Original Article

Association between Serum Ferritin and Insulin Resistance Markers with Distinct Glycemic Profiles in an Adult Bangladeshi Population

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ABSTRACT

Background: *Type 2 diabetes mellitus (T2DM) is a global health concern. Around 56% of subjects in Bangladesh go undiagnosed, increasing the risk of stroke and cardiovascular issues. Iron stores impact on diabetes is gaining attention, with ferritin as a key biomarker. Hyperferritinemia probably contributes to insulin resistance and subsequently to decreased insulin secretion, causing the development of insulin resistance. The link between ferritin and insulin resistance (IR) varies based on ethnicity, gender, and glycemic state.*

Objectives: *This study investigates serum ferritin levels in different glycemic stages and explores potential correlations between ferritin and insulin resistance markers (blood glucose, serum insulin and HOMA-IR)*

Methods*: The study was conducted at the Department of Biochemistry, Sir Salimullah Medical College, Dhaka, Bangladesh, from March 2018 to February 2019. A total of 140 subjects were included to conduct this study. Participants were categorized into three groups: those with normal fasting glucose (NFG group), impaired fasting glucose (IFG group), and newly diagnosed type 2 diabetes mellitus (Diabetic group) according to WHO (2006) criteria. A purposive convenient sampling method was used, focusing on adults aged 25 to 55 years. Exclusion criteria were applied to eliminate individuals with inflammatory diseases, chronic conditions, major cardiovascular events, anemia, or specific medications that could affect ferritin levels.*

Results: *The study found that serum ferritin levels significantly differed among three glycemic groups (NFG, IFG, and T2DM) with higher levels in T2DM. IFG and T2DM groups also had elevated serum insulin and HOMA-IR. Serum ferritin correlated strongly with fasting blood glucose, serum insulin, HOMA-IR, and BMI . The highest tertile of ferritin levels were associated with IFG & diabetic group.*

Conclusion. *Elevated serum ferritin levels in IFG and type 2 diabetes may significantly impact on glucose regulation. Compared to NFG, there are high insulin resistance markers (fasting insulin, glucose, HOMA-IR) in T2DM and IFG. They have crucial implications for both therapy and prognosis in these conditions.*

Keywords*: S.Ferritin, IR Markers, Glycemic state.*

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INTRODUCTION

As a predominant public and medical issue, the emergence of T2DM has increased dramatically in recent years and put a tremendous burden on medical, economic and social infrastructure. The International Diabetes Federation (IDF) estimates that in 2015, there were 415 million people with diabetes worldwide and indicates that the absolute number will reach 642 million by 2040[1]. For every diagnosed diabetic case, another one is undiagnosed; overall, undetected people are at significantly higher risk for stroke, coronary artery disease, and peripheral vascular disease. The proportion of undiagnosed DM

in Bangladesh is about 56.0 %[1]. While obesity and diabetes are reaching epidemic proportions in the developed world, the role of insulin resistance and its sequelae is gaining prominence[2]. Genetic and environmental factors cause IR & lead to impaired glucose tolerance, and play a crucial patho-physiological role in the development of diabetes[3]. Insulin resistance occurs before the development of T2DM and might be the best indicator for it[4]. Recent studies have shown Insulin resistance syndrome (IRS) correlated with increased serum ferritin, suggesting the pathophysiological link between the severity of IRS and serum ferritin levels[5].

There is an increasing interest in the adverse health outcomes associated with elevated body iron stores[6]. Through its oxidative properties, iron has also been suggested to play a role in IR and beta cell dysfunction, which are cardinal features of altered glucose homeostasis[7] Iron is the body's most abundant metal and an essential human nutrient. It is a cofactor for several enzymes involved in oxidation-reduction reactions due to its ability to exist in two ionic forms-ferrous and ferric[8]. Elevated iron stores may induce diabetes through various mechanisms, including oxidative damage to pancreatic beta cells, impairment of hepatic insulin extraction by the liver and interference with insulin's ability to supress hepatic glucose production. Long-term microvascular and macrovascular complications of diabetes may be due to raised serum ferritin[9-11]. Body iron stores are commonly assessed by serum ferritin, a widely available clinical biomarker to evaluate iron status[8,12]. Cross-sectional studies indicate an independent link between high iron stores and T2DM occurrence[13,14]. And found Ferritin, a reliable marker, has been linked to glycemic status and complications of Diabetes Mellitus, including Retinopathy, Nephropathy, Neuropathy, and Vascular dysfunction[15].

A few studies have demonstrated an association between markers of insulin resistance (fasting insulin, glucose and HOMA-IR) and ferritin[16,17]. They found that plasma ferritin concentrations positively correlate with fasting insulin and fasting glucose. Some observational studies have shown that elevated serum ferritin is associated with IR when assessed by HOMA-IR[18,19]. Another survey by Suarez-Ortegan[19] showed that serum ferritin did not significantly predict HOMA-IR in the healthy Colombian population. A study on the Finnish population suggests that the strength and direction of the association between serum ferritin and HOMA-IR depend on the population's glycemic state[7].

