International Journal of Diabetes in Developing Countries (April–June 2019) 39(2):362–368
https://doi.org/10.1007/s13410-018-0687-1

ORIGINAL ARTICLE

Ferritin and serum iron as surrogate markers of poor glycemic control and microvascular complications in type 2 diabetes mellitus

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Received: 27 March 2018 / Accepted: 6 August 2018 / Published online: 4 September 2018

Abstract

This study was designed to find the correlation of iron indices with HbA1c levels and microvascular complications among patients with type 2 DM. Results were consistent with our hypothesis that high iron indices (serum iron and serum ferritin) not high enough to cause hemochromatosis but were still associated with poor glycemic control and its complications. The mean age of study group was 58.57 ± 3.17 years; whereas, the mean age of control group was 53.95 ± 4.43. The mean HbA1c of study group was 9.46 ± 1.31; whereas, the mean HbA1c of control group was 6.42 ± 0.28. The duration of diabetes in study group was 9.69 ± 2.69 years; whereas, it is 5.26 ± 2.81 years in control group. The mean serum iron level in study group was 155.08 ± 22.13 μg/dl; whereas, it is 88.81 ± 38.04 μg/dl in control group. The mean serum ferritin level in study group was 284.79 ± 50.06 ng/ml; whereas, it is 181.31 ± 54.08 ng/ml in control group. The mean serum transferrin saturation in study group was 30.25 ± 9.94; whereas, it is 28.92 ± 6.03 in control group. Out of the 100 patients in the study group, 40 patients had nephropathy, 33 patients had retinopathy, and 31 patients had neuropathy. In 100 patients in the control group, 12 patients had nephropathy, 11 patients had retinopathy, and 12 patients had neuropathy. On applying Pearson's coefficient of correlation, a moderately significant correlation was obtained between serum iron, ferritin, and HbA1c in study group. However, no significant correlation was obtained with transferrin saturation. On applying regression analysis among HbA1c, serum ferritin, and serum iron, it was observed (the sum of squares of the group was 30.5) that variation is not due to chance.

Keywords Microvascular complications · Serum iron · Ferritin · Surrogate markers

Introduction

We have come a long way as far as our understanding about pathophysiology of type 2 diabetes is concerned from the core defects of impaired insulin secretion from β cells of pancreas and increasing peripheral insulin resistance to De Fronzo's famous ominous octet [1] and further to "Dirty Dozen" [2] involving a delayed incretin response from the gut, increased glucagon production from the α cells as well as insulin resistance in the brain and increased glucose reabsorption in the kidneys all playing a crucial role in worsening of hyperglycemia.

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The fact that the frequency of diabetes is increased in classic hereditary hemochromatosis gives us the clue that systemic iron overload could contribute to abnormal glucose metabolism [3].

The contribution of iron in the pathogenesis of diabetes is suggested by the following: (1) diverse causes of iron overload have been found to be associated with an increased incidence of type 2 diabetes and (2) improvement in glycemic burden with a reduction in iron load was achieved using either phlebotomy or iron chelation therapy.

It is recognized recently that increased body stores of iron have association with the development of insulin resistance syndrome, glucose intolerance, gestational diabetes, and type 2 DM [4–8]. Reduction in iron stores by repeated blood donations leads to decrease in postprandial hyperinsulinemia and improvement in insulin sensitivity [9]. Phlebotomy leads to fall in serum glucose, cholesterol, triglycerides, and improvement in both beta cell secretion

as well as peripheral insulin action in type 2 DM [10, 11]. Epidemiological studies also suggest the same correlation [12, 13]

Recent in vitro studies have shown that H-ferritin mRNA is four- to eightfold higher in rat islets treated with 20 mmol/l glucose than in islets treated with 1 mmol/l glucose [14]. The fact that beta cells are particularly sensitive to oxygen radicals and ferritin has some potential to exhibit antioxidant properties explains increased levels of ferritin in beta cells [15].

