Original Research Article

Evaluation of serum ferritin in in type II diabetes mellitus: a hospital based observational study from Dibrugarh, Assam, India

Meghna Borah[1]*, Rohini K. Goswami[2]

[1]Department of Biochemistry, North Eastern Indira Gandhi Regional Institute of Health and Medical Sciences, Mawdiangdiang, Shillong, Meghalaya-793018, India
[2]Department of Biochemistry, Assam Medical College and Hospital, Dibrugarh, Assam, India

*Correspondence:
Dr. Meghna Borah,
E-mail: drmeghnab@gmail.com

ABSTRACT

Background: Increased serum ferritin, reflecting body iron overload, is often associated with insulin resistance. The role of iron in the pathogenesis of diabetes is suggested by an increased incidence of type 2 diabetes mellitus in diverse causes of iron overload, and reversal or improvement in glycemic control) with a reduction in iron load achieved using either phlebotomy or iron chelation therapy.
Methods: This study was carried out to assess Serum Ferritin levels in Type 2 diabetes mellitus and to examine whether a correlation between Serum ferritin and HbA1c% and fasting blood glucose levels exists. 92 type 2 diabetes subjects (M:F - 52:40) were studied and compared with age and gender matched controls.
Results: The study showed that serum ferritin levels was significantly increased in diagnosed cases of type 2 diabetes mellitus in comparison with the age and gender matched healthy individuals (p value<0.01). A strong positive correlation was found between HbA1c% and serum ferritin levels and the correlation was found to be statistically significant (p<0.01). A strong positive correlation was also found between serum ferritin and fasting blood glucose levels
Conclusions: Therefore, the findings in the present study indicates that serum ferritin was increased in diabetes as long as glycemic control was not achieved and that this increase may contribute to the pathogenesis of this disease as well as in the development of complications. Thus, routine screening for serum ferritin concentration in pre-diabetes and diabetic patients can be done to assess the body iron stores.

Keywords: Diabetes mellitus, Glycated hemoglobin, Iron overload, Serum ferritin

INTRODUCTION

The explosive increase of diabetes mellitus, a chronic metabolic and endocrine disease worldwide is a major public health concern both in developing and developed countries. Diabetes mellitus (DM), one of the most prevalent endocrine disorders in the world, has now reached the proportions of a global pandemic. India, a forerunner in this regard, has a very high number of diabetics and has earned the dubious distinction of being the diabetes capital of the world.

The earliest evidence that systemic iron overload could contribute to abnormal glucose metabolism was that patients with classic hereditary hemochromatosis (HH) had increased frequency of diabetes mellitus.

However, with the discovery of novel disorders of iron metabolism, it is obvious that iron overload, irrespective of the cause, results in an increased incidence of type 2 diabetes. The role of iron in the pathogenesis of diabetes is suggested by 1) an increased incidence of type 2 diabetes in diverse causes of iron overload and 2) reversal

or improvement in diabetes (glycemic control) with a reduction in iron load achieved using either phlebotomy or iron chelation therapy. A causative link with iron overload is suggested by of the improvement in insulin sensitivity and insulin secretion with frequent blood donation and decreased iron stores.[1,2]

Recent studies have demonstrated that increased body iron stores are associated with the development of glucose intolerance, gestational diabetes, type-2 DM and insulin resistance syndrome.[3-7] Phlebotomy is followed by drop in serum glucose, cholesterol, triglycerides and improvement in both beta cell secretion and peripheral insulin action in type-2 DM.[7,8] Epidemiological studies also indicate the same correlation.[9,10] Patients with DM with increased glucose levels have also found to have increased ferritin levels which co-relates with complications of diabetes like retinopathy, nephropathy and vascular dysfunction.[11-13]

Hence this study was carried out to examine the relationship between serum ferritin and type 2 diabetes mellitus and to establish a correlation between serum ferritin and Fasting blood glucose (FBG) as well as with Glycated hemoglobin (HbA1c%).

METHODS

The present observational study was conducted for a period of one year in the Department of Biochemistry and Advanced Clinical Biochemistry Laboratory, Assam Medical College and Hospital, Dibrugarh. Ethical clearance from Institutional Ethical Committee and informed consent from patients and controls was taken. Out of a total of 132 diabetic patients, only 92 (52 males and 40 females) were included considering all relevant inclusion and exclusion criteria.

