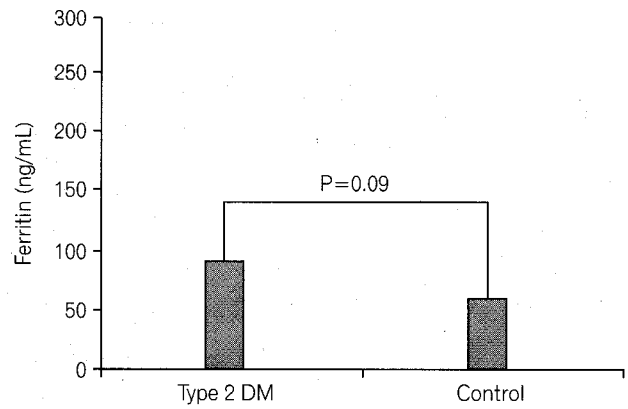

Fig. 1. Serum ferritin concentration in type 2 diabetic group and control group.

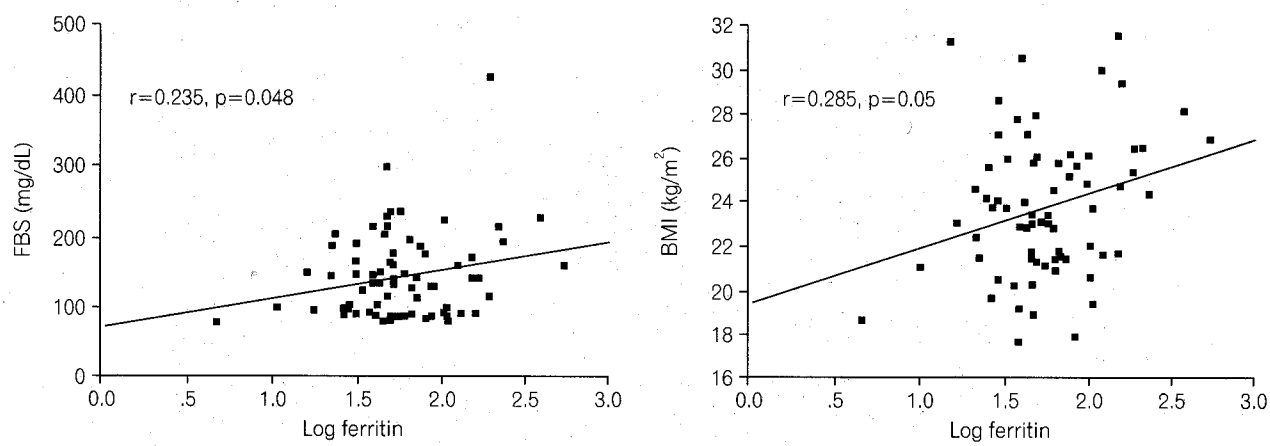

Fig. 2. Correlation of log ferritin with various parameters in the whole group.

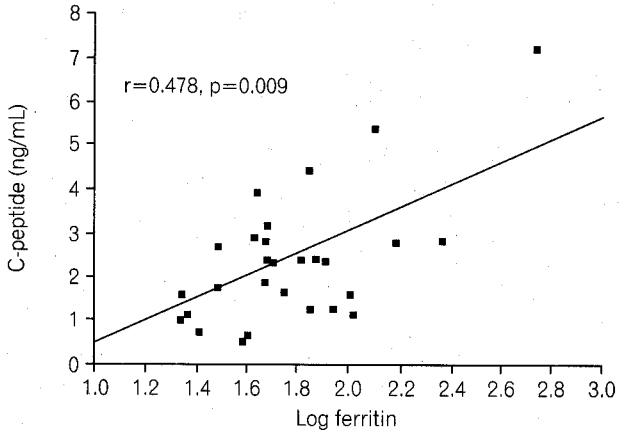

Fig. 3. Correlation of log ferritin with C-peptide in the type 2 diabetic patients.