In diabetes mellitus, increased glycation of transferrin decreases its ability to bind ferrous iron. Hence, there is an increased pool of free iron, which stimulates increased ferritin synthesis[20]. An increase in Ferritin synthesis is believed to result in the internalization of Insulin receptors, which contributes to Insulin resistance[11]. Thus, glucose metabolism is found to be interlinked with iron metabolism and measuring serum ferritin levels as part of diabetic management could aid in predicting the outcomes. Higher ferritin concentrations are observed in Asian populations, and even moderately increased iron stores are associated with diabetes[12,21]

A previous study observed positive correlation between serum ferritin concentration and HbA1c in T2DM patients in our country[22] But association of body iron stores and glucose homeostasis at different glycemic states were unclear.

Hence, We proposed this study to assess the association of serum ferritin to insulin resistance markers (fasting insulin, glucose and HOMA-IR) in three different glycemic states and tried to establish its role in glucose homeostasis.

METHODS

It was a cross-sectional, observational study conducted at the Department of Biochemistry of Sir Salimullah Medical College, Dhaka, Bangladesh, between March 2018 and February 2019. A total of 140 subjects were included to conduct this study divided into three groups: Group A having individuals with normal fasting glucose (NFG group) and Group B having individuals with impaired fasting glucose (IFG group), and Group C having individuals with newly diagnosed type 2 diabetes mellitus (Diabetic group). Adult people aged 25 to 55 years were included. Participants with acute or chronic inflammatory diseases, chronic liver diseases, major cardiovascular events, chronic alcoholism, and anaemia were excluded by history taking and clinical examinations. Conditions that altered ferritin level, e.g. Hemochromatosis, history of blood transfusion or iron therapy in the previous year and subjects with very high serum ferritin level (>800 ng/ml) were excluded. History of taking antidiabetic, lipid-lowering agents, or other medication that affect

carbohydrate, lipid or insulin metabolism were also excluded. Those with malignancy and pregnancy were excluded. All surveys were conducted after obtaining written informed consent. Anthropometric variables were measured accordingly, and a blood sample was collected to measure biochemical variables.

Subjects were selected from the outpatient department (OPD) of Medicine and Endocrinology of Sir Salimullah Medical College and Mitford Hospital, Dhaka, and National Academy for Educational Management (NAEM), Dhaka, as a part of their routine examination. Before collecting specimens, each eligible person was firmly approached and proper counselling about aims, objectives, and risks. benefit and procedure of the study were done. Only voluntary candidates were recruited as participants. Then they were interviewed, and relevant information was recorded systematically in a pre-designed standard datasheet, including general information and history of chronic diseases, and family history of diabetes. Data were checked and edited. All surveys were conducted after obtaining written informed consent. Anthropometric variables were measured accordingly, and a blood sample was collected to measure biochemical variables.

Diabetes was defined according to the WHO 2006 guidelines as having fasting blood glucose measurement e"7.0 mmol/L. Normal fasting glucose (NFG) indicates a healthy state where the blood glucose level is below 6.1 (mmol/L) after fasting. In impaired fasting glucose (IFG) signifies a condition in which the fasting plasma glucose falls between 6.1 and 6.9 mmol/L, suggesting a potential risk for diabetes. or having been diagnosed by a physician. Markers of insulin resistance as assessed by Fasting insulin, glucose and HOMA-IR[23]. BMI was calculated as weight in kilograms divided by the square of height in meters.

All statistical analyses were performed using SPSS (statistical package for social science) for Windows 22.0 version. The mean with standard deviation was determined to compare continuous variables. ANOVA test was done to compare continuous variables. Bonferonni test was performed for the comparison of groups. Serum ferritin concentrations were divided into tertiles to observe the trend of the related variables. The statistical significance, direction and strength of linear correlation between two quantitative variables were measured using Pearson's correlation coefficient test. $p<0.05$ was considered a test of significance in all statistical tests. Sample size was determined by applying the formula for a comparison of two means. Using IR values from Kim et al.[24]

The study was approved by the Ethical Review Committee of Sir Salimullah Medical College, Dhaka, Bangladesh.

RESULTS

The study is basically targeted to analyze the serum ferritin behaviour in the three groups representing different stages of glycemia. Table-I meticulously compiles mean values and standard deviations (SD) of baseline and biochemical parameters across three distinct glycemic groups. Notably, parameters such as age, BMI, SBP, DBP, WC, WHR, fasting plasma glucose, serum insulin, HOMA-IR, and serum ferritin exhibited significant differences among these groups ($p<0.001$), as validated by the ANOVA test.

