Iron Status in Type 2 Diabetes Patients With and Without Metabolic Syndrome

Sanda Kyaw, Hnin Oo Pwint**, Myat Thandar†, Mya Thandar Sein††, Cho Cho Aung*, Ohnmar**

Abstract

Elevated serum ferritin concentrations have recently been implicated in the pathogenesis of the metabolic syndrome (MetS).The present study aimed to investigate the iron status in type 2 diabetes mellitus (T2DM) patients with and without MetS. Serum ferritin levels were determined by enzyme-linked immunoassay, while serum iron was measured using a test kit, and hemoglobin or red cell indices were determined by a hematology analyzer. Serum ferritin levels of T2DM were significantly higher in those with MetS (n = 28) than those without MetS (n = 29) (132.97 ± 103.03 ng/ml *vs* 34.62 ± 15.30 ng/ml; $P < 0.001$). Hemoglobin concentration (Hb), total iron binding capacity (TIBC), total red blood cell count, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were not significantly different between the two groups, although values were in the lower normal range. Serum ferritin levels were inversely correlated with TIBC ($r = -0.49$, $P < 0.01$), MCV ($r = -0.41$, $P < 0.05$), MCH ($r = -0.5$, $P < 0.01$) and MCHC ($r = -0.46$, $P < 0.05$) only in those with MetS. Our data supported the link between iron status and MetS in T2DM. It is speculated that changes in iron status might be related to inflammation associated with metabolic syndrome in diabetes.

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Introduction

Serum ferritin, a marker for total body iron stores, is responsive to inflammatory stress,[1] so increased ferritin in diabetes could simply reflect the inflammatory component of that disease.[2] The ferritin molecule is an intracellular hollow protein shell, composed of 24 subunits surrounding an iron core that may contain as many as 4,000-4,500 iron atoms. In the body, small amount of ferritin is secreted into the plasma. The concentration of serum ferritin is positively correlated with the size of the total body iron stores in the absence of inflammation. A low serum ferritin value reflects depleted iron stores. However, ferritin is a positive acute phase response protein whereby its concentration increases during inflammation and thus it no longer reflects the size of the iron store.[3] Studies reported that increased body iron stores has been associated with the development of glucose intolerance, type 2 diabetes (T2DM) and micro- as well as macrovascular complications.[4] Moreover, elevated serum ferritin concentration has recently been implicated in the pathogenesis of the metabolic syndrome (MetS).[5] Increased serum ferritin, reflecting body iron overload is often associated with measures of insulin resistance, such as elevated blood glucose and insulin levels.[6] The role of MetS on serum ferritin and other iron monitoring molecules in diabetes mellitus has been under active consideration. Therefore, the present study was aimed to investigate the iron status in T2DM patients with and without MetS

*Department of Physiology, University of Medicine 1, Yangon;
**Department of Physiology, University of Dental Medicine, Mandalay;
†University of Nursing, Yangon;
††Department of Physiology, University of Medicine Magway; Myanmar.

Corresponding author:
Sanda Kyaw, MBBS, MMedSc (Physiology), PhD, DipMedEd
E-mail: sandakyaw.myanmar@gmail.com

Materials and Methods

Subjects

The study was carried out in 57 subjects (male = 17, female = 40), with age between 40 to 60 years old. History taking and physical examination were done and written informed consent was obtained. In history taking, each subject was asked for previous history of chronic infections, present acute infections, smoking and alcohol drinking. No history of regular iron supplementation and iron containing multivitamins were found in all subjects. Physical examination of neck, chest and abdomen examination (inspection, palpation, percussion and auscultation) were taken thoroughly. Among 57 patients, 28 were in with MetS and 29 in without MetS groups. MetS was identified according to the National Cholesterol Education Program, 2001.[7] A person has metabolic syndrome if he or she has three or more of the following criteria: abdominal obesity (waist circumference > 90 cm in men and > 80 cm in women), high blood pressure (> 130/85 mmHg), hypertriglyceridemia (≥ 1.7 mmol/l), low HDL cholesterol (< 1.03 mmol/l in men and < 1.29 mmol/l in women), and high fasting glucose (≥ 6.1 mmol/l).