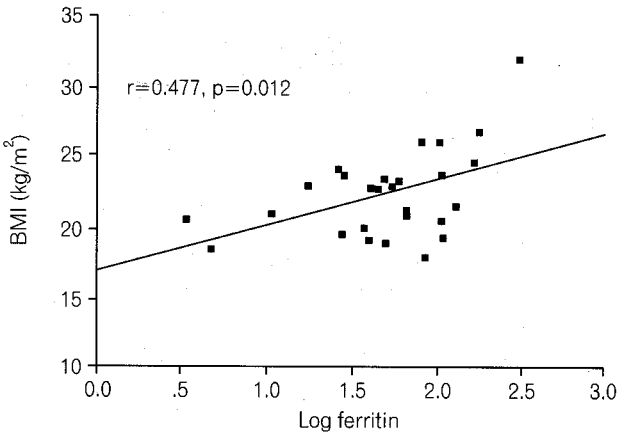

Fig. 4. Correlation of log ferritin with BMI in the control group.

treatment, there was no significant difference in ferritin concentration among the antihypertensive group (n=23), angiotensin converting enzyme inhibitor (ACEI) group (n=10), calcium channel blocker (CCB) group (n=8), ACEI+CCB group (n=5), and ACEI+CCB + α-blocker group (n=3) (108.96±107.9, 119.8±157.4, 69.8±54.5, 83.2±59.4, 147.9±163.6 ng/mL, $p>0.05$) Also, when the diabetic patients were divided into several groups according to treatment modality, the serum ferritin level showed no significant difference among the diet only group, oral hypoglycemic group and insulin group (95.3±108.9, 114.8±124.2, 78.5±50.4 mg/mL, $p>0.05$)

DISCUSSION

This study provides evidence that the serum ferritin level is higher for type 2 diabetic patients and that the ferritin concentration is correlated with several metabolic components of insulin resistance syndrome.

Diabetes mellitus is a common manifestation of severe iron overload in individuals idiopathic hemochromatosis[2] or patients treated by multiple transfusions for thalassemia major.[1] Overt diabetes in such cases is usually attributed to insulin deficiency due to the toxic effects of iron deposited in the pancreatic islets. However, some studies have reported increased, rather than reduced, insulin responses to oral glucose in some patients receiving hypertransfusion with thalassemia, suggesting that insulin resistance is also involved in the alterations to glucose metabolism that are observed in thalassemia.[9]

However, there are some controversies about the relationship between body iron content and the development of diabetes. Dinneen et al. compared hepatic iron stores in autopsy specimens from fifteen patients with NIDDM and 17 age-matched control subjects.[10] The two groups demonstrated no significant differences in either the distribution or the mean amount of hepatic iron. Their results suggest that NIDDM is not typically associated with a substantial level of iron overload.

The following two studies reported different responses of the iron chelating agent deferoxamine in diabetic patients. Culter reported that deferoxamine resulted in a decrease in glycosylated hemoglobin in patients with poorly controlled diabetes[4] but Redmon et al. were unable to confirm that observation.[11] Indeed, they reported that deferoxamine had no efect on glycemic control, or on basal or stimulated insulin and C-peptide levels.

In a recent epidemiological study, increased body iron stores even in healthy subjects, as measured by serum ferritin concentration, were associated with elevated serum insulin, blood glucose, and serum fructosamine concentrations in middle-aged men in eastern Finland.[12] This study showed a positive association between serum ferritin concentration and markers of glucose homeostasis. The results demonstrated that even though the observed elevation of ferritin concentration was not very large, it might be important for the population.