Study patients of DM have hyperferritinemia which corelates with diabetic retinopathy, diabetic nephropathy, and vascular dysfunction [16–18]. It is important to realize that raised levels of iron above physiological requirement do not seem to serve any useful purpose in these patients. Although few indirect evidences from the western region do suggest that iron overload influences DM in a negative way, but overall there is paucity of literature especially from our country showing any direct evidence between increased iron load and control of diabetes mellitus. Moreover, finding out such correlation in Indian population carries great clinical significance as anemia has very high prevalence in Indian population, and continuous efforts are being made at physician, community, and government level to prevent and treat anemia which might influence the coexisting diabetic state. Hence, the present comprehensive study was planned to find out the relationship between iron indices (serum iron, serum ferritin, and transferrin saturation) with HbA1c in type 2 DM and to study the influence of body iron stores on diabetic microvascular complications in patients coming to OPD and IPD of our hospital.

Aims and objectives

1. To study the correlation between serum ferritin, serum free iron, and transferrin saturation with HbA1c in patients of type 2 diabetes mellitus
2. To study the correlation between serum ferritin, serum free iron, and transferrin saturation with microvascular complications (neuropathy, nephropathy, retinopathy) in patients of type 2 diabetes mellitus

Sample size of 200 was calculated using the epi-info statistical software for cross-sectional comparative study. Taking reference from Mukesh Gohel study [19], using proportion of patients in each group with raised serum iron level, the sample size came out to be 84 in each group at 5% precision, but taking attrition into consideration, we took 100 patients in each group.

One hundred patients of type 2 diabetes mellitus with HbA1c greater than seven constituted study population. One hundred patients of type 2 diabetes mellitus with HbA1c less than seven constituted control population. Study duration was from March 2014 to November 2015.

Blood investigations

1. Glycated hemoglobin (normal range, < 6.5) was estimated by high performance liquid chromatography (HPLC).
2. Serum iron (normal range, 41–130 μg/dl) was estimated by *Bathophenthroline disulphonate assay* (BPS).
3. Transferrin saturation (normal range, 16–45%) was estimated by photometric color method.
4. Serum ferritin (normal range, males 29–248 μg/dl and females 10–150 μg/dl) was measured by CLIA (chemiluminescence immuno assay).

Tests for Micro vascular complications

Retinopathy was screened by fundus examination.

Nephropathy screening was done by *urine for albuminuria*. Patients were taken as positive for microalbumin (a ratio of albumin (mcg/l) to creatinine (mg/l) of less than 30 was taken as normal; a ratio of 30–300 signified microalbuminuria) if found in this range on two occasions separated by 3 months.

Neuropathy screening was done by bed side criteria which have been validated by Chawla et al. [20] using the following:

A) NSS (neuropathy symptom score)
B) NDS (neuropathy disability score)

A) *Diabetic neuropathy symptom score* (*NSS*): The questions should be answered "yes" (positive, 1 point) if a symptom (as mentioned in the table below) occurred more than two times a week during the last 2 weeks or "no" (negative, no point) if it did not.

Symptoms	No	Yes
Unsteadiness in walking	0	1
Burning feet	0	1
Numbness or tingling of hands and/or feet	0	1
Fatigue, cramping, aching, or nocturnal exacerbations	0	2

B) *NDS* (*neuropathy disability score*) is calculated as mentioned below.

Neuropathy disability score (NDS)

Vibration perception threshold using 128 Hz tuning fork Normal = can distinguish vibration	Normal = 0 Abnormal = 1	Right foot	Left foot
Temp perception on dorsum of foot Using cup full of cold or warm water or thermal tip	Normal = 0 Abnormal = 1	Right foot	Left foot
Pin prick Apply prick proximal to big toe nail just enough to deform the skin Trial pair = sharp/blunt Normal can distinguish sharp/not sharp	Normal = 0 Abnormal = 1	Right foot	Left foot
Achilles reflex	Present = 0 With reinforcement = 1 Absent = 2	Right foot	Left foot
	NDS total out of = 10	Right foot	Left foot

Statistical analysis

Data was entered into MS Excel and analyzed using the SPSS version 17. Descriptive statistics in the form of mean and standard deviations or proportions were used to characterize the study sample. For qualitative data, chi-square or Fisher's exact test was used to observe difference between proportions for independent groups. For continuous variables, student's *t* test was used to compare the two groups. Pearson correlation coefficients were calculated between the outcome and quantitative independent demographic and clinical factors. *p* value of less than 0.05 was considered to be statistically significant. Linear regression analysis was done to determine the models contributing to HbA1C/predictors of HbA1C.