Study procedures

After 10 hour overnight fasting, about 5 ml of venous blood was collected from the antecubital vein under aseptic conditions. Two ml of blood was kept in a test tube containing EDTA for determination of hemoglobin and red cell indices, and remaining 3 ml was kept in a plain test tube and serum separation was done. After that, 1/3 of serum and another 2/3 of serum were kept in two separate aliquots for serum ferritin, and serum iron and total iron binding capacity (TIBC). These aliquots were then capped and stored in -20°C until analysis. Serum ferritin level was determined by enzyme-linked immuno-assay (ELISA) method (Ferritin ELISA kit, VEDALAB, France) and read by a microtiter plate reader (HumaReader HS, HUMAN, Germany). Serum iron and total iron binding capacity (TIBC) was measured using a test kit (Ferentest) and read by a semi-automatic analyzer (HumaLyzer Primus 3500, HUMAN, Germany). Hemoglobin concentration (Hb) and red cell indices were determined by a hematology analyzer (Germany). Fasting blood sugar was determined by glucometer strip method. HbA1c levels and renal function tests were not determined in the present study.

Statistical analysis

Data were analyzed by using the Statistical Package for Social Science (SPSS) software version 16.0. The results were expressed as mean ± SD. Comparison of the variables between type 2 DM patients with MetS and those without MetS was done by Student's *t* test. The relationship between serum ferritin and red cells indices and % transferrin saturation were assessed by Pearson's correlation. *P* value less than 0.05 was considered significant.

Table 1 General characteristics of the studied population

Parameters	Without MetS (n = 29)	With MetS (n = 28)
Age (year)	51.16 ± 6.6	53.08 ± 5.6
Male	14 (48.28%)	3 (10.71%)***
Female	15 (51.72%)	25 (89.29%)***
Weight (kg)	56.40 ± 8.76	62.36 ± 15.48
Height (m)	1.57 ± 0.07	1.52 ± 0.1
BMI (kg/m^2)	22.79 ± 3.68	26.46 ± 5.07
Waist circumference (cm)	83.10 ± 8.95	91.82 ± 10.28
Resting SBP (mmHg)	120.0 ± 11.95	144.5 ± 5.92**
Resting DBP (mmHg)	75.69 ± 7.5	91.86 ± 3.52***
Fasting blood sugar (mg/dl)	131.48 ± 30.12	134.5 ± 29.20
Serum TG (mmol/l)	1.30 ± 0.30	1.75 ± 0.48*
Serum HDL (mmol/l)	1.12 ± 0.16	1.13 ± 0.23

Data were expressed as mean ± SD. *P < 0.05; **P < 0.01; ***P < 0.001. Abbreviations: DBP, diastolic blood pressure; HDL, high-density lipoprotein cholesterol; MetS, metabolic syndrome; SBP, systolic blood pressure; TG, triglyceride.

Table 2 Iron status of patients participated in this study

Parameters	Without MetS (n = 29)	With MetS (n = 28)
Serum ferritin (ng/ml)	34.62 ± 15.3	132.97 ± 103.03*
Serum iron (µmol/l)	24.79 ± 13.1	26.32 ± 11.8
% Transferrin saturation	122.07 ± 253.11	120.03 ± 256.3
RBC (million cells/µl)	4.93 ± 0.8	4.86 ± 0.6
Hb (g/dl)	12.47 ± 1.7	12.15 ± 1.1**
TIBC (µmol/l)	20.31 ± 5.1	21.92 ± 4.6
MCV (fl)	79.56 ± 8.5	77.47±7.1
MCH (pg)	26.12 ± 3.8	25.24 ± 2.9
MCHC (g/dl)	32.69 ± 1.7	32.42 ± 1.3
% of anemia (n)	41.37% (12)	42.85% (12)

Data were expressed as mean ± SD. *P < 0.05; **P < 0.01; ***P < 0.001. Abbreviations: Hb, hemoglobin concentration; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; MetS, metabolic syndrome; RBC, red blood cell count; TIBC, total iron binding capacity.

