Original Article

Hyperferritinemia: An Early Predictor of Insulin Resistance and Other Complications of Type 2 Diabetes Mellitus

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Abstract

Background: The incidence of type 2 diabetes mellitus is growing globally and has become a significant health burden accounting for 90% of total cases of diabetes. The present study was carried out to determine the interaction of insulin resistance and anemia in type 2 diabetes (T2D) patients. **Material & Methods:** A total of 110 type 2 diabetic patients were examined, and the laboratory investigations were carried out, including the lipid, anemia, and diabetic profile, along with the calculation of the Homeostatic Model Assessment for Insulin Resistance. **Results:** Serum ferritin and iron levels showed a significant positive association with Body Mass Index, fasting glucose, postprandial glucose, glycated hemoglobin (HbA1C), serum insulin levels, Homeostatic Model Assessment for Insulin Resistance (HOMA-IR), serum cholesterol, serum triglycerides and urinary protein/creatinine ratio. However, creatinine was positively associated with ferritin only. The significantly increased BMI, fasting & post-prandial blood glucose, HbA1c, serum ferritin, serum iron, total cholesterol, triglycerides, serum insulin, and urine protein/creatinine ratio were observed in patients with the HOMA-IR values of >3 than the patients with HOMA-IR values of ≤3. **Conclusion:** Increased serum ferritin levels reflect its role in insulin resistance, poor glycemic control, obesity, dyslipidemia, and complications of T2DM like diabetic nephropathy, diabetic neuropathy, and diabetic retinopathy. Therefore, hyperferritinemia can be used as an early tool to diagnose insulin resistance before the onset of overt diabetes.

Keywords: Type 2 diabetes mellitus, hyperferritinemia, insulin resistance, HOMA-IR, hyperglycemia

Introduction

Type 2 diabetes mellitus (T2DM), accounting for 90% of the diabetes cases globally, is a predominant public health concern worldwide. The interaction of genetic and environmental factors plays a vital role in the complex pathogenesis of T2DM. Individuals with T2DM show both insulin resistance and beta-cell defects[(1)].

Insulin resistance is defined as a normal or elevated insulin level that produces an attenuated biological response. Classically this refers to impaired sensitivity to insulin-mediated glucose disposal[(2)]. Therefore, the ability to respond to the normal circulating concentration of insulin is decreased in target organs like the liver, adipose tissues, and skeletal muscle. The primary cause of insulin resistance in T2DM is due to post-binding defects in insulin action[(3,4)].

The bidirectional association between T2DM and iron metabolism has been noted, with interest in research and clinical practice. Unsuspected influences between body iron stores, insulin resistance, and T2DM have been disclosed by the scientific evidence[(5)]. Even in the absence of significant iron overload, the glucose metabolism is affected by iron[(6,7)]. A significant incidence of insulin resistance and metabolic syndrome is found in patients with moderately elevated iron stores below the levels commonly found in genetic hemochromatosis[(8,9)]. Elevated iron stores reflected as elevated plasma ferritin levels induce baseline abnormalities that ultimately result in diabetes, or raised ferritin levels may be one of the several metabolic abnormalities related to insulin resistance and T2DM. Both of these abnormalities may also result from a third independent cause[(10)].

Patients with uncontrolled T2DM have abnormally increased ferritin levels, which correlates with diabetic retinopathy, diabetic nephropathy, and vascular dysfunction[(11-13)]. The chronic hyperglycemia of diabetes mellitus is also associated with long-term damage, dysfunction, and failure of various

organs, especially the eyes, kidneys, nerves, heart, and blood vessels. Increased body iron stores are associated with the development of glucose intolerance, T2DM, gestational diabetes mellitus, and insulin resistance syndrome[14].

A little indirect evidence from the western region suggests that iron overload negatively influences diabetes mellitus. However, overall, there is a paucity of literature, especially from India, showing direct evidence that there is difficult control of diabetes mellitus in patients with iron overload. Anemia is prevalent in the Indian population, and continuous efforts are being made at the physician, community, and government levels to prevent and treat anemia[15]. Therefore, finding out such an association in the Indian population carries great clinical significance, influencing the coexisting diabetic state. Hence, the present study aimed to find the association between insulin resistance and serum iron status in T2DM and to study the influence of body iron stores (reflected by serum ferritin levels) on various biochemical parameters with diabetic complications in an Indian population.

