ORIGINAL ARTICLE

Relation of iron stores to oxidative stress in type 2 diabetes

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Abstract

Background: Recent studies revealed elevated serum ferritin levels predict new-onset type 2 diabetes. Further studies proved ferritin to be an important and independent predictor of the development of diabetes. The link between hyperglycemia, enhanced free radical activity (oxidative stress) and serum iron and its stores (serum ferritin levels) levels is not clear.
Objectives: The present study is an attempt to understand the relationship between serum ferritin levels and oxidative stress (measured by malondialdehyde).
Materials and Methods: The study comprised of 30 apparently healthy controls and 30 type 2 diabetic patients who attended the outpatient and inpatient departments of Medical College, Kolkata. Levels of fasting blood glucose, postprandial blood glucose, serum iron, serum ferritin, glycosylated hemoglobin (HbA1c) and malondialdehyde (MDA), serum urea and creatinine were estimated. The statistical software SPSS 11.0 and Systat 8.0 were used for the analysis of the data and Microsoft Word and Excel have been used to generate tables and graphs.
Results: Serum iron (82.16 ± 13.24 µg/dl), serum ferritin (224.53 ± 96.06 µg/L), HbA1c (8.62 ± 1.79%), MDA (2.66 ± 0.76 nmol/ml) levels were significantly higher in type 2 diabetics compared with apparently healthy controls. Elevations in serum iron, ferritin and HbA1c are accompanied by a parallel increase in blood glucose. Based on groups of glycemic control, i.e. HbA1c levels >8%, serum ferritin levels were highest, 258.63 ± 22.67 µg/dl. There is an inverse correlation of serum ferritin levels to MDA levels in the diabetic cases of longer duration of more than 10 years.
Conclusion: Serum ferritin level in the present study is found to be higher in the newly diagnosed cases and lower in those patients suffering from diabetes for more than 10 years. This study probably suggests that serum ferritin can represent either as a pro-oxidant or as an antioxidant in a time-dependent manner.

Key words: Oxidative stress, serum ferritin, serum malondialdehyde, type 2 diabetes mellitus

Date of Acceptance: 25-Apr-2012

Introduction

Elevated iron stores may increase the risk of developing diabetes.[1] Emerging scientific evidence has revealed unsuspecting influences between iron metabolism and NIDDM. The relationship is bi-directional, iron affects glucose metabolism, and glucose metabolism impinges on several iron metabolic pathways. It is increasingly recognized that iron influences glucose metabolism, even in the absence of significant iron overload. Serum ferritin is known as an index for body iron stores and also as an inflammatory marker. Increased serum ferritin concentration is associated with an increased risk of diabetes mellitus. Serum ferritin levels predict new-onset type 2 diabetes.[2] Elevated iron stores could enhance oxidation of lipids, especially of free fatty acids; through accelerated production of free radicals. The complex process of advanced glycation end product formation

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Access this article online

Quick Response Code:

Website: www.njcponline.com

DOI: 10.4103/1119-3077.106776

PMID: *******

produces reactive oxygen species (ROS) by metal catalyzed reactions. Advanced glycation end products themselves bind transition metals, potentiating their toxic effects, including insulin resistance. ROS interfere with insulin signaling at various levels, impairing insulin uptake through a direct effect or insulin receptor functions and inhibiting the translocation by GLUT4 in the plasma membrane. Iron through Fenton's reaction participates in the formation of highly toxic free radicals such as hydroxide and the superoxide anion that are capable of inducing lipid peroxidation. Hydroperoxides react with transition metals to form stable aldehydes, such as malondialdehyde (MDA). ROS can stimulate vascular smooth muscle cell growth and protooncogene expression.

In patients with type 2 diabetes mellitus, higher levels of MDA, a marker of lipid peroxidation is found. Oxidative stress induces both insulin resistance by decreasing internalization of insulin and increased ferritin synthesis.[3] Both at transcriptional and posttranscriptional levels, synthesis of apoferritin are induced by the presence of free iron. The heavy chain in the apoferritin molecule exerts ferroxidase activity, catalyzing the oxidation of Fe2+ to Fe3+, which prevents iron-induced cyclic redox reaction that would spread and amplify the oxidative damage. The ferroxidase activity in the heavy chain is down regulated when concentration of antioxidants are low, Therefore, there is a rapid release of iron from ferritin as well as there is a decrease in the incorporation of iron into ferritin. The present study is an attempt to understand the relation between serum iron and ferritin to oxidative stress (MDA).

