Increased Serum Ferritin Levels in Type 2 Diabetes Mellitus Patients: A Hospital Based Cross-Sectional Study

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Abstract

Background: Type 2 diabetes mellitus is a metabolic disorder of multiple etiology characterized by chronic hyperglycemia with a derangement in carbohydrate, fat and protein metabolism resulting from defects in insulin secretion and action. Ferritin is a ubiquitous intracellular protein complex that reflects the iron stores of the body. Studies have shown that the increased body iron stores are associated with the development of glucose intolerance often leading to metabolic syndrome and type 2 diabetes (T2DM). The objective of the study was to find out association of serum ferritin level with T2DM and assess the correlation between serum ferritin and HbA$_{1c}$.

Methods: A hospital based comparative cross-sectional study was conducted in 43 diabetic patients and 42 age and sex matched healthy controls. Fasting blood glucose (FBG), postprandial blood glucose (PBG), Glycated hemoglobin (HbA$_{1c}$) and serum ferritin were estimated in cobas c311 autoanalyser using standard protocol.

Results: Mean age of healthy control and T2DM were found 54.83 ± 6.48 and 55.95±10.92 years respectively. Mean FPG (mg/dl) (170.41 ± 71.7 v/s 98.38 ± 9.7), PBG (mg/dl) (266.16 ± 110.09 v/s 123.20 ± 17.0), HbA1c (%) (8.17 ± 1.83 v/s 4.9 ± 0.29 and median ferritin (μg/L) 207.90 (138, 306.0) v/s 127.95 (85.75, 210.25) were significantly higher in T2DM compared to the healthy controls. Spearman's correlation depicted that ferritin level was positively correlated with HbA1c level but the correlation was statistically insignificant.

Conclusion: Serum ferritin level was found significantly increased in T2DM compared to healthy age and sex matched controls in our study.

Introduction

Type 2 diabetes mellitus (T2DM) is increasingly gaining importance as public health problem in our country. The pathogenesis of T2DM is not fully understood as multiple factors appear to be involved. One of these factors may be an excessive absorption and storage of dietary iron [1]. Increased body iron store has been emerging as putative risk factor for development of insulin resistance and cardiovascular diseases (CVD). Basic, clinical, and epidemiological studies have generated enough scientific evidence to suggest that iron overload is proinflammatory and proatherosclerotic [2].

Evidence suggests that Diabetes mellitus (DM) is an early complication of idiopathic hemochromatosis. This comprises of about 60% of the total persons affected by this disease. Glycemic status improvement is seen in the patients when the decrement in body iron stores by phlebotomies was performed. Hence, this provides an insight that diabetes mellitus in these patients is a secondary and reversible manifestation of iron overload [1]. A study report from Finland suggested a positive association between serum ferritin level and DM which has consequently encouraged investigating iron as a risk factor for DM [3]. Serum ferritin is an excellent biomarker of body iron stores and has been proposed as a component of the insulin resistance syndrome. In addition, excess iron deposition in the liver may cause insulin
resistance by interfering with the ability of insulin to suppress hepatic glucose production [4, 5]. Estimation of ferritin in our setup in T2DM patients may establish as a risk factor which may help in proper management of T2DM.

The main objectives of the study were to outline the association of serum ferritin level between ages and sex matched healthy control and T2DM and to determine the correlation between HbA₁c and serum ferritin.

**Methods**

A Hospital based cross-sectional study was conducted in the Department of Biochemistry in collaboration with Department of Internal Medicine at B.P. Koirala Institute of Health Sciences (BPKIHS), Dharan, Nepal between January 2017 and December 2017. Patients aged 18 year and above, with T2DM and taking anti-diabetic drugs were enrolled. Patients having overt thyroid dysfunction, chronic kidney disease, chronic liver disease and on corticosteroid therapy were excluded. Considering 36.63 ug/L difference in serum ferritin between T2DM and normal healthy control, the sample size was calculated to be 80 at 5% type 1 error and 90% probability using power and sample size program version 3.0.34 [6]. Study participants were divided as 40 case and 40 control.

Patients with T2DM (n = 43) and age/sex matched healthy controls (n = 42) were enrolled in the study after obtaining the written informed consent.