Results

The present comparative study evaluated the relationship between iron indices, Hb1Ac levels, and microvascular complications among patients with type 2 diabetes mellitus. This cross-sectional comparative study comprised of 200 patients (100 patients each categorized under the control and study type 2 diabetes). The overall statistics of the study subjects are shown in Tables 1 and 2.

Serum Iron and HbA1c

The present study states that there is a significant positive correlation of serum iron and HbA1c ($r = 0.46$; $p = 0.001$*) in the study diabetic group (Table 3). Similar results have been found in a study conducted by Shetty et al. [21] where a positive correlation between free iron and HbA1c was found in uncontrolled type 2 diabetes ($r = +0.513$; $p < 0.01$)

Table 1 Overall sample statistics

	Mean	SD	N
Hb1Ac	7.943	1.7911	200
IRON	121.95	45.466	200
Ferritin	233.05	73.432	200
Transferrin saturation	29.58	8.219	200

Serum ferritin and HbA1c

Our study shows that there is a significant positive correlation between serum ferritin levels with HbA1c ($r = 0.43$; $p = 0.001$*) which coincides with a study conducted by Sumeet et al. [22] (Fig 1). Their study showed significant positive correlation between serum ferritin with HbA1c ($p = 0.04$) and also positive correlation between raised ferritin levels in study diabetics. In a study conducted by Sun et al. [23], elevated-circulating ferritin concentrations were associated with higher risk of type 2 diabetes and metabolic syndrome in middle-aged and elderly Chinese independent of obesity, inflammation, adipokines, and other risk factors which support our association of ferritin with HbA1c.

Microvascular complications

In the present study, there is a significant positive correlation between serum iron, serum ferritin levels, and microvascular complications; nephropathy, retinopathy, and neuropathy ($p = 0.001$) (Tables 4, 5, 6, 7, and 8). HbA1c and duration of diabetes also correlated well with microvascular complications ($p = 0.001$) which were significantly higher in the study diabetes group in comparison to the control diabetes group (Table 9). Correlation with HbA1c and duration is a well-known fact, and our study also showed the same consistent results. Dymock et al. [10] reported influence of the increase body iron stores on diabetic nephropathy and vascular dysfunction. In a study by Canturk et al. [16], patients of diabetes had hyperferritinemia, and they found correlation between ferritin levels and diabetic retinopathy. These findings are in accordance with our study.

Table 2 Evaluation of study parameters among the control and study diabetics

Parameter		Study	Control	Statistic
Male	*N*	56	62	Fisher exact = 0.744;
	Percent	47.5	52.5	*p* value = 0.388
Female	*N*	44	38	
	Percent	53.7	46.3	
OHA (oral hypoglycemic agent)	*N*	73	97	Fisher exact = 22.588;
	Percent	42.9	57.1	*p* value = 0.001
OHA + INS (oral hypoglycemic agent + insulin)	*N*	27	3	
	Percent	90.0	10.0	
Iron (normal)	*N*	14	81	Fisher exact = 90.095;
	Percent	14.7	85.3	*p* value = 0.001
Iron (increased)	*N*	86	19	
	Percent	81.9	18.1	
Transferrin saturation (normal range)	*N*	95	99	Fisher exact = 7.749;
	Percent	49.0	51.0	*p* value = 0.097
Transferrin saturation (increased)	*N*	5	1	
	Percent	83.3	16.7	
Ferritin (normal)	*N*	16	68	Fisher exact = 55.501;
	Percent	19.0	81.0	*p* value = 0.001
Ferritin (increased)	*N*	84	32	
	Percent	72.4	27.6	

Transferrin saturation and HbA1c

We also observed the relationship of transferrin saturation with HbA1c; however, we found weak negative correlation in the study diabetic group which was insignificant ($r = -0.05$; $p = 0.6$) consistent with a study conducted by Montonen et al. [3]. But in study conducted by Thomas et al [24], they observed that higher transferrin saturation was observed in diabetics compared to healthy individuals.