Materials and Methods

The study was conducted after obtaining usual permission from ethical committee, and consent from subjects and controls were taken before commencing the study. The study comprised of 30 apparently healthy controls and 30 type 2 diabetic patients, who attended the outpatient and inpatient departments of Medical College, Kolkata. The inclusion criteria were male subjects in the age group of 40–60 years with type 2 diabetes mellitus without complications. Exclusion criteria included anemia, any other disease or drugs, anemia therapy that could affect ferritin levels. Patients having renal disease were excluded from the study determining the serum levels of urea and creatinine. Patients suffering from hemochromatosis were excluded from the study. History and physical data was obtained from both cases and controls. Determination of serum malondialdehyde (TBARS method),[4] serum iron (colorimetric method),[5] serum ferritin (Enzyme Immunoassay)[6] along with estimations of both fasting blood glucose (FBG) and postprandial blood glucose (PPBG) by (glucose oxidase method)[7] and glycosylated hemoglobin (immunoinhibition method),[8] serum creatinine (Jaffe's method),[9] serum urea (UV method)[10] were carried out.

Statistical analysis

Chi-Square test has been used to find the homogeneity of sex distribution between apparently healthy controls and diabetic cases. Student's *t* test (independent two tailed) has been used to find the significance of serum ferritin and other biochemical parameters between these two groups. The effect sizes due to Hedges (Bias Corrected) have been computed to find the effect of diabetes on biochemical parameters over the control group. Pearson's correlation coefficient (*r*) has been calculated. The statistical software SPSS 11.0 and Systat 8.0 were used for the analysis of the data and Microsoft Word and Excel have been used to generate the tables and graphs.

Results

There was a significant elevation in the cases in the levels of FBG (138.47 ± 30.51 mg/dl), PPBG (232.37 ± 51.13 mg/dl) and is of statistical significance ($P < 0.001$). Mean values of HbA_{1c} was found to be (8.62 ± 1.79%) in cases compared with mean values of HbA_{1c} i.e. (5.54 ± 0.22%) with controls and is statistically significant ($P < 0.001$). Serum iron content (82.16 ± 13.24 μg/dl) in the cases is found to be higher than controls (77.81 ± 13.05 μg/dl) but not statistically significant ($P < 0.204$). Mean values of serum ferritin is higher in cases (224.53 ± 96.06 μg/l) compared with controls (78.09 ± 14.60 μg/L) and is statistically highly significant ($P < 0.001$). Mean values of MDA were higher in cases (2.66 ± 0.76 nmol/ml) compared with controls (1.59 ± 0.36 nmol/ml) and is of statistical significance ($P < 0.001$). Serum levels of ferritin, iron and MDA and HbA_{1c} in cases and controls are depicted in Table 1.

When cases were grouped based on glycemic control (HbA_{1c} values) i.e. good control (6-7% HbA_{1c}), moderate control (>7-8% HbA_{1c}) and poor control (>8% HbA_{1c}), the serum ferritin level was highest 258.63 ± 22.67 μg/l in groups with poor glycemic control. The same was much lower 183.43 ± 75.36 μg/l, in group of cases with good glycemic control. Percentage of diabetic cases and serum ferritin levels based on groups of glycemic control are depicted in Table 2.

Serum ferritin and MDA levels are found to be higher in diabetic cases with longer duration of diabetes and are 168.38 ± 43.25 μg/l and 3.58 ± 0.53 nmol/ml, respectively. Percentage of diabetic cases, serum ferritin levels and MDA levels based on duration of diabetes are depicted in Table 3.

Pearson's correlation co-efficient (*r*) has been calculated and the (*r* value) with *P* value are depicted in Table 4.

