Original Research Article

To Determine Serum Ferritin and Lipid Profile in Type 2 Diabetes Mellitus

Shilpa Sunil Shende[1*], Vishakha Vivek Mahajan[2], Arun Tadas[3], Mangesh Tekade[4]

[1]Assistant Professor, Indira Gandhi Government Medical College, Nagpur, Maharashtra, India
[2]Assistant Professor, Indira Gandhi Government Medical College, Nagpur, Maharashtra, India
[3]Professor and Head of the Department, Indira Gandhi Government Medical College, Nagpur, Maharashtra, India
[4]Assistant Professor, Indira Gandhi Government Medical College, Nagpur, Maharashtra, India

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Abstract

Background: Type 2 diabetes mellitus is a major growing health concern worldwide. In recent years much is talked about the role of serum ferritin, a marker of iron stores in the body in the development of type 2 diabetes mellitus and its complications. This study was carried out to assess the levels of serum ferritin in type 2 diabetes mellitus and to find its correlation with glycaemic control. **Method:** Total 188 subjects were enrolled in the study, including 94 clinically diagnosed patients of type 2 Diabetes Mellitus in the age group of 35-60 years and 94 normal age and sex matched non diabetic control. All subjects were clinically examined, had laboratory investigations including fasting and 2 hour post prandial blood glucose, HBA1c%, serum ferritin and lipid profile. Statistical analysis was carried by using student's 't' test and Pearson's correlation coefficient (r) was used to assess correlation between measured parameters. **Results:** The study showed that serum ferritin levels were significantly increased in diagnosed cases of type 2 diabetes mellitus as compared to age and gender matched healthy control (P = < 0.01). A strong positive correlation was observed between serum ferritin and HBA1c % (r= 0.78, P= <0.01). Serum total cholesterol, triglycerides, LDL-C and VLDL-C was significantly increased in cases as compared to control while serum HDL-C was significantly decreased in cases as compared to control. (P= <0.01) Significant positive correlation was observed between serum ferritin and parameters of lipid profile. **Conclusion:**The study showed increase serum ferritin levels and dyslipidaemia in diagnosed cases of diabetes mellitus as compared to healthy control which suggest that increase iron may contribute to pathogenesis and complications of diabetes mellitus. Our results provide evidence that serum ferritin levels are significantly associated with major lipid parameters and this association represents a cardiometabolic risk factor.

Key words: Diabetes mellitus, Glycated haemoglobin, fasting blood glucose, serum ferritin, lipid profile.

Introduction

Diabetes Mellitus is a major public health concern involving about 463 million population worldwide. It is a group of complex metabolic disorder involving interaction of both genetic and environmental factors and characterized by high blood glucose level over a prolonged period of time[1]. Early diagnosis and treatment of DM is of particular importance in the prevention of comorbidities and mortality.

Iron an essential trace mineral element is involved in many metabolic processes essential for life such as differentiation and growth of living cells, participates in electron transfer between cells, transport of oxygen to various parts of the body. However, iron also act as a strong pro-oxidant and tissue iron excess can generate hydroxyl radicals via Fenton reactions and the induced oxidative stress may impair insulin secretion and sensitivity[2]. Damage caused by iron also triggers the events of chronic diabetes complications such as dyslipidaemia, retinopathy, neuropathy, endothelial dysfunction[3,4].

The first and clearest evidence for a relation between iron and human diabetes came from clinical observations of individuals with pathologic iron overload in patients with hereditary hemochromatosis[5]. Ferritin, an ubiquitous intracellular protein that is key in the regulation of iron homeostasis, is an accepted biomarker to evaluate body iron stores[6]. Several epidemiological studies have demonstrated that systemic iron overload identified by elevated serum ferritin concentration may play role in pathogenesis and complications of diabetes mellitus[7,8].

*Correspondence
Dr. Shilpa Sunil Shende
Assistant Professor, Indira Gandhi Government Medical College, Nagpur, Maharashtra, India.

Although the exact mechanism of iron-induced diabetes is uncertain, it is likely to be mediated by β-cell failure, insulin resistance and hepatic dysfunction through oxidative damage[9]. A causative link of T2DM with iron overload is suggested by of the improvement in insulin sensitivity and insulin secretion with frequent blood donation and decreased iron stores[10,11].