These patients were compared with an equal number of age and gender matched controls selected randomly. To find out the influence of body iron stores on various biochemical parameters, both diabetics and controls underwent the following biochemical investigations: Serum Ferritin levels, fasting and postprandial blood glucose, and glycosylated Hemoglobin (Hb) levels using standard methods.

Serum ferritin was measured by MAG-16 kit, which is a immunoradiometric assay kit of the sandwich type based on two monoclonal antiferritin antibodies: one 125I-labelled anti-ferritin antibody in liquid phase and other monoclonal antiferritin antibody is coupled to magnetizable cellulose.

Blood glucose was determined by the GOD-POD method (glucose oxidase peroxidase method, Randox reagent) and glycosylated hemoglobin by the Ion-Exchange Resin method (Tulip group). Patients were evaluated with detailed history, meticulous examination and laboratory investigations. Since serum ferritin is an acute phase reactant and may be elevated in presence of inflammation, an attempt was made to minimize this potential source of confounding by excluding those patients with suspected infection, inflammation and liver disease and with positive CRP levels. Most of the patients were selected when they had come for treatment for diabetes.

Patients receiving iron supplements, patients with anemia (Hemoglobin levels less than 12g/dl in women and less than 13g/dl in males) or receiving treatment for anemia in the past three months, patients with history of blood donation in the last three months, with diagnosed type 1 diabetes mellitus, patients who do not give consent to the study, pregnant women, patients with hepatic disorders, renal disorders, malignancies, acute infections, fever, myocardial infarction, bleeding disorders or on medication with possible influence on serum ferritin levels were excluded from the study.

Under all aseptic and antiseptic conditions 5 ml of blood sample was collected from each subject from a suitable peripheral vein (preferably antecubital vein) by venipuncture using a sterile disposable syringe and divided into a sterile empty vial and an EDTA vial. EDTA vials are used for estimation of glycated hemoglobin. The rest of the sample was then allowed to stand for some time and then centrifuged for separation of serum. This serum was used for estimation of the other parameters.

Statistical analysis

Arithmetic mean and standard deviation were worked out to assess the levels of various parameters in both groups under study. Students 't' test was used for comparison of quantitative variables. Co-relation between serum ferritin and HbA1c% in patients and co-relation between serum ferritin and fasting blood glucose levels was evaluated using Pearson Co-relation Co-efficient. All tests were considered statistically significant if the p-value was <0.05.

RESULTS

In the present study carried out in diagnosed cases of type 2 diabetic mellitus, the youngest case study was found to be 30 years of age, and the oldest to be 78 years. The highest prevalence was found in the age group 41-50 years (28%) followed by the age group of 51-60 (24%). It was observed that 56.5% of the cases are male and 44.5% female with a male female ratio of 1.3:1.

The age of onset of diabetes in 78% of patients was between 40 and 59 years. 76% patients were on oral hypoglycemic agents and 21% on insulin therapy and remaining (2%) were on diet control. The duration of diabetes was between 5- 10 years in 35% and 10-15 years in 37% more than 15 years were 20 %. 8% of patients were diagnosed with diabetes mellitus in the last five

years. In the present study it was observed that the mean serum ferritin level in males with type 2 diabetes was 147.76±82.66 ng/mL, and that in females cases, 153.93±63.11 ng/mL. Here, the difference between male and female cases is very minimal and is statistically not

significant (p>0.05). In control group also, the difference between male and female cases is very minimal and is statistically not significant (p>0.05). The above findings indicate the serum ferritin levels are independent of the gender of the study groups.

Table 1: Comparison of means of the different anthropometric, clinical and biochemical characteristics between the test and control groups.