Fernandez-Real et al. [25] documented in general population increased body iron stores associated with increased occurrence of glucose intolerance, type 2 diabetes, and gestational diabetes which is consistent with our study.

Elevated iron and diabetes

In the last few decades, the impact of transition metals, and iron in particular, on human physiology has been explored. Iron, being a first-line prooxidant, seems to regulate the clinical manifestations of various systemic diseases, including diabetes and atherosclerotic vascular diseases. Iron regulation of the cell oxidative stress can explain, to some extent, its close association with abnormalities in insulin sensitivity.

Table 3 Correlation values among study diabetes correlations

		Iron	Ferritin	Transferrin sat
Hb1Ac	Pearson correlation	0.462	0.436	− 0.050
	p value	0.001	0.001	0.620

The mechanisms by which elevated iron stores may induce diabetes include oxidative damage to pancreatic beta cells, impairment of hepatic insulin secretion by the liver, and interference with insulin's ability to suppress hepatic glucose production.

Elevated ferritin and diabetes

Oxidative stress and chronic inflammation are being postulated as mechanisms involved in the pathophysiology of diabetes and its complications. Being an inflammatory biomarker and a reflector of iron stores, higher ferritin levels in correspondence to higher iron levels in our study might have been associated with the positive correlation observed in our study with both poor glycemic control and long-term microvascular complications.

Transferrin saturation and diabetes

Based on previous studies, it was speculated that Se transferrin levels may increase as a compensatory mechanism for a reduction in free iron levels that may occur secondary to oxidative stress, and thus, it may serve as a biomarker of some other factor that is causally related to diabetes, and possibly not related to iron load. In the present study, we did not find any association between Se transferrin and diabetes incidence. Interestingly, Se transferrin seemed to be associated with

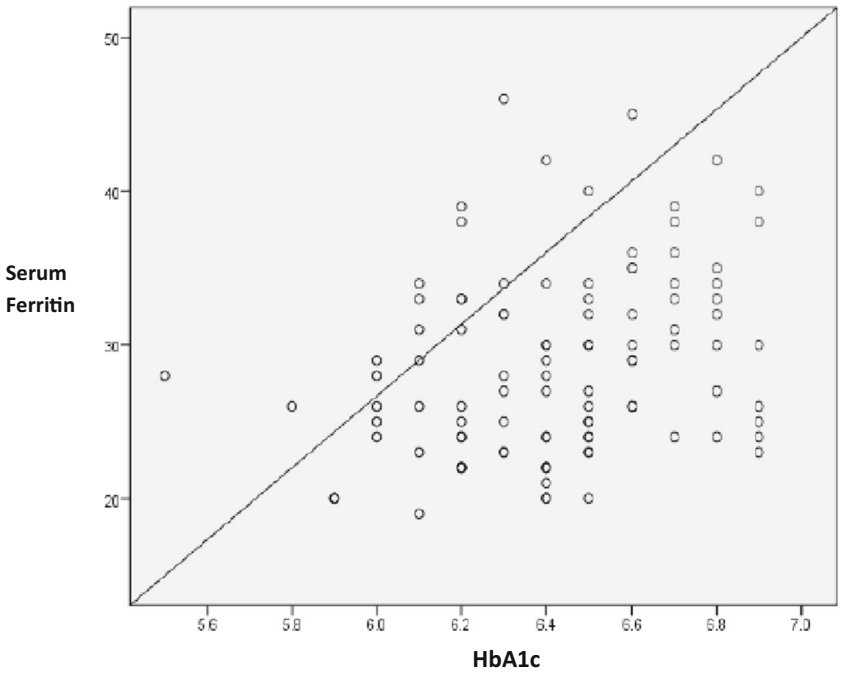

Fig. 1 Scatter graph depicting strength between serum ferritin and HbA1c in control diabetics

Table 4 Glycosylated hemoglobin (HbA1c) and its correlation with microvascular complications in the study group