Correlation of serum ferritin to MDA in cases and controls is projected in scattered graphs in Figures 1 and 2.

Table 1: Serum levels of ferritin, iron and MDA and HbA_{1c} in cases and controls

Biochemical Parameter	Control	Cases	*P* value
FBG (mg/dl)	82.23±10.04	138.47±30.51	<0.001
PPBG (mg/dl)	128.53±8.49	232.37±51.13	<0.001
Serum MDA (nmol/ml)	1.59±0.36	2.66±0.76	<0.001
HbA_{1c} (%)	5.54±0.22	8.62±1.79	<0.001
Serum Ferritin (µg/l)	78.09±14.60	224.53±96.06	<0.001
Serum Iron (µg/dl)	77.81±13.05	82.16±13.24	<0.2.4

Table 2: Percentage of diabetic cases and serum ferritin levels based on groups of glycemic control

Classification of cases based on HbA_{1c}	% of cases	Serum ferritin (µg/l)
Good control (6-7% HbA_{1c})	13.33	183.43±75.36
Moderate control (>7-8% HbA_{1c})	43.33	203.08±59.24
Poor control (>8 HbA_{1c})	43.33	258.63±22.67
Significance		*P*<0.227

Table 3: Percentage of cases, serum ferritin and MDA levels based on duration of diabetes

Duration of type 2 diabetes	% of cases (*n*=30)	Serum Ferritin (µg/dl)	MDA (nmol/ml)
≤5 years	46.67	281.38±105.63	2.08±0.51
6-10 years	33.33	178.63±55.17	2.92±0.43
>10 years	20.00	168.38±43.25	3.58±0.53
Significance		*P*<0.006	*P*<0.001

Table 4: Correlation of serum ferritin with other parameters in controls and cases

Correlation of serum ferritin levels to other parameters	Control (*r* value)	Case (*r* value)
FBS vs. Serum ferritin	0.608	0.493
	P<0.001	*P*<0.006
PPBS vs. Serum ferritin	0.502	0.460
	P<0.001	*P*<0.011
HbA_{1c} vs. Serum ferritin	0.230	0.318
	P<0.221	*P*<0.086
MDA vs. Serum ferritin	0.500	−0.579
	P<0.005	*P*<0.001
Iron vs. Serum ferritin	0.055	0.235
	P<0.771	*P*<0.285

Discussion

Significant hyperglycemia-both FBG and PPBG–was observed in cases. Glycosylated hemoglobin (HbA_{1c}) used in long-term monitoring of diabetes mellitus is higher in cases ($P < 0.001$). Many studies have reported that post-translational modification of ferritin with glucose causes change of its immunochemical properties, and changes in iron metabolism affects the level of HbA_{1c} in diabetics.[11] Among the cases with poor glycemic control (>8% HbA_{1c}), serum iron and serum ferritin levels were higher and is in agreement with several other studies. Serum ferritin level is elevated in type 2 diabetes ($P < 0.001$). A recent finding with 1013 Finnish men is that there is a positive association between ferritin and type 2 diabetes.[12] Another study has also shown a strong association between serum ferritin and incidence of diabetes.[13] Elevated ferritin levels are indicated to be due to elevated body iron stores or ferritin is an acute-phase reactant and elevated ferritin may reflect inflammation or delayed clearance of glycosylated ferritin. It is also suggested that ferritin level is increased due to lack of glycemic control. One study suggested that treating diabetic patients with deferoxamine lowered ferritin concentrations and improved the management of diabetes, but these findings were not confirmed in subsequent studies. Serum ferritin level in the present study is found to be higher in the newly diagnosed cases and low in those patients suffering from diabetes for more than 10 years. The results are in agreement with the previous studies. Elevated serum ferritin could be a marker of insulin resistance and this may account for elevated FBG and PPBG in cases. Obviously, further research is needed on the associations of serum ferritin concentration with insulin resistance and other components of the insulin resistance syndrome.