Parameters	Control group	Diabetic group	p value
Age (years)	53.72±10.17	54.14±9.76	>0.05
BMI (Kg/m2)	23.42±2.246	25.589±2.112	<0.01
FBG (mg/dL)	84.98±11.45	168±65.01	<0.01
PPBG (mg/dL)	117.30±14.41	270.08±98.62	<0.01
B.Urea (mg/dL)	23.54±7.93	24.35±7.64	0.60
S.Creatinine(mg/dL)	0.98±0.17	1.01±0.19	0.48
Glycated haemoglobin (%)	5.19±0.61	9.00±2.08	<0.01
Serum ferritin (ng/mL)	47.42±21.71	150.35±74.43	<0.01

Table 2: Mean and standard deviation of serum ferritin in different levels of HbA1 C%.

HbA1C Range (%)	No.	Mean±SD	
		HbA1C%	Ferritin (ng/mL)
6.00-7.50	24	6.64±0.44	89.15±45.34
7.51-9.00	30	8.28±0.45	114.02±18.58
9.01-10.50	18	9.81±0.52	179.17±37.10
≥ 10.51	20	12.11±1.02	249.32±8.50

The mean level of serum ferritin in diabetic cases was found to be 150.35±74.43 ng/mL and in the control group it was found to be 47.42±21.71 ng/mL.

The serum ferritin levels in the diabetic cases was found to be higher than that in the control group and it was statistically highly significant (p<0.01) (Table 1).

Table 3: Correlation between glycated hemoglobin (HbA1C%) and serum ferritin levels in diabetic cases.

HbA1C range%	Serum ferritin	
	r value	p value
6.00-7.50	0.62	<0.05
7.51-9.00	0.55	<0.05
9.01-10.50	0.83	<0.01
≥ 10.51	0.73	<0.05
Overall	0.89	<0.01

In the present study, in cases with type 2 diabetes mellitus it was seen that the highest serum ferritin levels (i.e. 249.32±8.50 ng/mL) were found in the cases with the highest HbA1C % levels of >10.51%. On the contrary, cases with the HbA1C % range of 6.00-7.50 % had the lowest serum ferritin levels of 89.15±45.34 ng/mL.

Hence, the cases with a higher HbA1c% had a higher serum ferritin level (Table 2).

It was also observed in the present study that serum ferritin levels showed a positive correlation with HbA1c%, that was statistically highly significant (p<0.01). The Pearson correlation coefficient "r" found to be 0.89 established the strong positive correlation between the two parameters (Table 3).
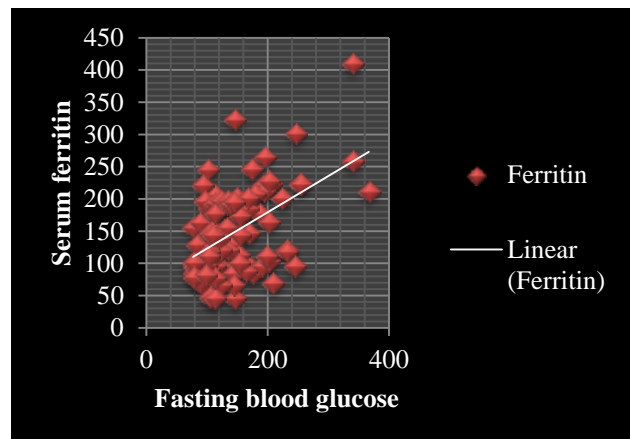

Figure 1: Correlation of fasting blood glucose with serum ferritin in diabetic cases.

Regression analysis

In the equation $y = a + bx$

Taking y = serum ferritin level and x = HbA_{1c} %

Regression analysis revealed that a= (-137.10) and b= 31.93

Now, the equation becomes,

Serum ferritin level = (-137.10) + 31.93 x HbA_{1c}%

Thus, the serum ferritin level can be calculated from the HbA1c %. It was also observed that serum ferritin levels showed a positive correlation with fasting blood glucose level, that was statistically highly significant (p<0.01). The Pearson correlation coefficient "r" found to be 0.75 established the strong positive correlation between the two parameters (Figure 1).

DISCUSSION

In the present study carried out in diagnosed cases of type 2 diabetic mellitus, the highest prevalence was found in the age group 41-50 years (28%) followed by the age group of 51-60 (24%). Similar findings were also observed Scott and Fischer, Mc Nair et al, and Yoon.[14-16] They found the highest prevalence of type 2 DM in 41-50 years of age.