Patients with type-2 diabetes have increased risk of cardiovascular disease associated with atherogenic abnormalities and dyslipidaemia. It is well established that dyslipidaemia is a major risk factor for macrovascular complications in patients with type-2 diabetes mellitus (T2DM). Dyslipidaemia in diabetes commonly manifests as raised low density lipoprotein cholesterol (LDL-C), decreased high-density lipoprotein cholesterol (HDL-C) levels, or elevated triglyceride (TG) levels[12]. Early detection and treatment of hyperlipidaemia in diabetic patients reduces the risk for cardiovascular and cerebrovascular diseases.

Hence this study was carried out to analyse the level of serum ferritin and lipid profile in Type 2 Diabetes Mellitus Patients and a group of healthy controls and to establish their correlation with glycaemic control in Type 2 Diabetes Mellitus patients.

Material and Method

The present case control study was conducted in Clinical Biochemistry Laboratory, Indira Gandhi Government Medical College and Hospital, Nagpur from march 2021 to Dec 2021. The study protocol was approved by the institutional ethical committee. An informed written consent was obtained from all the study subjects who were enrolled in the study.

Total 188 subjects were enrolled in the study, including 94 clinically diagnosed patients of type 2 Diabetes Mellitus in the age group of 35-60 years and 94 normal age and sex matched non diabetic control. The data on family history and personal history of diabetes, smoking

habits, alcohol consumption, dietary habits, recent blood donation, hypertension and treatment for any other diseases were collected through a standard questionnaire.

Inclusion criteria

Clinically diagnosed cases of type 2 Diabetes Mellitus of more than 6 months duration irrespective of gender between 35-60 years of age. Type 2 DM was diagnosed using American Diabetes Association criteria, fasting serum glucose (FSG) ≥126 mg/dl (normal value 70–110 mg/dl) or 2hour postprandial glucose (PPG) ≥200 mg/dl (normal value <140 mg/dl) with glycated haemoglobin level between 6.5% and 10.5%.

Exclusion criteria

Subjects with type 1 diabetes mellitus, pregnant women, heart disease, chronic disorders like Chronic kidney disease, Chronic liver disease and other states associated with altered serum ferritin like hemochromatosis, bleeding disorder, chronic alcoholics, history of repeated blood transfusions, iron deficiency anaemia were excluded from the study.

All subjects were clinically examined, and arterial blood pressure and body parameters, including height, weight and waist circumference, were measured. Body Mass Index (BMI) was calculated as weight in kilograms divided by squared height in meters (m2).

Laboratory Assays

Under all aseptic precautions 5 ml of blood samples were drawn in the morning after 10-12 hrs of fasting using standard venepuncture techniques and divided into a fluoride vial, sterile empty vial and an EDTA vial. EDTA vials are used for estimation of glycated Haemoglobin (HbA1c %). Samples for PPBS were collected after 2 hours of taking a meal. Samples were allowed to clot for 30 minutes and then centrifuged. The separated serum was analysed for the following biochemical parameters on fully automated biochemistry analyser XL-640 by using kits of Erba Diagnostic Ltd.

- Plasma fasting and post prandial blood glucose by GOD-POD method.
- Blood HbA1c by immunoterbidimetric method.
- Serum ferritin by Turbilatex method.
- Serum creatinine by Jaffes method
- Serum total cholesterol by enzymatic method
- Serum TG by enzymatic method
- Serum HDL Cholesterol by direct enzymatic method.
- Serum LDL Cholesterol by Friedwald formula
- Serum VLDL Cholesterol by Friedwald formula

For adequate quality control both normal, abnormal reference control solutions and calibrators were run before each batch.

Statistical analysis

All statistical analyses were performed by using SPSS Software. The data was expressed as Mean ± SD. Statistical analysis was carried by using student's 't' test and P<0.05 was considered as statistically significant. Pearson's correlation coefficient (r) was used to assess correlation between measured parameters.

Results

Table 1: Anthropometric parameters in cases and control

	Non-diabetic control N= 94 Mean ±SD	Cases with T2DM N= 94 Mean ±SD
Age in years	53.09 ± 6.14	55.51 ±6.38
Male	55 (58%)	58 (61%)
Female	39 (42%)	36 (39%)
BMI	25.02 ± 4.18	28.99 ±4.92

The demographic data does not reveal any difference between age and sex of study participants. The study included total 94 patients of type 2 DM with mean age of 55.51±6.38 & majority of patients were male (61% vs 39%). The mean age in non-diabetic healthy control group was 53.09 ± 6.14 having male predominance similar to cases (58% Vs 42%). The duration of diabetes was between 5-10 years in 38%, 10-15 years in 32% and more than 15 years in 30 % patients. Body mass index was significantly increased in cases as compared to control (P< 0.001).