**For Cases**

Diagnosis of diabetes mellitus was made as per the guidelines of American Diabetes Association criteria (FBS ≥ 126 mg/dl and/or 2- hour PPBS ≥ 200 mg/dl or HbA1c ≥ 6.5%) [7]. A structured self-designed proforma was used to collect the relevant data.

A baseline evaluation of all participants was conducted including a clinical history followed by complete physical examination. The screening of the patients was carried out in the OPD of Internal Medicine department after consultation and physical assessment by the co-investigators and the collection and analysis of the sample was done by the investigator from department of Biochemistry.

**For Control**

Age-matched apparently healthy individuals with FBS < 100 mg/dl plus 2-hour PBS < 140 mg/dl plus HbA1c < 5.6% with no history of T2DM, no history of medication use and any other systemic illness were enrolled as controls.

Patients with chronic kidney disease, chronic liver disease, those on corticosteroid therapy and other states associated with altered serum ferritin like haemochromatosis, bleeding disorder, chronic alcoholics and anemia were excluded. Patients were considered as anemic if hemoglobin level is less than 13 gm/dl in male and 12 gm/dl in females. Individuals with repeated blood transfusion were also excluded.
Anthropometric Measurement

Height (cm), Weight (Kg), Blood Pressure (mmHg) and Waist circumference (Cm) of each participant were measured.

Biochemical Measurements

Blood Glucose was estimated by hexokinase method in cobas c311 autoanalyser (Roche Diagnostics), HbA1c by turbidimetric immuno-inhibition method (TINIA) in cobas c311 autoanalyser (Roche Diagnostics) and serum Ferritin by particle enhanced immunoturbidimetric assay in cobas c311 autoanalyser (Roche Diagnostics).

Statistical Analysis

Data were entered in MS Excel 2007. Descriptive statistics mean, standard deviation (SD) and percentage were calculated. Normality test was performed using Kolmogorov Smirnov test. Comparison of mean FPG, PBG, HbA\textsubscript{1c} between the T2DM and control group was done by Independent t-test. Comparison of median ferritin between T2DM and control group was done by Mann Whitney 'U' test. Spearman's correlation was used to correlate ferritin level with HbA1c. With P < 0.05 considered as statistically significant. Statistical Package for Social Sciences version 11.5 (SPSS Inc., Chicago, USA) was used to analyze the data.

Results

The mean age of healthy control and T2DM were found 54.83 ± 6.48 and 55.95 ± 10.92 years. Significantly higher mean SBP, DBP and BMI were found in T2DM compared to healthy controls (p value < 0.05) as depicted in Table 1.

Mean FPG, PBG, HbA\textsubscript{1c} and median ferritin were significantly higher in T2DM compared to healthy controls (p value = 0.001) as depicted in Table 2.

A significant positive correlation was found between HbA1c, FBS and PBS among T2DM and total participants but no significant correlation between HbA1c and ferritin was observed in cases as illustrated in Tables 3 and 4. However statistically significant week positive correlation was found between HbA1c and serum ferritin levels in total participants (r = 0.20, p < 0.05) as shown in Table 4.
### Table 1
Clinical and Demographic Profile of the study participants

| Variables                        | T2DM       | Control    | p value | Value  |
|----------------------------------|------------|------------|---------|--------|
| Mean age (years)                 | 55.95 ± 10.92 | 54.83 ± 6.48 | 0.568  |        |
| Gender                           |            |            |         |        |
| Male                             | 19         | 20         | 0.548  |        |
| Female                           | 24         | 23         |         |        |
| Systolic Blood Pressure (SBP) (mm Hg) | 134 ± 5.5  | 115 ± 4.0  | 0.003a*|        |
| Diastolic Blood Pressure (DBP) (mm Hg) | 98 ± 6.2   | 82 ± 4.0   | 0.02 a*|        |
| Body Mass Index (BMI) (kg/m²)    | 28.52 ± 2.4 | 22.70 ± 2.7 | 0.01 a*|        |

a = Independent t-test; *p value < 0.05 is considered to be statistically significant

