

## A study on estimation of serum ferritin in type 2 diabetes mellitus cases on oral hypoglycemic agents

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### Abstract

**Introduction:** Diabetes mellitus is a metabolic disorder characterized by hyperglycemia and associated micro and macro vascular complications on long duration. Insulin resistance is associated with onset of type 2 DM and various studies point its connection to iron overload in the body. Hyperinsulinemia due to resistance to insulin may be responsible for increasing serum Ferritin.

**Objectives:** The present study was carried out to determine the relationship between serum Ferritin and type 2 diabetes mellitus and to establish correlation between serum Ferritin and fasting blood glucose as well as with HbA1c.

**Materials and Methods:** An observational study for one year at a tertiary care hospital was carried on 50 diabetic male cases and 50 age matched controls. BMI, serum ferritin, HbA1c, FBS and PPBS were estimated on all the cases and controls as per standard guidelines and compared between cases and controls. P value less than 0.05 was considered statistically significant.

**Results:** In the present study, 41-50 years was the common age group, and BMI (kg/m<sup>2</sup>), Fasting blood sugar, Post prandial blood sugar parameters were statistically significant between cases and controls. The mean HbA1c% in cases was  $9.48 \pm 2.65$  and greater than controls and significant correlation with serum ferritin levels which was  $359.11 \pm 87.18$  was observed. Greater levels of Serum ferritin was observed in cases with elevated levels of HbA1c%. A strong positive correlation was found between serum ferritin and HbA1c, FBS and PPBS levels in the study.

**Discussion:** This study clearly explains that there is a significant increase in the serum ferritin in cases of diabetes mellitus compared to normal controls and hyperferritinemia may be one of the causes for decreased insulin production and development of insulin resistance in diabetic individuals. Study of iron and related parameters may be a useful offshoot of the conventional studies on diabetes and its complications. Hence monitoring the prevalence of serum ferritin is beneficial in long run among the diabetics.

**Keywords:** Diabetes mellitus, Glycated hemoglobin, serum ferritin, Insulin resistance.

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### Introduction

A rise in the non communicable diseases is a serious threat globally. Among them diabetes mellitus ranks the top. By 2030, it is estimated that 79.4 million people will be in the grip of diabetes. It will be the seventh leading cause of death by 2030, as per the projection of WHO. An ICMR funded study in India pointed out that, south Indian population are more affected by diabetes than north Indian people.<sup>1</sup> Cytokines and inflammatory factors play a pivotal role in producing Diabetes mellitus as said by Pick up JC.<sup>2</sup> Metabolic disturbances associated with DM include hyperglycemia, insulin resistance, malnutrition, and other risk factors like hypertension, polycystic ovarian syndrome and dyslipidemia. Insulin resistance is associated with onset of type 2 DM and various studies point its connection to iron overload in the body. Catalytic action of free iron is instrumental to insulin resistance in the beginning and later on to

reduced insulin release which subsequently results in development of Type 2 DM. Long standing co morbidities due to diabetes are also moderated by iron mediated deterioration.<sup>3</sup> Different studies across the globe and India have given inconsistent results regarding iron profile status in diabetics. Regulation of blood iron levels is mediated by Ferritin protein, Ferritin functions as a buffer in iron overload and iron overload.<sup>4</sup> Ferritin is a complex globular protein that stores iron as soluble and non toxic component. Increasing concentration of iron and Ferritin in cells could cause resistance to insulin and dysfunction of  $\beta$  cells of pancreas. Hyperinsulinemia due to resistance to insulin may be responsible for increasing serum Ferritin. Abnormalities in the Ferritin metabolism following glycation in chronic hyperglycemic state might be a cause of hyperferritinemia in T2DM as mentioned in many studies universally.<sup>5</sup> It is surmised that this increase may contribute to the pathogenesis of the disease and

as well as development of complications like retinopathy, nephropathy etc. Serum concentrations of Ferritin are usually increased in poorly controlled type 1 and type 2 diabetic subjects, and Ferritin has been shown to predict HbA1c independently of glucose reflecting increased oxidative stress.

The present study was carried out to determine the relationship between serum Ferritin and type 2 diabetes mellitus and to establish correlation between serum Ferritin and fasting blood glucose as well as with HbA1c.

## Materials and Methods

The present observational study was conducted at a tertiary care hospital in the department of biochemistry and research laboratory for a period of one year from October 2015 to September 2016. The study proposal was approved by the institutional ethical committee and all the ethical guidelines were followed as per the protocol. Written consent was obtained from all the cases and controls of the study after explaining the objectives of the study. The study population consisted of 50 cases of clinically diagnosed diabetics who were receiving oral hypoglycemic drugs and attending the department of endocrinology and general medicine and 50 controls of normal healthy subjects. The controls were age matched and all the cases and controls were males only in the study. All the cases and controls were evaluated with detailed clinical history, meticulous examination and laboratory investigations. The anthropometric measurements were noted i.e., age, weight, hip circumference and BMI was calculated. The data was collected and entered in a separate proforma sheet.