Upon applying the Bonferroni test for intergroup comparisons, it emerged that serum ferritin levels were notably higher ($p<0.001$) in individuals with Type 2 Diabetes (T2DM) compared to those in the Impaired Fasting Glucose (IFG) group. Furthermore, subjects with IFG and T2DM displayed significantly elevated ($p<0.001$) serum insulin and HOMA-IR levels compared to the Normal Fasting Glucose (NFG) group. Specifically, the mean ferritin concentration was markedly higher in subjects with IFG (84.04±80.79) and newly diagnosed T2DM (158.1± 44.9) in comparison to those with NFG (40.50±28.512) (Fig. 1).

Table-II shows insightful correlations between serum ferritin and key variables—fasting blood glucose, serum insulin, HOMA-IR—across the studied groups. Notably, strong correlations existed between serum ferritin and fasting blood glucose, serum insulin, and HOMA-IR levels. Furthermore, a significant correlation was found between serum ferritin and participants' Body Mass Index (BMI). These correlations were consistent across groups, lacking significant differences.The normal reference interval of serum ferritin has a wide range because of age and gender variations9 The reference range for adult male is between 20-270 µg/L and that of adult female is 10-120 µg/L. In the current study, we also observed that ferritin distribution was positively skewed. Higher tertiles of serum ferritin were notably (Â0.01) associated with Type 2 Diabetes (T2DM) Illustrated in Figure 2.

Table-I: *Characteristics of the studied population (n=140)*

Variables	Group A (NFG) (n=60)	Group B (IFG) (n=50)	Group C (T2DM) (n=30)	p-value
Age (years)	35.60±8.77	42.64±8.54	49.73±5.13	<0.001
BMI (kg/m^2)	23.25±2.18	27.74±2.29	27.75±2.29	<0.001
SBP (mmHg)	113.88±10.65	115.20±7.57	118.9±10.7	<0.001
DBP (mmHg)	75.50±8.61	83.60±5.69	87.7±4.17	<0.001
WC (inch)	85.05±7.77	97.56±7.31	94.4±9.0	<0.001
WHR	0.83±0.10	0.95±0.10	0.95±0.08	<0.001
FPG (mmol/L)	5.14±0.40	6.26±0.17	9.69±2.41	<0.001
Serum Insulin (µU/ml)	5.98±1.90	11.64±2.62	12.67±4.27	<0.001
HOMA -IR.	0.78±0.25	1.60±0.31	1.89±0.64	<0.001
Serum ferritin (ng/mL)	40.50±28.51	84.04±80.79	158.1±44.9	<0.001

	Age	BMI	Serum Insulin (µU/ml)	HOMA -IR.	Serum ferritin (ng/mL)
Group A vs B	0.002	<0.001	<0.001	<0.001	<0.05
Group A vs C	<0.001	<0.001	<0.001	<0.001	<0.001
Group B vs C	<0.05	1.000	0.758	0.067	<0.001

Data were expressed as mean±SD; ANOVA test was performed to compare the three groups.

Table-II : *Correlation of serum ferritin with BMI, FPG, serum insulin and HOMA IR*

Name	Overall		Group A (NFG) (n=60)		Group B (IFG) (n=50)		Group C (T2DM) (n=30)	
Variables	r	p-value	r	p-value	r	p-value	r	p-value
BMI	0.297	0.007	0.384	0.515	0.185	0.376	0.383	0.159
FPG	0.520	<0.001	0.448	0.554	0.303	0.141	0.243	0.382
S.insulin	0.461	<0.001	0.191	0.238	0.156	0.456	0.129	0.648
HOMA -IR	0.504	<0.001	0.156	0.336	0.181	0.387	0.191	0.495

Correlation is significant at the 0.05 level (2-tailed); Pearson correlation coefficient test was performed.

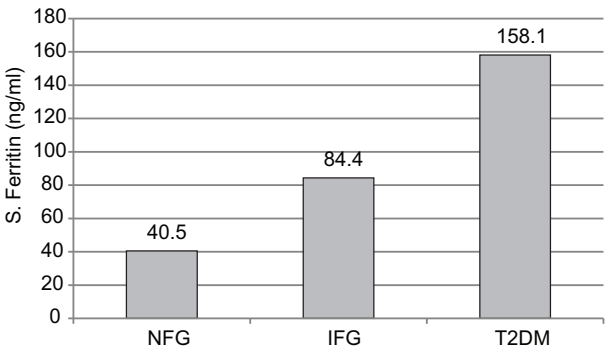

Figure 1: *Bar diagram showing mean ferritin concentration between three groups, showing increased serum ferritin levels in type 2 DM compared to the NFG and IFG groups.*