MDA is one of the lipid peroxidation products frequently used to assess the state of oxidative stress in diabetics. In the

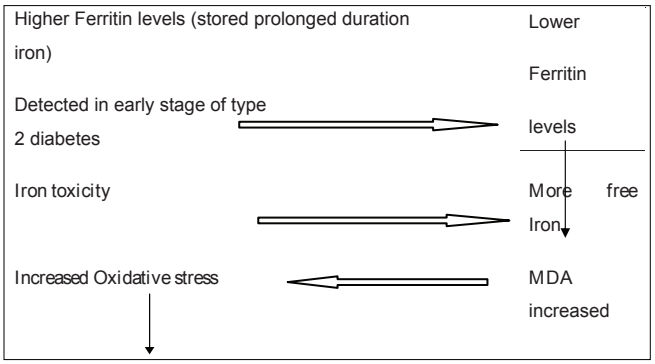

Figure 1: Correlation of serum ferritin to MDA levels in controls

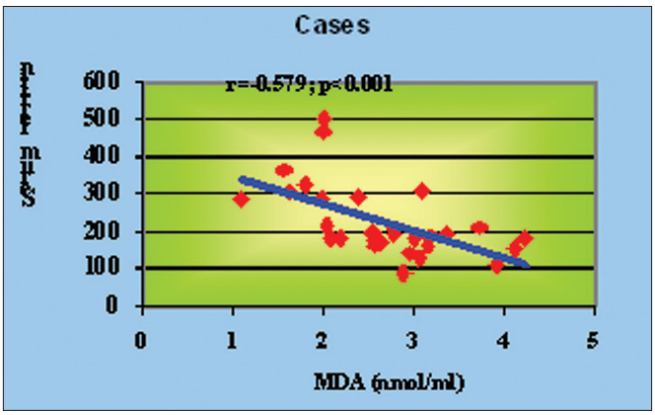

Figure 2: Correlation of serum ferritin to MDA levels in cases

present study, lipid peroxidation is significantly increased in type 2 diabetics compared with controls ($P < 0.001$). Although increased levels of MDA has been reported in both type 1 and type 2 diabetes in many studies, some studies have failed to detect any significant elevation in lipid peroxidation.[14] We have found that elevated MDA level correlates with poor glycemic control and its finding is the same with several other studies. Jain *et al*. reported that poor glycemic status could produce permanent chemical alterations in proteins and increase lipid peroxidation in experimental models of hyperglycemia. Hyperglycemia itself is suggested to stimulate platelet aggregation and auto-oxidation of glucose leading to free radical production in diabetics. The source of free radicals in diabetes is not well understood but glycation of proteins can lead to oxidative damage. Mean serum ferritin levels are highest in individuals with diabetes of shorter duration and are significantly different from levels of serum ferritin in individuals with previously diagnosed diabetes. Because serum ferritin levels reflect the sum of body iron stores and the production of acute-phase reactants, changes in either can alter ferritin levels. Thus, the lower mean ferritin levels in individuals with previously diagnosed diabetes may be due to changes in body iron stores or may be due to changes in factors that affect the production of acute-phase reactants.[15] It is said that ferritin and its formation can protect against iron toxicity, thus causing decrease in oxidative stress. But as the duration of diabetes increases there is an increase in oxidative stress and other complications. This leads to the lowering of ferritin levels and release of free iron contributing to further increase in oxidative stress.[16] This clearly explains our observation of inverse relation between the ferritin level and the MDA level in the cases of longer duration of diabetes.

This study has several limitations. Firstly, it is a cross-sectional study comprising a small group; therefore, the directionality of associations cannot be clearly established. Secondly, although ferritin is considered a good measure of body iron stores, it is not a "gold standard". Thirdly, ferritin is also an acute phase reactant and may be artificially elevated in the presence of inflammation. Similarly, residual confounding by unmeasured factors that may be related both to the prevalence of type 2 diabetes and to ferritin levels cannot be ruled out. This study seems to suggest that elevated ferritin can act as a pro-oxidant at the early onset of type 2 diabetes. So large-scale clinical trials to establish a relation between elevated serum ferritin levels and type 2 diabetes is worth undertaking. Further, this study suggests that this putative activity of ferritin may constitute a missing link in the regulatory loop between iron and reactive oxygen species.