Furthermore, Salonen et al. found that serum

ferritin was correlated with diastolic blood pressure, HDL_2/HDL_3 quotient, glucose area under the curve, and S_I.[6]

In our study. Serum ferritin was associated with glucose homeostasis and a few components of insulin resistance syndrome in both the diabetic and control subjects. The ferritin level had a positive correlation with fasting blood sugar, BMI and fasting C-peptide. Most importantly of all, the serum ferritin concentration was most significantly influenced by the fasting C-peptide level, which is another indicator of hyperinsulinemia. Because we included diabetic patients using insulin, the fasting insulin level could not precisely reflect endogenous insulin secretion. We were not able to demonstrate the relationship between the ferritin level and the other markers of insulin resistance syndrome; blood pressure, triglyceride, total cholesterol, HDL cholesterol etc. It was impossible to evaluate the insulin resistance exactly because we did not measure the insulin resistance by euglycemic clamp study or frequently sampled intravenous glucose tolerance test (FSIGT). But we could indirectly estimate the degree of insulin resistance by the levels of serum insulin, C-peptide, fasting blood sugar, BMI, etc.

Another research study reported that serum ferritin is a determinant of metabolic control and insulin resistance syndrome.[6] Thus, we initially hypothesized that serum ferritin would be higher for diabetic patients with insulin resistance syndrome than those without it. So we divided the diabetic patients into two groups. Those with 4 or 5 of the following were considered to have insulin resistance syndrome ($n=23$): hypertriglyceridemia (VLDL triglyceride >200 mg/dL); low serum HDL cholesterol (<35 mg/dL); hypertension; coronary heart disease; and obesity (BMI >25 kg/m^2). We expected that serum ferritin would be higher for those with insulin resistance syndrome than those without it. The results seem to suggest that the ferritin concentration is significantly higher for diabetic patients without insulin resistance syndrome than the control subjects. And the serum ferritin level was also higher in diabetic patients with insulin resistance syndrome than those without it, but the difference was not statistically significant. We cannot rule out the possibility that such insignificance is due to the small number of subjects included in our study. Excess iron could be related to disturbed glucose homeostasis in at least two different ways. Firstly, iron deposition in the pancreas can lead to defects in insulin synthesis and secretion.[13,14] However, in an epidemiologic study, insulin secretion responded similarly to the oral glucose load both in the highest and the lowest serum ferritin quintiles, suggesting that modest elevations in body iron levels do not affect the pancreatic capacity to secrete insulin.[12] Secondly, accumulated iron could interfere with the insulin-extracting capacity of the liver resulting in hyperinsulinemia.[15] Iron deposition in the liver may also cause insulin resistance by interfering with the ability of insulin to suppress hepatic glucose production. In our study, both blood glucose and serum insulin concentrations were elevated at high serum ferritin concentrations.

Oxidant stress is increased in diabetes because of the generation of oxygen free radicals during protein glycation and glucose autooxidation.[16] This reaction is catalyzed by iron, so it might increase the risk of coronary heart disease.[6,17-19] Salonen et al. presented a study with the first empirical evidence that serum ferritin is a strong risk factor for acute myocardial infarction.[6] That study confirmed that serum ferritin is a strong risk factor for ischemic heart disease at levels previously regarded as normal.

Similarly in diabetic patients, serum ferritin might be related to oxidant stress, especially atherosclerotic vascular complications. In our study, the serum ferritin concentration seemed to be higher in diabetic patients who had atherosclerotic coronary heart disease or cerebrovascular accidents than in diabetic patients without these complications (91.7 ± 130 vs. 75.2 ± 55.8 ng/mL). But it was not statistically significant.

In summary, the serum ferritin concentration was significantly correlated with fasting C-peptide in type 2 diabetic patients and was significantly correlated with fasting sugar level in the control group. So, serum ferritin could be a marker of not only glucose homeostasis but also some components of insulin resistance syndrome in both diabetic and control subjects. However, this finding must be confirmed by additional studies consisting of more subjects and employing a more direct method of measuring insulin resistance such as euglycemic clamp study or frequently sampled intravenous glucose tolerance test (FSIGT).

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