In the present study it was also seen that there was a slight male preponderance in cases of diabetes mellitus with a male: female ratio of 1.27:1. WHO Expert Committee on diabetes mellitus in the second report in 1980 mentioned that there is a slight male preponderance in south East Asian races. Caixas et al in their study of 60 patients had observed a male to female ratio of 1.14:1. Shah S.K et al reported the similar findings in the state of Assam. Recently a study carried out by Khalid A et al also revealed a male preponderance of diabetes mellitus.[17-19]

It is also observed in the present study that the mean HbA1c % in the diabetic cases is 9.00±2.08 % and in the control group it was found to be 5.19±0.61 %. Statistically, this difference was found to be highly significant (p<0.01). This is almost consistent with the observations made by Trivelli LA et al, who observed that HbA1c% was a highly significant indicator of diabetes and that the highest values for HbA1c% were found in those diabetics who had the poorest control.[20]

Koenig RJ et al also reported increased concentration of HbA1c% in diabetic cases as compared to controls and concluded that HbA1c% probably reflects the mean daily blood glucose concentration and the degree of carbohydrate imbalance better than fasting blood glucose levels and glucose tolerance tests and might provide a better index of glycaemic control in diabetic cases

without resorting to a glucose loading procedure.[21] Nathan DM et al concluded that the glycosylated hemoglobin assay provides information about the degree of long-term glucose control that is not otherwise obtainable in the usual clinical setting.[13]

The serum ferritin levels in the diabetic cases was found to be higher than that in the control group and it was statistically highly significant (p<0.01). Similar findings were obtained by Ford et al.[10] They had examined the cross-sectional associations among ferritin concentration, glucose tolerance status, and concentrations of insulin, glucose, and glycosylated hemoglobin in 9,486 U.S. adults aged greater than or equal to 20 years from the third national health and nutrition examination survey (1988-1994).

All multiple linear regression coefficients between ferritin concentration and concentrations of insulin, glucose, and glycosylated hemoglobin were found to be positive and significant for both men and women. The workers in this study found that ferritin concentration and the percentage of individuals with elevated ferritin concentrations were lowest for individuals without diabetes, somewhat higher for individuals with impaired fasting glucose and highest for individuals with diabetes.

A study carried out in Korea University Hospital from 1997 to 1998 by Kim et al showed that the value of serum ferritin was higher in the type 2 diabetes patients than the control subjects.[22] They concluded that serum ferritin can be employed as a marker of not only glucose homeostasis but also insulin resistance both in type 2 diabetic and control subjects.

In a study carried out by Smotra S, et al in a tertiary care hospital in North India found that in those with increased level of Serum Ferritin, more number of patients had poor glycemic control reflected by higher levels of HbA1c % as compared to those with normal levels and was found to be statistically significant (p<0.05).[23] It was surmised from the study that increased Serum Ferritin levels are associated insulin resistance, poor glycemic control and also associated with complications of type-2 DM like nephropathy, retinopathy, neuropathy and hypertension.

A study carried out by Jiang et al found that the mean ferritin concentration was significantly higher (P<0.001) in the cases than in the healthy controls.[24] Because ferritin concentration reflects both the storage of iron and acute-phase inflammation, the workers further adjusted for CRP to reduce potential confounding by inflammation.

The associations between ferritin concentration and diabetes risk remained virtually unchanged. Similar findings were reported by Kaye et al, Gallou G et al, Canturk Z et al.[11,12,25] These studies revealed hyperferrtinemia in poorly controlled diabetic cases. The findings in the present study were consistent with the

reports of the above workers. It was also observed in the present study that serum ferritin levels show a positive correlation with HbA1c%, that is statistically highly significant (p<0.001). The Pearson correlation coefficient "r" found to be 0.89 establishes the strong positive correlation between the two parameters. Serum ferritin levels also had a positive correlation with fasting blood glucose levels with a Pearson correlation coefficient "r" of 0.75

Eschwege et al also reported similar findings in their study.[26] The workers in this study reported a positive correlation between increased serum ferritin levels and poor glycemic control, reflected by higher HbA1c% in diabetic subjects. Similar findings were also reported by Ford and his colleagues who found that ferritin concentration was positively and significantly related to concentrations of insulin, glycosylated hemoglobin, and glucose among all the study participants.[10]