Results

Table 1 shows general characteristics of the subjects. There was significant difference in male and female percentages between without MetS and with MetS groups (P < 001). There was significant difference in resting systolic blood pressure and diastolic blood pressure between without MetS and with MetS groups (P < 0.01 and P < 001, respectively). Serum triglyceride was significantly higher in the patient with MetS group than the patient without MetS. Iron status of the patients was shown in Table 2. Although serum ferritin concentration was significantly higher in the patient with MetS than the patient without MetS groups (P < 0.05), hemoglobin concentration was significantly lower in the patient with MetS than the patient without MetS group (P < 0.01).

There was no significant correlation between serum ferritin and iron status in the patients without MetS (Figure 1). However, a significant negative correlation between serum ferritin *vs* TIBC (r = -0.49, P < 0.01), *vs* MCV (r = -0.41, P < 0.05), *vs* MCH (r = -0.5, P < 0.01), and *vs* MCHC (r = -0.46, P < 0.05) was found in the patients with MetS (n = 28; Figure 2).

There was a significant positive correlation between serum ferritin and % transferrin saturation found in the patient with MetS group (r = 0.41, P < 0.05, n = 28). There was no significant positive correlation between serum ferritin and % transferrin saturation in the patient without MetS group (r = 0.07, P > 0.05, n = 29) (Figure 3). There was no significant correlation of anthropometric parameters and serum iron in either the group with or without MetS (data were not shown).

Discussion

Although all iron status parameters were at the lower limit of normal range in those with and without MetS, there was no significant difference in these iron status parameters between the two groups. However, serum ferritin was significantly higher in those with MetS than those without MetS. In addition, the present study demonstrated that although serum ferritin levels

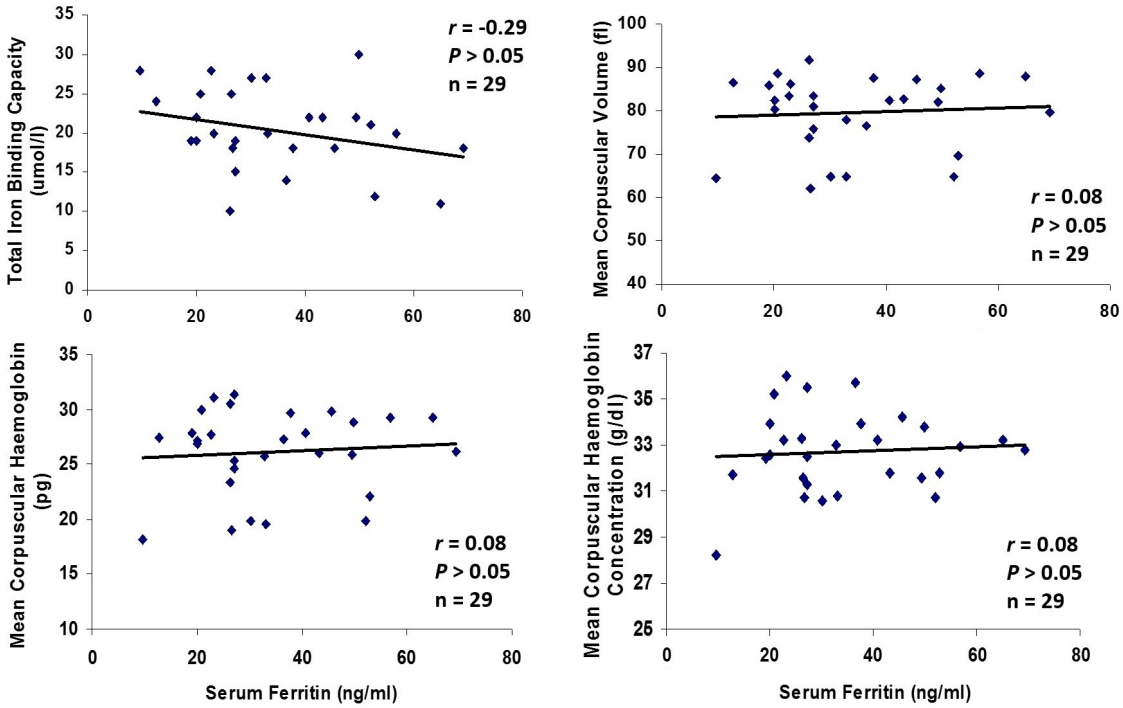

Figure 1 Correlation between serum ferritin and TIBC, MCV, MCH and MCHC in patients without MetS group.