Material and Methods

The present cross-sectional study was conducted in a Bharati Vidyapeeth (Deemed to be University) Medical College & Hospital, Pune. A total of 110 patients of either gender of age ≥ 30 years presenting with T2DM, from OPD and IPD of Bharati Hospital and Research Centre, Pune, over 18 months and fulfilling the inclusion criteria were included in the study after taking informed written consent. Institutional ethical committee clearance for the study was obtained. All patients >30 years and <80 years with T2DM and hemoglobin >13 gm% for males and >12 gm% for females were included in this study. The patients with pregnancy, acute inflammatory conditions affecting serum ferritin levels like recent liver disease, renal disease, malignancy, autoimmune disease, history of gastrointestinal blood loss, blood transfusion / donation in last year, and hemochromatosis ingestion of haemanitics were excluded from the study. The sample size of 104 was calculated from the prevalence of 7.3% diabetes mellitus with a 95% confidence interval and 5% margin error[16].

After inclusion history of patients was taken, physical examination was done, and body mass index was calculated. After 8-10 hours of overnight fast, 10 ml of venous blood was collected under aseptic conditions. The hemoglobin level, WBC, and platelet were estimated from the Ethylene Diamine Tetraacetic Acid (EDTA) vacutainer. The biochemical parameters viz. blood glucose level (fasting and postprandial), serum creatinine, cholesterol, triglyceride, Iron, Total Iron-Binding Capacity (TIBC), and ferritin levels were measured by fully automated analyzer with commercially available kits (Randox Laboratories, United Kingdom).The insulin level was estimated by Chemiluminescence Microparticle Immuno Assay (CMIA) (Abbott Laboratories, United States) method. Values of TIBC and Transferrin Saturation were calculated using the below formulae.

$$\text{TIBC} = \text{Serum ferritin} + \text{Serum Unsaturated Iron-Binding Capacity (UIBC)}$$

$$\text{Transferrin Saturation} = \text{Serum Iron} \times \frac{100}{\text{Serum TIBC}}$$

Urine Protein/Creatinine ratio to evaluate diabetic nephropathy was calculated after estimation of urine protein and creatinine. Insulin resistance was calculated as Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) from fasting blood glucose (mg/dl) and fasting serum insulin levels (IU/L)[16] using the below formula-

$$\text{HOMA-IR} = \frac{\text{Fasting Insulin (IU / L) x Fasting glucose (mg/dl)}}{405}$$

The influence of body iron stores was evaluated for diabetic complications like diabetic retinopathy (using Indirect Ophthalmoscope), diabetic neuropathy (using relevant tests), and diabetic nephropathy (Urine protein/creatinine ratio) and its association with increased serum ferritin levels.

Statistical Analysis

The data was analyzed for statistical significance using SPSS software. The categorical variables were classified as 'n' (% of patients). The continuous variables were presented as mean ± standard deviation (SD). The inter-group statistical comparison of categorical variables was done using Chi-square test. The correlation analysis of iron & ferritin with biochemical markers was studied using Pearson's correlation coefficient. The independent predictor of Insulin resistance (HOMA-IR) was obtained using multivariate linear regression analysis. The p-value < 0.05 was considered to be statistically significant.

Results

The mean ± SD age of the study group was 53.6 ± 8.7 years. The minimum and maximum age range among the patients studied was 36 & 72 years. Out of 110 T2DM patients studied, 53 patients (48.2%) were males, and 57 patients (51.8%) were females. The male to female sex ratio of the patients studied in

the entire study group was 0.93: 1. The mean values of demographical, hematological, and biochemical parameters were calculated along with their respective standard deviation (Mean ± SD) values, shown in table 1.