Table 2: Biochemical parameters in cases and control

Biochemical Parameter	Non-diabetic control N= 94 Mean +SD	T2DM N= 94 Mean +SD	P value
Fasting blood glucose(mg/dl)	83.63 ±11.07	175.41±66.5	< 0.001
Post prandial blood glucose (mg/dl)	114.14 ±12.95	248.03 ±102.92	< 0.001
Glycated Haemoglobin (HbA1c %)	5.74 ± 0.52	8.03 ± 1.32	< 0.001
Serum ferritin	24.2 ±13.5	100.35 ±52.86	< 0.001
C- reactive protein	1.06 ±0.92	13.3±15.0	< 0.001
Serum creatinine (mg/dl)	0.6 ±0.2	2.2 ±1.53	< 0.001
Serum total cholesterol(mg/dl)	154 ±18.6	249.43 ±80.88	< 0.001
Serum Triglycerides(mg/dl)	119.04 ±26.8	238.04 ±90.25	< 0.001
Serum HDL (mg/dl)	51.33 ±5.1	41.15 ±3.86	< 0.001
Serum LDL (mg/dl)	79.06 ±18.6	160.66 ± 74.7	< 0.001
Serum VLDL (mg/dl)	23.80 ±5.3	47.06 ±18.05	< 0.001
LDL/HDL	1.55 ± 0.4	4.02 ±2.16	< 0.001

In our study, cases were selected on the basis of increased fasting blood glucose and HbA1c % level. Subjects with T2DM had significantly increased level of fasting and post prandial blood glucose as compared to non-diabetic control (P< 0.001). HbA1c % was also significantly increased in cases as compared to control showing poor glycaemic control in T2DM patients. Highly significant difference was observed in serum ferritin level in subjects with T2DM and control group (P< 0.001). Serum creatinine levels in T2DM subjects were significantly increased as compared to control (P< 0.001). we also observed significant increase in serum total cholesterol (TC), triglycerides (TG), LDL-cholesterol and VLDL-cholesterol level in T2DM cases as compared to control while serum HDL-cholesterol was significantly decreased in cases as compared to control (P< 0.001). LDL/HDL was significantly increased in cases as compared to control.

Table 3: Correlation between serum ferritin with glycaemic index and lipid profile

	r	P value
HBA1C%	0.780**	<0.001
FBG	0.679**	<0.001
PPBG	0.815**	<0.001
Total cholesterol	0.674**	<0.001
TG	0.884**	<0.001
LDL-C	0.554**	<0.001
VLDL-C	0.884**	<0.001
HDL-C	-0.760**	<0.001

In our study positive correlation was observed between HbA1c levels and serum ferritin levels, with a highly significant p value (r =0.84, p < 0.001). Similarly, serum ferritin levels were positively and significantly correlated with fasting and post prandial blood glucose levels (r =0.67and r =0.81). Another correlation study revealed significant positive correlations between serum ferritin levels and parameters of lipid profile such as serum cholesterol, triglycerides, LDL-cholesterol and VLDL-cholesterol and significant negative correlation was observed between serum ferritin levels and HDL-Cholesterol level