### Table 2
Comparison of biochemical parameters of T2DM and healthy control

| Variables          | T2DM (n = 43)       | Control (n = 42)      | p-value |
|--------------------|---------------------|-----------------------|---------|
| Mean FBG (mg/dL)   | 170.41 ± 71.76      | 98.38 ± 9.70          | 0.001a* |
| Mean PPG (mg/dL)   | 266.16 ± 110.09     | 123.20 ± 17.20        | 0.001a* |
| Mean HbA1c (%)     | 8.17 ± 1.8          | 4.96 ± 0.29           | 0.001a* |
| Median Ferritin (µg/dL) | 207.90 (138–306)  | 127.95 (85.75-210.25) | 0.001b* |

aIndependent t-test; bMann-Whitney U test; *p value < 0.05 is considered to be statistically significant
Table 3
Spearman’s rho correlation between various biochemical parameters in T2DM (n = 43)

| Variables | FBG | PPG | HbA1c | Ferritin |
|-----------|-----|-----|-------|----------|
| FBG       | -   | r = 0.76** | r = 0.71** | r = -0.01 |
|           |     | p = 0.001 | p = 0.001 | p = 0.94 |
| PPG       | r = 0.76** | -   | r = 0.58** | r = 0.08 |
|           | p = 0.001 |     | p = 0.001 | p = 0.59 |
| HbA1c     | r = 0.71** | r = 0.58** | -   | r = 0.08 |
|           | p = 0.001 | p = 0.001 |     | p = 0.59 |
| Ferritin  | r = -0.01 | r = -0.06 | r = 0.08 | - |
|           | p = 0.94 | p = 0.68 | p = 0.59 |     |

**Statistically not significant at p value < 0.05

Table 4
Pearson’s correlation between variables in total study participants (n = 85)

| Variables | FBG | PBG | HbA1c | Ferritin |
|-----------|-----|-----|-------|----------|
| FBG       | -   | r = 0.86 | r = 0.76 | r = 0.09 |
|           |     | p = 0.001 | p = 0.001 | p = 0.37 |
| PBG       | r = 0.86 | -   | r = 0.69 | r = 0.05 |
|           | p = 0.001 |     | p = 0.001 | p = 0.69 |
| HbA1c     | r = 0.76 | -   |       | r = 0.20 |
|           | p = 0.001 |     |     | p = 0.05* |
| Ferritin  | r = 0.09 | r = 0.05 | r = 0.20 | - |
|           | p = 0.37 | p = 0.69 | p = 0.05* |     |

*Statistically not significant at p value < 0.05

We found significantly higher levels of median serum ferritin in males (238 (151.8, 323.4) as compared to females (158 (109, 233.5); p value = 0.02) in T2DM as depicted in Fig. 1.

**Discussion**

The incidence and prevalence of T2DM is rapidly increasing in our part of world. There are multifactorial etiologies for development of T2DM. Increased iron level or iron overload and high serum ferritin level is
considered to be one of them [8]. Therefore, we conducted this comparative cross-sectional study to delineate the association of serum ferritin with T2DM and correlation between and HbA1c, FBS, PBS and HbA1c in T2DM as well as healthy controls.

Elimam et al. have reported positive correlation between serum ferritin, HbA1c and CRP levels and highlighting the direct association of inflammation and glycemic control ongoing in T2DM patients [9]. There has not been a very consistent finding between elevated body iron stores and serum insulin and blood glucose levels [10]. A study conducted on 9,486 participants out of total 16573 individuals in the United State by Ford et al in 1999 reported that serum ferritin levels were lowest in non-diabetic, higher in pre-diabetic and highest in diabetic patients [11]. Similarly, a study from China by Liu et al. found an increase in the HOMA-IR values in parallel to the increases in serum ferritin levels and the decrease in insulin secretion from the pancreas [12]. In accordance to the present study, Wolide et al. reported significantly higher ferritin, waist circumference, BMI, and blood pressure values in patients with type 2 DM compared to the healthy control (p < 0.0001) [13]. A study by Andrews et al. conducted on diabetic obese and non-obese patients and healthy controls depicted higher serum ferritin and inflammatory status in obese patients than the control group reflecting a significant positive association of serum ferritin levels with type 2 DM and obesity [14].

Increased ferritin levels have been recognized as the marker of inflammation in T2DM patients [15]. Raised serum ferritin and CRP levels are a primary risk factor for the development of chronic diseases [15]. Our study depicts increased ferritin level in T2DM patients compared to healthy control which is in accordance to the studies reported from China, Japan, Bangladesh, Egypt, Spain, and Korea [16, 17, 18, 19, 20, 21 & 22]. Our findings are in accordance with the study of Raj et al. from India where serum ferritin was found higher in T2DM compared to healthy controls [8]. This possibly reflects the subclinical hemochromatosis developing in a long-standing diabetic patient.