Table 1: Levels of hematological and biochemical parameters in T2DM patients

Parameters	Type 2 diabetic patients
Age	53.6 ± 8.7
Gender Males n(%)	53 (48.2%)
Females n(%)	57 (51.8%)
BMI (kg/m^2)	25.07 ± 2.26
Hemoglobin (g%)	12.74 ± 0.74
Serum Creatinine (mg%)	0.95 ± 0.19
Blood glucose fasting (mg/dL)	230.83 ± 110.86
Blood glucose PP (mg/dL)	271.68 ± 124.05
HbA1c (%)	8.05 ± 2.46
Serum Ferritin	272.52 ± 94.76
Serum Iron	45.83 ± 11.12
TIBC	36.65 ± 6.15
Total cholesterol (mg/dL)	210.28 ± 57.74
Triglycerides (mg/dL)	141.99 ± 36.71
Serum Insulin µU/mL	6.88 ± 5.99
HOMA-IR	4.52 ± 4.77
Urine protein/creatinine ratio (mg/g)	0.48 ± 0.63

The association of ferritin and iron levels was evaluated using Pearson's correlation coefficient. Serum ferritin and iron levels showed a significant positive association with BMI, fasting glucose, postprandial glucose, HbA1C, serum insulin levels, HOMA-IR, serum cholesterol, serum triglycerides, and urinary protein/creatinine ratio. While serum creatinine was only positively associated with ferritin levels, not with serum iron. The correlation coefficient 'r' and corresponding 'p' values are depicted in table no. 2.

Table 2: Correlation analysis showing association of serum ferritin and iron with biochemical parameters

Parameters	Ferritin		Iron	
	'r' value	p value	'r' value	p value
BMI	0.371	0.001*	0.315	0.001*
Fasting glucose	0.755	0.001*	0.558	0.001*
Post prandial glucose	0.738	0.001*	0.575	0.001*
HbA1C	0.579	0.001*	0.351	0.001*
Serum Insulin levels	0.551	0.001*	0.272	0.004*
HOMA-IR	0.635	0.001*	0.345	0.001*
Serum Cholesterol	0.608	0.001*	0.461	0.001*
Serum Triglyceride	0.580	0.001*	0.487	0.001*
Serum Creatinine	0.218	0.022*	0.099	0.3050
Urine Protein/Creatinine ratio	0.354	0.001*	0.301	0.001*

*statistically significant

On multivariate analysis, BMI, Serum ferritin, serum triglycerides, and HbA1C were the independent and significant predictors of insulin resistance (HOMA-IR). The results of the multivariate analysis are presented in table no. 3.

Table 3: Multivariate analysis showing predictors of insulin resistance (HOMA-IR)

Variables in the model	t-value	p-value
Age (years)	0.778	0.439
Sex	0.573	0.568
BMI (kg/m2)	2.054	0.042*
Serum Ferritin (mg/dL)	2.875	0.005*
Serum Triglycerides (mg/dL)	2.142	0.035*
Serum Creatinine (mg/dL)	0.393	0.695
HbA1C (%)	15.421	0.001*
*statistically significant		

The distribution of prevalence of patients for the biochemical parameters was done based on abnormal levels in respective groups. Among the 110 patients studied, 89 (80.9%), 28 (25.5%) & 110 (100%) patients had normal level of serum iron, ferritin & TIBC, and 21 (19.1%) & 82 (74.5%) patients had abnormal serum iron and ferritin level respectively. A significant (p<0.0001) number of patients were normal for serum iron levels, while a significantly (p<0.0001) higher number of patients were abnormal for serum ferritin levels. The indicator of insulin resistance HOMA-IR was categorized to be mild (<3), moderate (3-5), and severe (>5), with the corresponding number of patients 49 (44.5%), 27 (24.5), and 34 (31%), there was no any significant change in the distribution of the number of patients reported. The number of patients with abnormal HbA1c level 80 (72.7%) was significantly (p<0.0001) higher than the normal HbA1c level 30 (27.3%). The values of BMI were also classified into three groups, viz. normal, overweight, and obesity, with corresponding numbers 67 (60.9%), 39 (35.5%), and 4 (3.6%), respectively. The numbers of patients in overweight (p=0.0002) and obesity (p<0.0001) subgroups were significantly lower when compared with the number of patients with normal BMI. The results of the same are presented in table 4.