Acknowledgments

We would like to thank the following technical staff for their active participation in our study, Mr Arup Ratan Chowdhury, Mr. Subir Mondal. Special thanks to the management of Medical College, Kolkata, for their active participation and patronage.

References

1. Pankaj B, Puja B, Akshay R, Kansal HM. Is serum ferritin associated with type ii diabetes mellitus: A clinical study in a representative indian population. J Med Sci Res 2011;2:20-24.
2. Forouhi NG, Harding AH, Allison M, Sandhu MS, Welch A, Luben R, *et al*. Elevated serum ferritin levels predict new-onset type 2 diabetes: Results from the EPIC-Norfolk prospective study. Diabetologia 2007;50:949-56.
3. Bertelsen M, Anggard EE, Carrier MJ. Oxidative stress impairs insulin internalization in endothelial cells *in vitro*. Diabetologia 2001;44:605-13.
4. Premanand R, Kumar S, Mohan A. Study of thiobarbituric reactive substances and total reduced glutathione as indices of oxidative stress in chronic smokers with and without chronic obstructive pulmonary disease. Indian J Chest Dis Allied Sci 2007;49:9-12.
5. Williams J, Jeevithan S, Thirunavukkarasu D. Estimation of serum ferrium in HIV/AIDS patients. Indian J Sex Transm Dis 2007;28:23-5.
6. Zamani F, Razmjou S, Akhlaghpoor S, Eslami SM, Azarkeivan A, Amiri A. T2* magnetic resonance imaging of the liver in thalassemic patients in Iran. World J Gastroenterol 2011;17:522-5.
7. Sacks DB. Carbohydrates. In: Burtis CA, Ashwood ER, Bruns DE, editors. Tietz textbook of clinical chemistry and molecular diagnostics. St. Louis: Elsevier Saunders;2006. p. 837-902.
8. Hanas R, John G. International HbA consensus committee. 2010 Consensus statement on the worldwide standardization of the hemoglobin A_{1c} measurement. Pediatr Diabetes 2010;11:209-11.
9. Chromy V, Rozkosna K, Sedlak P. Determination of serum creatinine by Jaffe method and how to calibrate to eliminate matrix interference problems. Clin Chem Lab Med 2008;46:1127-33.
10. Tayal D, Chawla R, Arora S. Dynamic changes in biochemical markers of renal function with thyroid status -A study in indian population. Internet J Med Update 2009;4:36-41.
11. Ikeda Y, Suehiro T, Yamanaka S, Kumon Y, Takata H, Inada S, *et al*. Association between serum ferritin and circulating oxidized low-density lipoprotein levels in patients with type 2 diabetes. Endocr J 2006;53:665-70.
12. Touraine TP, Nyyssonen K, Salonen R, Tervahauta A, Korpela H, Lakka T, *et al*. Body iron stores are associated with serum insulin and blood glucose concentrations: Population study in 1013 eastern Finnish men. Diabetes Care 1997;20:426-8.
13. Smotra S, Kudyar RP. Relationship between serum ferritin and type-2 diabetes mellitus. Journal Knowledge Science, published in Jammu and Kashmir, 2008;10:170-4.
14. Kalaivalam KN, Dharmalingam M, Marcus SR. Lipid peroxidation in type 2diabetes mellitus Int J Diab Dev Ctries 2006;26:30-2.
15. Ford ES, Cogswell ME. Diabetes and serum ferritin concentration among U.S. adults. Diabetes Care1999;22:1978-83.
16. Zhang F, Wang W, Tsuji Y, Torti SV, Torti FM. Post-transcriptional modulation of iron homeostasis during p53-dependent growth arrest. J Biol Chem 2008;283:33911-8.

How to cite this article: Kundu D, Roy A, Mandal T, Bandyopadhyay U, Ghosh E, Ray D. Relation of iron stores to oxidative stress in type 2 diabetes. Niger J Clin Pract 2013;16:100-3.

Source of Support: Medical College, Kolkata and KIMS, Bangalore, **Conflict of Interest:** None declared.