HbA1c		*N*	Mean	SD	SE	*t* statistic c	*p* value e
Nephropathy	Yes	40	8.96	1.00	0.12	− 5.145	0.001
	No	60	10.19	1.38	0.21		
Retinopathy	Yes	33	9.13	1.18	0.14	− 3.823	0.001
	No	67	10.13	1.32	0.23		
Neuropathy	Yes	31	9.20	1.28	0.15	− 2.986	0.004
	No	69	10.02	1.20	0.21		
Iron	Normal	14	8.59	1.12	0.30	− 2.751	0.007
	Increased	86	9.60	1.29	0.13		

Table 5 Glycosylated hemoglobin (HbA1c) and its correlation with microvascular complications in the study group

Hb1Ac		*N*	Mean	SD	SE	*t* statistic	*p* value
Nephropathy	Yes	12	6.43	0.22	0.06	0.082	0.935
	No	88	6.42	0.29	0.03		
Retinopathy	Yes	11	6.49	0.32	0.09	0.794	0.429
	No	89	6.41	0.27	0.02		
Neuropathy	Yes	12	6.52	0.27	0.07	1.286	0.202
	No	88	6.41	0.28	0.03		
Iron	Normal	81	6.41	0.28	0.03	− 0.709	0.480
	Increased	19	6.46	0.29	0.06		

Table 6 Comparison of nephropathy in the study and control groups

Diabetes type		Patients with nephropathy	Mean	SD	SE	*t* statistic	*p* value
Iron	Study	40	161.98	21.26	3.36	5.828	0.001
	Control	12	107.00	46.15	13.32		
Ferritin	Study	40	304.68	49.11	7.76	7.896	0.001
	Control	12	177.08	49.02	14.15		
Transferrin sat	Study	40	30.83	11.11	1.75	0.885	0.381
	Control	12	27.83	6.46	1.86		

Table 7 Comparison of retinopathy cases in the study and control groups

Diabetes type		No. of patients with retinopathy	Mean	SD	SE	*t* statistic	*p* value
Iron	Study	33	158.18	19.64	3.42	6.335	0.001
	Control	11	99.36	41.85	12.62		
Ferritin	Study	33	308.30	34.06	5.93	6.912	0.001
	Control	11	205.45	62.90	18.96		
Transferrin sat	Study	33	28.67	10.96	1.90	− 0.069	0.945
	Control	11	28.91	6.53	1.97		

Table 8 Comparison of neuropathy cases in the study and control groups

Diabetes type		No. of patients with neuropathy	Mean	SD	SE	*t* statistic	*p* value
Iron	Study	31	165.42	19.15	3.44	6.578	0.001
	Control	12	102.83	43.80	12.64		
Ferritin	Study	31	288.16	53.05	9.52	4.625	0.001
	Control	12	195.58	72.40	20.90		
Transferrin sat	Study	31	29.77	11.21	2.01	1.384	0.174
	Control	12	25.17	3.76	1.08		

Table 9 Correlation values among study diabetes correlations

		Iron	Ferritin	Transferrin sat
Hb1Ac	Pearson Correlation	0.462	0.436	− 0.050
	p value	0.001	0.001	0.620

diabetes risk in individuals with HbA1c values in the control group, but not in those with higher HbA1c values.

Limitations

1. Sample size of the study is small, thereby preventing us from drawing strong conclusions.
2. Diabetes mellitus is a state of chronic inflammation. Though ferritin is a marker of chronic inflammation, the causal relationship between raised ferritin and the study diabetes group is a matter of debate and requires further exploration in establishing the authenticity of causal hypothesis.

Summary and conclusion

In the present cross-sectional, comparative study, we have found significant positive correlation between iron indices (serum iron, serum ferritin) and HbA1c levels in the study group with type 2 diabetes mellitus. We also found that iron indices (i.e., serum iron, serum ferritin, and transferrin saturation) have significant correlation with microvascular complications in them. However, we did not observe any significant correlation between transferrin saturation and HbA1c levels in the present study ($p = 0.62$).

We conclude that serum ferritin and serum iron may be used as surrogate markers of poor glycemic control and microvascular complications in association with HbA1c. Though, the causal association of iron indices is evident by the regression analysis in our study, further larger trials need to be undertaken to establish this causal relationship.

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