Table 4: Prevalence of abnormal iron levels, ferritin, total iron-binding capacity (TIBC), HOMA-IR, HbA1c, serum creatinine, and BMI among the patients studied.

Parameters	Status	Number of patients	Percentage (%)	P value
Serum Iron (mg/dl)	Normal	89	80.9	< 0.0001*
	Abnormal (Deficiency)	21	19.1	
Serum Ferritin (mg/dl)	Normal	28	25.5	< 0.0001*
	Abnormal (Raised)	82	74.5	
Serum TIBC (%)	Normal	110	100.0	-
	Abnormal	0	0.0	
HOMA-IR	Mild (<3)	49	44.5	0.1036
	Moderate (3-5)	27	24.5	
	Severe (>5)	34	31.0	
HbA1C	Normal (<6.5%)	30	27.3	<0.0001*
	Abnormal (e”6.5%)	80	72.7	
BMI (Kg/m2)	Normal	67	60.9	-
	Overweight (25.0 – 29.9)	39	35.5	0.0002*
	Obesity (>30.0)	4	3.6	<0.0001*
*statistically significant				

The patients were divided based on the cut-off value of HOMA-IR; the first group was with HOMA-IR values of ≤3 (n=49), and the second group with HOMA-IR values of >3. The differences in the means of biochemical parameters were compared between these two groups. The results indicated the significantly (p<0.0001) increased levels of BMI, FBG, PPBG, HbA1c, serum ferritin, serum iron, total cholesterol, triglycerides, serum insulin, and urine protein/creatinine ratio in patients with the HOMA-IR values of >3 than the patients with HOMA-IR values of ≤ 3. The results are shown in table no. 5.

Table 5: Effect of HOMA-IR (insulin resistance) on biochemical parameters

Parameters	T2DM patients with HOMA-IR ≤3 (n=49)	T2DM patients with HOMA-IR >3(n=61)	P-value
BMI	23.88 ± 1.66	26.03 ± 2.24	<0.0001*
Serum Creatinine	0.91 ± 0.19	0.98 ± 0.20	0.0649
Fasting blood glucose	130.14 ± 67.30	311.70 ± 61.45	<0.0001*
Post-prandial blood glucose	160.51 ± 78.77	360.98 ± 69.22	<0.0001*
HbA1c (%)	6.42 ±0.53	9.36 ± 2.62	<0.0001*
Serum Ferritin	196.47 ± 85.01	333.60 ± 44.88	<0.0001*
Serum Iron	39.80 ± 9.0	50.67 ± 10.30	<0.0001.*
TIBC	36.61 ± 5.80	36.69 ± 6.47	0.9463
Total cholesterol	174.59 ± 48.85	238.95 ± 47.65	<0.0001*
Triglycerides	118.18 ± 29.04	161.11 ± 30.65	<0.0001*
Serum Insulin	3.38 ± 1.13	9.69 ± 6.81	<0.0001*
Urine protein/creatinine ratio	0.18 ± 0.18	0.72 ± 0.75	<0.0001*

*statistically significant

The patients further were classified based on the presence or absence of diabetic complications. The number of patients with the presence (78 (70.9%)) of diabetic nephropathy was significantly (p<0.0001) higher than with absence (32 (29.1%)). The number of patients based on diabetic retinopathy and dyslipidemia (62 (54.4%), 62 (56.4%)) respectively did not differ significantly. The number of patients in respective groups with percentages and p values is shown in table no. 6.