Canturk Z et al also confirmed in their studies that poorly controlled diabetes patients, reflected by higher HbA1c%, had hyperferritinemia.[25] This showed that serum ferritin was increased in diabetes as long as glycemic control was not achieved. In a study carried out by Smotra S et al that those with increased level of Serum Ferritin, more number of patients had poor glycemic control reflected by higher levels of HbA1c % as compared to those with normal levels and was found to be statistically significant (p<0.05).[23]

Although the exact mechanism for association of elevated serum ferritin with type 2 diabetes mellitus is yet to be established, there are a 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. Pancreatic damage due to some degree of subclinical hemochromatosis has been considered at least in some cases of diabetes.[11]

Iron is auto-oxidized to form highly reactive, lipid-soluble iron–oxygen complexes. These free radicals are powerful pro-oxidants, which can change membrane properties and result in tissue damage.[27,28]

Oxidative stress can also lead to hyperglycemia through disturbed glucose metabolism. Conversely, insulin stimulates cellular iron uptake through increased transferrin receptor externalization Insulin resistance coupled with poor glycemic control can also increase ferritin levels.[2] Thus, insulin and iron can mutually potentiate their effects leading after a vicious cycle to insulin resistance and diabetes.

CONCLUSION

The study showed that serum ferritin levels was significantly increased in diagnosed cases of type 2 diabetes mellitus in comparison with the age and gender matched healthy individuals. Moreover it was seen that the poorly controlled diabetic patients, reflected by higher HbA1c % had significant hyperferritinemia. Therefore, the findings in the present study indicates that serum ferritin was increased in diabetes and this increased ferritin levels positively correlated with poor glycemic control, as reflected by elevated HbA1c%.

It is surmised that this increase may contribute to the pathogenesis of this disease as well as in the development of complications. In summary, there is suggestive evidence that iron plays a pathogenic role in diabetes and its complications such as microangiopathy and atherosclerosis. 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 to prevent diabetes-related complications.

To conclude, the major issue arises whether to estimate Serum ferritin levels routinely in all type 2 diabetes patients and whether to set a cutoff value of serum ferritin for good glycemic control. In a nation like India where anemia is prevalent and recognized, there is often widespread and injudicious dispensing of Iron and Folic acid tablets, irrespective of the hemoglobin status of the person even by grass root level workers. In a person with preexisting type 2 DM, this might hamper an already critical situation if it leads to iron overload. Though our study is a pointer in this direction, yet elaborate studies would help in shedding greater light in this aspect.

REFERENCES

1. Jiang R, Ma J, Ascherio A, Stampfer MJ, Willett WC, Hu FB. Dietary iron intake and blood donations in relation to risk of type 2 diabetes in men: a prospective cohort study. Am J Clin Nutr. 2004;79:70-5.
2. Fernandez-Real JM, Lopez-Bermejo A, Ricart W. Iron stores, blood donation, and insulin sensitivity and secretion. Clin Chem. 2005;51:1201-5.
3. Fernandez-Real JM, Lopez-Bermejo A, Ricart. Cross talk between iron metabolism and diabetes. Diabetes. 2002;51:2348-54.
4. Dmochowski K, Finegood DT, Francombe W, Tyler B, Zinman B. Factors determining glucose tolerance in patients with thalassemia major. J Clin Endocrinol Metab. 1993;77:478-83.
5. Cavallo-Perin P, Pacini G, Cerutti F, Bessone A, Condo C, Sacchetti L, et al. Insulin resistance and hyperinsulinemia in homozygous ß-thalassemia. Metabolism. 1995;44:281-6.