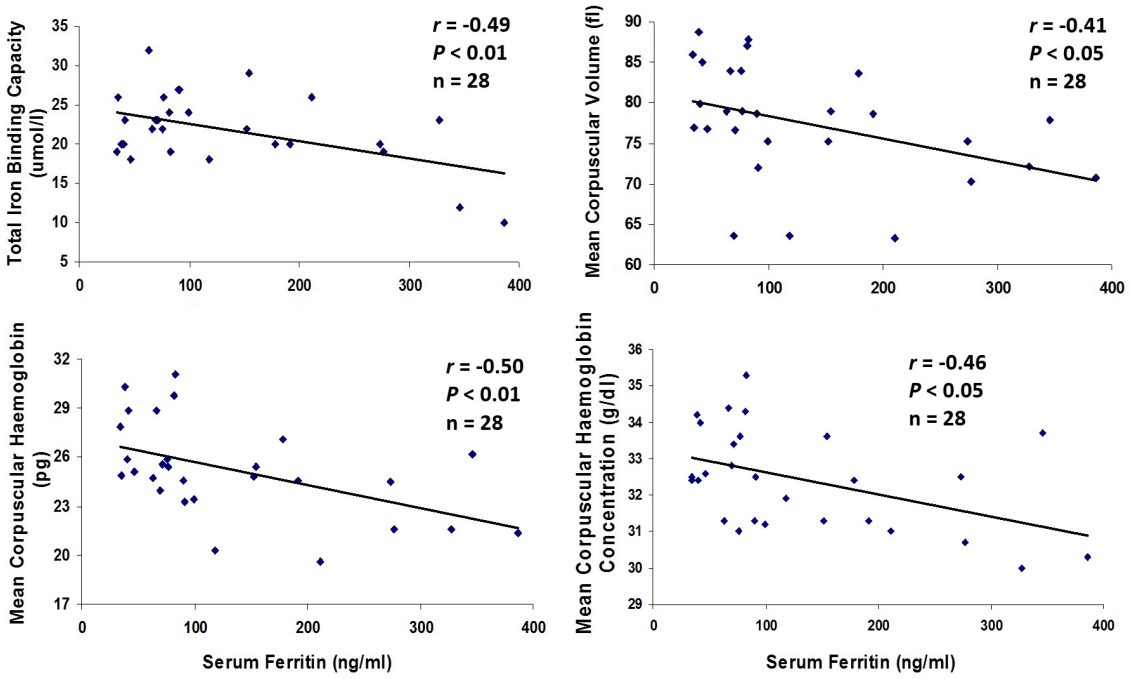

Figure 2 Correlation between serum ferritin and TIBC, MCV, MCH and MCHC in patients with MetS group.

increases, microcytic anemia is present in some patients with MetS. Out of 29 patients in the without MetS group, 12 were anemic. Out these 12 patients, 4 were male. Out of 28 patients with MetS, 12 were anemic and all were female. Although, having unequal proportion of male patients could be a confounding factor, their average hemoglobin concentration was 11.1 g/dl. Therefore, the present significant difference in hemoglobin concentration between the two groups were not due to disparity in gender composition.