Table 6: Distribution of prevalence of diabetic complications and metabolic risk factors among the patients studied (n=110)

Diabetic Complications	Status	Number of patients	Percentage (%)	P-value
Diabetic Nephropathy	Absent	32	29.1	<0.0001*
	Present	78	70.9	
Diabetic Retinopathy	Absent	48	43.6	0.0582
	Present	62	56.4	
Dyslipidemia#	Absent	48	43.6	0.0582
	Present	62	56.4	

*statistically significant
#Dyslipidemia: (Cholesterol >200mg/dl OR Triglyceride >150mg/dl)
@Obesity: BMI>25.0

The distribution of prevalence of severity of insulin resistance according to serum iron, ferritin, and BMI status was evaluated. The significantly (p=0.022) higher numbers of patients with abnormal serum iron levels showed mild insulin resistance. In contrast, a significantly (p=0.001) higher number of patients had moderate to severe insulin resistance with abnormal serum ferritin levels and were overweight as per the BMI values. The results are shown in table no. 7.

Table 7: Distribution of the number of T2DM patients for serum iron, ferritin, and BMI status as per the severity of insulin resistance

HOMA IR	Serum Iron status				Serum ferritin status				BMI* (kg/m^2)					
	Normal (n=89)		Abnormal (n=21)		Normal (n=28)		Abnormal (n=82)		Normal (n=67)		Overweight (n=39)		Obesity (n=4)	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Mild	34	38.2	15	71.4	28	100	21	25.6	45	67.2	4	10.3	0	0.0
Moderate	24	27.0	3	14.3	0	0.0	27	32.9	12	17.9	15	38.5	0	0.0
Severe	31	34.8	3	14.3	0	0.0	34	41.5	10	14.9	20	51.3	4	100
P value		P=0.022*				P=0.001*						P=0.001*		

*statistically significant
*BMI: Normal (18.5 – 24.9), Overweight (25.0 – 29.9), Obesity (>30.0)

Discussion

Scientific evidence suggests there are unsuspected influences between the metabolism of iron status and T2DM. Since iron affects glucose metabolism, and glucose metabolism impinges on several iron metabolic pathways, the relationship between them is bidirectional[5]. These relationships are influenced by oxidative stress and inflammatory cytokines, which amplify, potentiate, and aggravate the initiated events[18].

In the present study, the association of iron and ferritin with the biochemical parameters and the diabetic complications was studied. The serum iron and ferritin levels were positively associated with BMI, fasting blood glucose, postprandial glucose, HbA1C, serum insulin levels, HOMA-IR, serum cholesterol, serum triglycerides, and urinary protein creatinine ratio. In contrast, serum creatinine was positively associated only with ferritin levels.

The iron from the blood enters the cellular compartment via its attachment to serum ferritin and then binding to its specific transferrin receptors by endocytosis [19]. This can be used to synthesize essential cellular components[20]. Insulin rapidly stimulates iron uptake by fat cells and is also responsible for increasing serum ferritin levels[21]. Insulin action of inhibiting glucose production by the liver is interfered by serum iron. As iron stores increase insulin metabolism, hepatic extraction is reduced, leading to peripheral hyperinsulinemia[22]. Liver insulin resistance is the initial and common abnormality in iron overload conditions[23].

The exact mechanism regarding the association of serum ferritin with T2DM remains unclear. Firstly, serum ferritin is regarded as a biomarker of body iron stores, whose catalytic effects could induce lipid peroxidation[24], responsible for developing insulin resistance[25]. Secondly, excess body iron may be directly involved in insulin signaling[26], leading to highly reactive free radicals causing disturbed glucose metabolism and subsequent hyperglycemia[27]. In a study conducted by Gonz'alez et al.[28], it was observed that hepcidin, a peptide made in the liver, was elevated in response to hypoxia or inflammation and was correlated with an increase in serum ferritin. It was suggested that leptin was responsible for inducing hepcidin's expression via the JAK2/STAT3 pathway[29,30].

The levels of ferritin were found to be increased in the T2DM patients with BMI >25 kg/m2 than the normal BMI in various studies[31-35]. In a cross-sectional study conducted by Zhan et al., it was observed that elevated serum ferritin levels were associated with higher risks of diabetes and higher levels of HbA1c and HOMA-IR[36]. However, the observations of the present study are in contrast to Gupta et al., who observed that serum ferritin was not a strong risk factor in the pathogenesis of obesity and diabetes[37]. Padwal MK et al. identified that high serum ferritin levels within normal range are significantly associated with metabolic syndrome and insulin resistance[38].