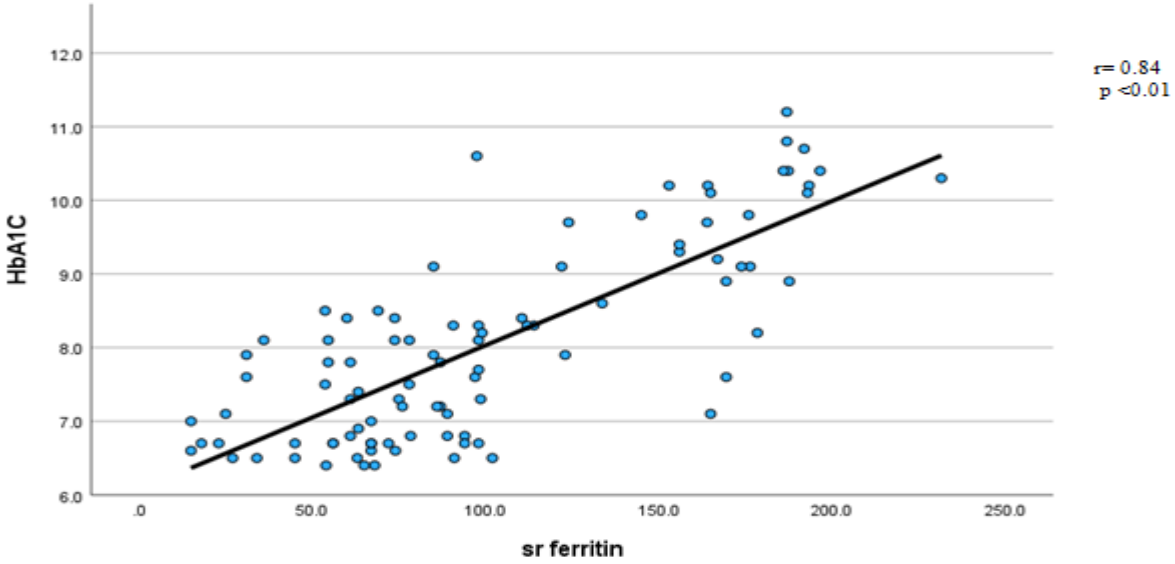

Fig 1: Correlation of HbA1C with serum ferritin

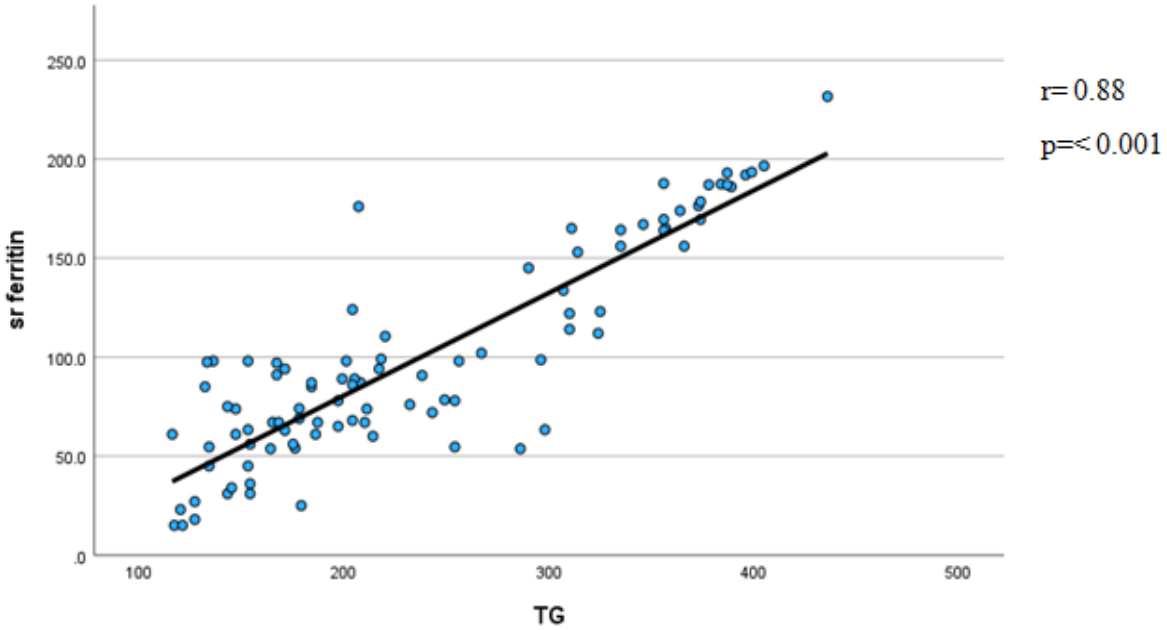

Fig 2: Correlation of serum ferritin with TG

Discussion

In the present study carried out in diagnosed cases of type 2 diabetic mellitus, it is observed that the mean HbA1c % in the diabetic cases is 8.03 ±1.32 and in the control group it was found to be 5.74 ± 0.52%. Statistically, this difference was found to be highly significant ($p < 0.001$). This is consistent with the observations made by Trivelli LA et al, who observed that HbA1c% was a highly significant indicator of diabetes[13].

In the present study we estimated the level of serum ferritin as a reflector of body iron status. In our study, serum ferritin level was significantly increased in patients with T2DM as compared to healthy control ($p < 0.001$). This finding is consistent with the previous studies performed by Ford etal[2] and similar other studies[14,15].