In the present study, the number of male patients with and without MetS was 3 and 14 (10.71% and 48.28% of the respective group). Therefore, significantly higher serum ferritin levels in the patient with MetS might not be due to confounding effect of gender difference. Serum ferritin concentration normally reflects body iron stores and it is well known that serum ferritin level is high in iron overload. In conditions of chronic inflammatory diseases, at least three sources may contribute to elevated ferritin in the circulation: tissue ferritin

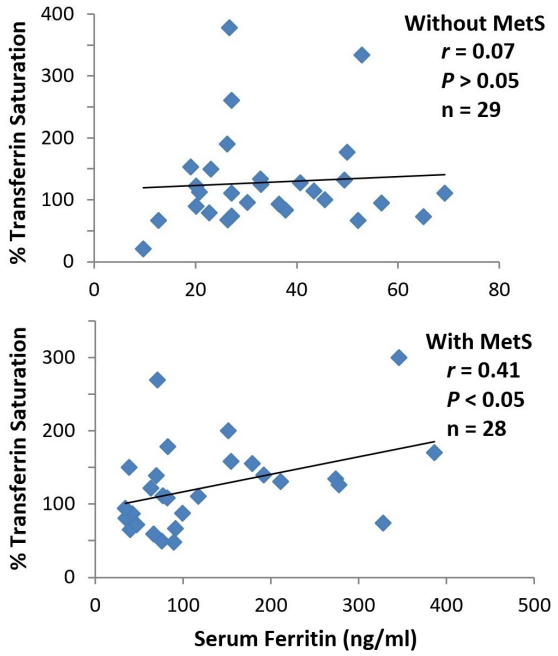

Figure 3 Correlation between serum ferritin and TIBC, % transferrin saturation in patients without MetS group (*upper*) and with MetS group (*lower*).

secreted by the Kupffer cells or macrophages;[8] tissue ferritin released from damaged hepatocytes;[9] and serum ferritin induced by inflammatory-related mechanisms such as IL-6 and TNF.[10,11]

Direct IL-6 stimulation has been shown to significantly increase hepcidin via the JAK/STAT3 signaling pathway.[12,13] Hepcidin controls the flux of iron into plasma by post-translational regulation of the body's sole cellular iron exporter, ferroportin-1.[14] Acutely high hepcidin inhibits both the absorption of iron from the diet and iron release from storage sites by obliterating ferroportin-1 expression. Martinelli *et al.*[15] provided the first evidence for chronic hyperhepcidinemia as an additional feature of MetS. Hepcidin, a peptide hormone made in the liver, causes tissue iron retention, resulting in decreased serum iron levels and contributing to the development of anemia of inflammation, probably as a host defence mechanism to limit the availability of iron to invading microorganisms.[14]

In 2014, Aigner *et al.*[16] pointed out that iron homeostasis is affected by obesity and obesity-related insulin resistance in a many-facetted fashion.[16] According to findings of researchers, iron deficiency and anemia are frequent findings in subjects with progressed stages of obesity.[16] In the previous findings, hyperferritinemia with normal or mildly elevated transferrin saturation is observed in approximately one-third of patients with metabolic syndrome (MetS) or nonalcoholic fatty liver disease (NAFLD). The researchers suggested that not only elevated body iron stores but also iron deficiency are unfavorable to health and to the course of obesity-related conditions. This has been named as "Dysmetabolic Iron Overload Syndrome (DIOS)."[16] The possible mechanism may be related to obesity-associated inflammation and it is tightly linked to iron deficiency, involving impaired duodenal iron absorption associated with low expression of duodenal ferroportin (FPN) along with elevated hepcidin concentrations.[16]

The present study agrees with the suggestion of Aigner *et al.*[16] because we found that high ferritin levels were inversely correlated with blood indices in the patients with MetS. Although, the percentage of anemia patients was not quite different between two groups, hemoglobin concentration was significantly lower in the patients with MetS compared with those without MetS. Moreover, serum ferritin levels and % transferrin saturation were positively correlated in patients with MetS and such a correlation was absent in patients without MetS. Therefore, it could be hypothesized that hepcidin might be a key regulator of obesity-associated inflammation and is linking to iron deficiency in DM patients with MetS.

Study Limitations

The present study could not determine the levels of cytokines and hepcidin to explain the mechanism of action of hyperferritinemia in low/normal iron parameters.

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