Laaksonen et al. observed that the positive association of serum ferritin with plasma glucose, triglyceride, and apolipoprotein B negatively affects serum HDL cholesterol levels, all of which are components of the insulin-resistant syndrome[39]. In agreement with the present study results, Nan Hee Kim et al. in T2DM patients also showed a positive association of serum ferritin with fasting plasma glucose, BMI, fasting C-peptide, hemoglobin, serum iron, and Total Iron Binding Capacity (TIBC)[18]. The results are also consistent with Orban et al.[40] and Ford et al.[41].

Multiple linear regression analysis showed that elevated serum ferritin levels and serum triglyceride levels were significant and independent predictors for higher HOMA-IR, independent of age and sex. Serum ferritin levels were significantly correlated with Body Mass Index, serum creatinine, and urinary protein to creatinine ratio.

A significantly more number of patients were found to be abnormal values for ferritin and HbA1c. In comparison, the

number of patients with abnormal values of iron and BMI were significantly lower than with normal levels. The levels of biochemical parameters based on the HOMA-IR cut-off value were compared, with higher levels of all the biochemical markers except creatinine and TIBC in patients with HOMA-IR values >3 than <3.

Among the complications studied, only diabetic nephropathy and obesity were present in more patients significantly out of total T2DM patients. Kim et al. similarly reported an increase in iron stores, i.e., serum ferritin level was associated with the risk of developing T2DM and related complications[18].

Serum ferritin is an independent determinant of poor metabolic control in diabetic patients. Diabetic microangiopathy is associated with an abnormal increase in serum ferritin levels. In a study conducted by Jehn et al., it was observed that men with moderately higher ferritin levels had a significantly worse coronary risk profile as compared to men with lower ferritin levels[9].

In a study conducted by Cantur et al., it was observed that patients with uncontrolled diabetes had hyperferritinemia, which was associated with diabetic retinopathy[12]. In a study conducted by Baer et al.[42] and Ascherio et al.[43], it was observed that there was no association between serum ferritin levels and coronary heart disease.

In diabetes mellitus there is an increase in Reactive Oxygen Species (ROS) in tissues leading to a cascade of events resulting in diabetes and its complications[44,45]. Iron converts reactive free radicals into highly reactive ones. As the serum ferritin level increases, it affects insulin synthesis and secretion in the pancreas and interferes with the insulin extracting capacity of the liver. Pancreatic β-cells act as a target of oxidative stress-mediated tissue damage[46]. Deposition in muscles leads to muscle damage and decreases glucose uptake.

Advanced glycation end products themselves bind transition metals[43], potentiating their toxic effects, including insulin resistance. Decreasing iron stores would ameliorate insulin resistance by reducing this cascade of events. Therefore, reactive oxygen species interfere with insulin signaling at various levels, impairing insulin uptake through a direct effect on insulin receptor function and inhibiting the translocation of GLUT 4 in the plasma membrane. Perhaps this could be one of the reasons for the increased incidence of T2DM in people with relatively higher levels of serum ferritin. The patients with abnormal iron levels significantly fall into the HOMA-IR group with mild insulin resistance. The higher numbers of patients were within the moderate to severe insulin resistance group with abnormal serum ferritin levels, and those were overweight.

Conclusion

There was a significant association of serum ferritin and iron with insulin resistance in patients with T2DM. Increased serum ferritin levels reflect insulin resistance, poor glycemic control, obesity, dyslipidemia and complications of T2DM like diabetic nephropathy, diabetic neuropathy and diabetic retinopathy. Hyperferritinemia could be an early tool to diagnose insulin resistance before the onset of overt diabetes. Reliable and sensitive methods need to be developed to precisely measure the free/ catalytic iron that participates in oxidative injury. Iron chelation therapy may present a novel way to interrupt the cycle of catalytic iron-induced oxidative stress and tissue injury and consequent release of catalytic iron in diabetes and prevent diabetes-related complications. Therefore, it is recommended that more studies be performed regarding the role of ferritin in the pre-diabetic stage and type 2 diabetics, preferably using a larger sample size.

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