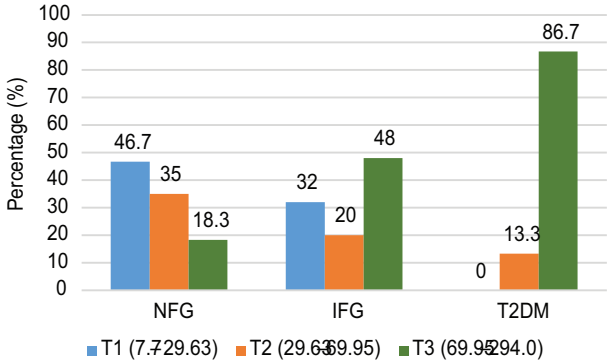

Figure 2: *Showing serum ferritin tertile with the association of glycemic status, where 86.7% of T2DM patients belonged to the highest tertile, which is statistically significant.*

DISCUSSION

The impact of type 2 diabetes mellitus (T2DM) on Bangladesh's economic and social well-being is significant, with macrovascular complications appearing before the disease manifests. Screening for T2DM is crucial for surveillance and identifying individuals with prediabetes for timely interventions[25]. Body iron stores are commonly assessed by serum ferritin, a key protein that regulates iron homeostasis, a widely available clinical biomarker to evaluate iron status[12]. The study analyzed serum ferritin due to its interconnection with iron and glucose metabolism[13]. A total of 140 subjects were included in this study based on predefined enrollment criteria. The subjects comprised 60 normoglycaemic, 50 with impaired fasting glucose and 30 newly diagnosed type 2 diabetes mellitus. Therefore, this cross-sectional study evaluated the association between serum ferritin concentration and IR markers (fasting insulin, glucose and HOMA-IR) in Bangladeshi adult subjects of different glycemic states.

Serum ferritin levels were significantly higher in IFG and diabetics compared to the NFG group, consistent with previous research findings[11,23]. In the present study, subjects with impaired fasting glucose (IFG) and type 2 diabetes mellitus (T2DM) exhibited a notable rise in serum insulin and HOMA-IR levels when compared to the normal fasting glucose (NFG) group, as indicated in Table I. This finding aligns with the results reported by a previous study conducted over six years in France. The French study demonstrated that individuals with elevated serum ferritin levels faced an increased risk of hyperinsulinemia and high HOMA-IR values in both genders [26].

The exact mechanism linking elevated serum ferritin with insulin resistance IR is not fully understood, but several theories have been proposed. Iron's pro-oxidant nature can induce oxidative stress, disrupting insulin signaling at the cellular level[13]. Secondly, ferritin can increase pro-inflammatory cytokines, potentially mediating its association with IR[27]. Thirdly, iron accumulation in the liver may interfere with insulin extraction, impairing glucose tolerance[27] Lastly, iron might hinder insulin action and glucose utilization in adipocytes, contributing to IR. According to Pramiladevi et al.[9]elevated serum ferritin levels might contribute to insulin resistance (IR) even before the onset of diabetes. A prospective study by Jung et al.[28] demonstrated that the risk of IR increased proportionately to serum ferritin levels, independent of metabolic factors, suggesting the predictive value of serum ferritin in IR. Frequent blood donation and phlebotomy therapy have been linked to improved insulin sensitivity, as reported in studies[13]. In this study, higher serum ferritin tertile levels were significantly associated with impaired glycemic states (IFG and T2DM) (Fig II), aligning with findings from a survey by Koo et al. [29,] which suggested that hyperferritinemia might increase the risk of diabetes mellitus through heightened insulin resistance rather than dysfunctional beta cells. In a prospective study, Nakamura et al.[30] observed that clinically high-normal and mildly elevated iron storage in the body may cause diabetes, mainly by inducing insulin resistance, regardless of race. This influence is likely independent of the potential pathway between obesity and insulin resistance.

It was evident from the study that high serum ferritin, serum insulin and HOMA-IR were observed in IFG and type 2 diabetic subjects, and hyperferritinemia was significantly associated with insulin resistance markers like fasting insulin, glucose and HOMA-IR.

We found a positive association between ferritin and BMI and showed that higher serum ferritin is associated with increased BMI. However, we did not consider obesity status, which is positively correlated with the degree of insulin resistance.

CONCLUSION

To conclude, our data reveals a positive correlation between serum ferritin levels and glycemic status, particularly in IFG and diabetes mellitus type 2, compared to normoglycaemic subjects., IFG and type 2 diabetic subjects had higher insulin resistance markers (fasting insulin, glucose and HOMA-IR) levels than the NFG group, which presents significant opportunities in diagnosing and managing diabetes. This finding holds promise for predicting an individual's tendency to develop diabetes and related complications. Integrating serum ferritin assessments into screening programs could identify high-risk individuals, enabling targeted preventive measures. Lowering elevated serum ferritin levels through interventions offers a potential avenue for reducing

the morbidity and mortality associated with type 2 diabetes mellitus.

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