In several epidemiological studies increased iron stores predicted the development of diabetes while decrease in iron is protective. Although the exact mechanism for association of elevated serum ferritin with type 2 diabetes mellitus remains unclear, there are number of prevailing theories. Iron overload is believed to be associated with insulin resistance. Iron deposition in the liver may cause insulin resistance by interfering with the ability of insulin to suppress hepatic glucose production[16]. In addition, iron is a transition metal capable of causing oxidative stress-induced tissue damage by catalysing the conversion of hydrogen peroxide to free radicals that attack cellular membranes, proteins, and DNA[17,18], leading to derangements in glucose homeostasis such as insulin resistance and pancreatic β-cell dysfunction through iron toxicity[19,20]. Furthermore, reactive oxygen species interfere with insulin signalling 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.

Fernandez etal studied the effect of ferritin reduction by bloodletting on insulin sensitivity and HBA1c levels in diabetic patient. In this study positive effect of ferritin reduction on blood glucose control was used for the confirmation of probable role of ferritin in pathogenesis of diabetes mellitus[21].

Liu B W[22] and Koo etal[23] concluded that elevated serum ferritin levels are associated with insulin resistance and may not be associated with the decline in beta cells of pancreas. Smotra and Kudyar reported that increased ferritin levels had positive correlation with serum insulin level[24] which suggest the association of serum ferritin with insulin resistance in patients with T2DM.

In our study serum ferritin level was significantly correlated with HBA1c%, fasting and post prandial blood glucose levels. These results are consistent with the findings from the study conducted by Borah etal[25], Momeni Aetal[26], AK Al-Miraj[27]. However, our results contradict the findings by Sharifi and Sazandeh[28], Pramiladevietal[29] who did not observe a correlation between serum ferritin and HbA1c levels in patients with T2DM and normal controls.

It is recognized that dyslipidemia is an independent risk factor for cardiovascular disease. Elevated blood glucose level along with dyslipidemia increases atherosclerosis related inflammation and makes person more prone for cardiovascular disease[30]. Chronic oxidative stress caused by increased iron is associated with disruption of mitochondrial β-oxidation of long chain fatty acids and β-cell dysfunction in the pancreas[31]. Abnormal mitochondrial β-oxidation leads to hypertriglyceridemia, and excessive triglyceride accumulation in muscle and liver tissue[32].

Present study shows dyslipidemia in T2DM patients as compared to healthy control. Serum total cholesterol, Triglycerides, VLDL and LDL cholesterol were significantly increased in cases as compared to control ($p < 0.01$). serum HDL cholesterol was significantly decreased in cases as compared to control ($p < 0.001$). LDL/HDL ratio was also significantly increased in cases as compared to healthy control ($p < 0.001$) which suggest increased cardiovascular risk in T2DM patients. These findings are in agreement with the Framingham heart study[33] and similar other studies[34,35].

We observed significant positive correlation between serum ferritin and parameters of lipid profile such as serum total cholesterol, Triglycerides, VLDL and LDL cholesterol and negative correlation with HDL cholesterol (table no.). These findings are in accordance with the findings of previous studies[36,37].

A large cross-sectional study (China Health and Nutrition Survey 2009) found that elevated serum ferritin levels were associated with the prevalence of hyperlipidemia among Chinese adults. This study also showed that subjects with hyperlipidemia and diabetes had higher serum ferritin levels than subjects without hyperlipidemia and diabetes. [38] These results are in agreement with the results of correlation analysis conducted as part of our study.

Conclusion

- The study showed increase serum ferritin levels and dyslipidemia in diagnosed cases of diabetes mellitus as compared to healthy control which suggest that increase iron may contribute to pathogenesis and complications of diabetes mellitus.
- There is significant positive correlation between serum ferritin and HBA1c in patients with T2DM which shows positive association of serum ferritin with glycemic control.
- Our results provide evidence that serum ferritin levels are significantly associated with major lipid parameters and this association represents a cardiometabolic risk factor.
- Hence routine estimation of serum ferritin can help in early detection of patients prone for diabetes and early intervention in these patients helps in prevention of comorbiditits.

Further large-scale studies are needed to verify the importance of screening of hyperferritinemia in pre-diabetics and to define cut off level of serum ferritin for possible early detection of type 2 diabetes mellitus and prevention of its complications.

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