6. Wrede CE, Buettner R, Bollheimer LC, Scholmerich J, Palitzsch KD, Hellerbrand C. Association between serum ferritin and the insulin resistance syndrome in a representative population. Eur J Endocrinol. 2006;154(2):333-40.
7. Suvarna J, Ingle H, Deshmukh CT. Insulin resistance and beta cell function in chronically transfused patients of thalassemia major. Ind Pediatr. 2006;43:393-400.
8. Ascherio A, Rimm EB. Blood Donation and risk of coronary heart disease in men. Circulation. 2001;103:52-7.
9. Dymock IW, Cassar J, Pyke DA, Oakley WG. Observations on the pathogenesis, complications and treatment of diabetes in 115 cases of haemochromatosis. Am J Med. 1972;52:203-10.
10. Facchini FS. Effect of phlebotomy on plasma glucose and insulin concentrations. Diabetes Care 1998;21:2190.
11. Rajpathak S, Ma J, Manson J, Willett WC, Hu FB. Iron intake and the risk of type 2 diabetes in women: a prospective cohort study. Diabetes Care. 2006;29:1370-6.
12. Jiang R Ma J, Ascherio A, Stampfer MJ, Willett WC, Hu FB. Dietary iron intake and blood donations in relation to risk of type 2 diabetes in men: a prospective cohort study. Am J Clin Nutr. 2004;79:70-5.
13. Canturk Z, Cetinarslan B, Tarkun I. Serum ferritin levels in poorly- and well-controlled diabetes mellitus. Endocr Res. 2003;29:299-306.
14. Scott DA, Fisher AM. The insulin and the zinc content of normal and diabetic pancreas. J Clin Invest. 1938;17(6):725-8.
15. McNair P, Christiansen C, Christensen MS, Madsbad S, Faber OK. Development of bone mineral loss in insulin-treated diabetes: a 1 1/2 years follow-up study in sixty patients. Eur J Clin Invest. 1981;11(1):55-9.
16. Yoon KH, Kim HS. A short message service by cellular phone in type2 diabetic patients for 12 months. Diabetes Res Clin Pract. 2008;79(2):256-61.
17. Caixas A, Pérez A, Qrdóñez-Llanos J, Bonet R, Rigla M, Castellví A, et al. Lack of Change of Lipoprotein(a) Levels by the Optimization of Glycemic Control With Insulin Therapy in NIDDM Patients. Diabetes Care. 1997;20(9):1459-61.
18. Shah SK, Saikia M, Burman NN, Snehalatha C, Ramachandran A. High prevalence of type 2 diabetes in urban population in north eastern India. Int J Diab in Developing Countries. 1999;19:144-7.
19. Khalid A. Alqurashi, Khalid S. Aljabri, and Samia A. Bokhari. Prevalence of diabetes mellitus in a Saudi community. Ann Saudi Med. 2011;31(1):19-20.
20. Trivelli LA, Ramsey HM, Lai HT. Hemoglobin components in patients with diabetes mellitus. New Eng.J.Med. 1971;284:353-7.
21. Koenig RJ, Peterson CM, Kilocharles, Jones RL, Saudek C, Lehrman M, et al. Correlation of glucose regulation and hemoglobin A1c in diabetes mellitus. N Engl J Med. 1976;295:417-20.
22. Kim NH, Oh JH, Choi KM, Kim YH, Baik SH, Choi DS, et al. Serum ferritin in healthy subjects and type 2 diabetes mellitus. Yonsei Med J. 2000;41(3):387-92.
23. Smotra S, Kudyar R.P. Relationship between Serum Ferritin and Type-2 Diabetes Mellitus. JK Science. 2008;10(4):170-4.
24. Jiang R, Manson JE, Meigs JB, Ma J, Rifai N, Hu FB. Body iron stores in relation to risk of type 2 diabetes in apparently healthy women. JAMA. 2004;291(6):711-7.
25. Canturk KZ, Cetinarslay B, Tarkun I, Canturk NZ. Serum ferritin levels in poorly- and well-controlled diabetes mellitus. Endocr Res. 2003;29:299-306.
26. Eschwege E, Saddi R, Wacjman H, Levy R, Thibult N, Duchateau A. Haemoglobin AIc in patients on venesection therapy for haemochromatosis. Diabete Metab. 1982;8:137-40.
27. Oberley LW. Free radicals and diabetes. Free Radic Biol Med. 1988;5(2):113-24.
28. Wolff SP. Diabetes mellitus and free radicals. Free radicals, transition metals and oxidative stress in the aetiology of diabetes mellitus and complications. Br Med Bull. 1993;49(3):642-52.