Epidemiological studies have reported a strong association between elevated serum ferritin concentration and increased risk for diabetes [11]. Raised serum ferritin may possibly be related to the occurrence of micro and macro-vascular complications of diabetes. Optimum amount of iron is essential for all cellular metabolism and growth [8]. Iron is toxic when it is released from ferritin. Few studies have shown that high iron leading to the oxidative stress can induce T2DM [8, 11]. It is mediated by three key mechanisms: insulin deficiency, insulin resistance and hepatic dysfunction. Ferritin has been referred as a surrogate marker for insulin resistance possibly due to iron deposition in the liver leading to hepatic insulin resistance and increased hepatic glucose production [23, 24]. Elevated serum ferritin levels results in raised level of intra-hepatic oxidative stress and hepatic fibrosis which may further impair insulin extraction and insulin ability to suppress glucose production [23, 24].

Our findings revealed that median ferritin levels in diabetic patients was significantly higher in males compared to females 238 (151.8, 323.4) versus 158 (109, 233.5); p value = 0.02. This is in accordance with the studies reported by Chen et al. [16] and Han et al. [25] which have showed statistically significant positive association of serum ferritin levels with diabetes, metabolic syndrome and obesity in male
patients than in female patients. In contrast to the present study, Dekker et al. worked on different ethnic groups which included 508 patients from the Netherlands, 597 African Surinamese patients, and 339 South Asian Surinamese patients aged between 35 to 60 years have reported positive correlation between serum ferritin levels and FBG in patients with type 2 DM. whereas a stronger positive correlation between FBS and serum ferritin was found in females than males patients among all ethnic groups [26].

A cohort study by Chen et al. evaluated the relationship between serum ferritin levels and the risk of developing T2DM in the 2,225 Chinese population. The researchers compared the findings between diabetic and non-diabetic patients which revealed a higher baseline serum ferritin levels, BMI, HOMA, blood pressure, HbA1c, cholesterol, HDL-C, ALT, and TAG values in T2DM patients compared to the non-diabetic group. This study also reported a significantly higher incidence of T2DM corresponding to one standard deviation increase in serum ferritin levels in Chinese males and concluded that serum ferritin levels could potentially be used as a biomarker in risk prediction for development of type 2 DM in males [16].

The study by Zhan et al., on 8,235 participants out of which 644 (7.8%) diabetics and 7,591 (92.2%) non-diabetics have shared their findings of higher serum ferritin levels in T2DM patients and a significant relationship with the HbA1c and HOMA-IR values. The researchers postulated that an elevated serum ferritin levels could be a marker of risk of developing DM [27].

This is a hospital based cross-sectional study conducted in a small group of T2DM patients, thus the result could not be generalized. Further, additional biomarkers for inflammation like HS-CRP, IL-6, TNF-α could have been done for more precise conclusion. It could not be assayed due to resource constraints.

**Conclusions**

Our study showed significantly higher levels of serum ferritin in T2DM specifically in males compared to healthy controls. Ferritin is an acute phase reactant and could reflect ongoing inflammation in T2DM patients.

**Declarations**

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**Authors’ contributions:**

RKC and AN conceived the idea, enrolled the patients and analyzed the data; RKC, RM, AN and BG contributed to data interpretation; RKC, ML, DPS, AN drafted the manuscript and revised the manuscript; RKC, AN, JKB ML, RM provided valuable insight in data interpretation and critical revision of the
manuscript; RKC revised and prepared the final version of the manuscript. All authors read and approved the final manuscript.

**Ethics approval and consent to participate:** The study was done in accordance with Declaration of Helsinki. This study was ethically approved by Institutional Review Committee (IRC) of BPKIHS, Dharan, Nepal (Code No: IRC/070/014). Written informed consent was obtained from all participants.

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**Availability of data and materials:** The data of the patients included and analyzed in the present study could not be incorporated here for maintenance of confidentiality but can be made available from the corresponding author on reasonable request.

**Consent for publication:** Not Applicable

**Competing interests:** The authors declare that they have no conflict of interest

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Figures
Figure 1

Serum Ferritin levels in male and female T2DM patients