### Inclusion criteria

Uncomplicated Type-2 diabetic male patients on oral hypoglycemic agents, with a history of diabetes less than 10 years and age greater than 25 years were included in the study.

### Exclusion criteria

As Ferritin is an acute phase reactant, cases and controls with possible or suspected infection, inflammation or any other disease with a possible cause of elevation of serum Ferritin were excluded by estimation of CRP. Any case with raised CRP levels was excluded from the study. Patients with anemia, on iron supplementation therapy by any means, who underwent blood transfusion or donation, bleeding disorders and other diabetic complications (e.g. nephropathy, neuropathy etc) were excluded from the study.

## Sample collection

5ml of venous blood was collected after a period of 12 hours overnight fasting and FBS, Hb%, Serum Ferritin, Glycated hemoglobin (HbA1c) was estimated by standard protocols. 1ml of venous blood was collected only from cases after 2 hours post prandial and PPBS was measured.

1. Blood glucose was determined by Hexokinase G-6-PDH method and carried out on auto analyzer –Roche/Hitachi Cobas 6000 system. [Normal range : 70-110mg/dl] Post prandial blood glucose was also calculated by using the same auto analyzer. [Normal range 7-140 mg/dl]
2. Serum Ferritin was estimated by using DRG Ferritin ELISA kit which is a immune radiometric assay kit of the sand witch type using two monoclonal antibodies. Using the mean absorbance value, the corresponding concentration of Ferritin in ng/ml was calculated from the standard curve. [Normal range: 20-250ng/ml]
3. HbA1c was measured by the Ion-exchange resin method, [Tulip group] [Normal value: <6.5%]
4. Hb% was measured in whole blood by the cyanide free sodium lauryl sulphate method. [Normal range in males: 13-17 gm%].

## Statistical Analysis

Descriptive and inferential statistical analysis was carried out. Mean and standard deviation was carried out to assess the levels of various parameters in both groups. Student 't' test was carried out for comparison of quantitative Co-relation between serum Ferritin and variables. Co-relation between serum Ferritin and HbA1c in patients and co-relation between serum Ferritin and FBS was evaluated by using Pearson correlation coefficient. P-value <0.05 was considered statistically significant.

## Results

In the present study conducted on diagnosed type 2 diabetic cases of males and controls, the highest age of diabetic male was 75 years and lowest age was 38 years, The most common age group was 41-50 years in both cases and controls. The mean age of diabetic cases was  $48.56 \pm 6.75$  and control group was  $46.28 \pm 9.75$  and there was no significant age difference in cases and control group. The BMI of all the diabetic cases were  $>18.5 \text{ kg/m}^2$  and the mean BMI was  $32.16 \pm 1.88 \text{ kg/m}^2$  and the BMI of control group was  $28.22 \pm 2.68 \text{ kg/m}^2$  and less than the diabetic cases. Nearly 54% of diabetic

cases had BMI between 25-30 kg/m<sup>2</sup> and was found statistically significant (p value<0.01). The waist circumference and waist hip ratio of diabetics were higher than controls in the study indicating diabetics had central obesity. The waist to hip ratio is significantly higher (p value >0.001) in diabetics (1.12 ± 0.12) than controls (0.98 ± 1.1) and diabetics are associated with central obesity. In the present study all the cases were diagnosed diabetics with FBS levels >126mg/dl and the mean value of FBS was 198 ± 46.1 mg/dl and in the controls was 98.6 ± 2.1 mg/dl and was found to be statistically highly significant (p value < 0.001). The mean PPBS in the diabetic cases was higher (286.12± 2.65) than the control group (118.12 ± 2.1) and was found significant (p value >0.01). There was no much difference in the Hemoglobin % between the cases and controls in the study with a mean value of 15.12 ± 1.1 in cases and 14.98 ± 1.4 in controls and was not statistically significant. (p value <0.01) . The mean percentage of Glycated hemoglobin (HbA1c) in the controls was less (5.01 ± 0.45) than the percentage of HbA1c in

cases (9.48 ± 2.65) and was found to be strongly significant in the study. The level of Serum Ferritin was higher in diabetic cases than controls in the study. Diabetics with a mean value of 359.11 ± 87.18 and in controls with a value of 182.12 ± 26.14 and showed a statistically significant association (p value <0.01). In our study a rise in serum Ferritin levels were observed in diabetics even though the hemoglobin levels were in the normal physiological range. [Table-1]

In our study it was observed, serum Ferritin levels were higher (214.14± 5.85ng/ml) in diabetic cases with highest HbA1c levels of >10.5%. In contrary lower levels were observed (89.45± 2.14ng/ml) in cases with HbA1c levels between 6.0 – 7.5%. [Table-2] Hence it was observed that levels of HbA1c had a strong positive correlation with levels of serum Ferritin in diabetic cases in our study and was statistically significant. The value of Pearson correlation coefficient overall was 0.98 and established a strong positive correlation between the two parameters in the study. [Table-3]

**Table1: Comparison of means of anthropometric measurements, clinical and biochemical characteristics between test and control groups**

Parameters	Control group	Diabetic group	p value
Age (Years)	46.28 ± 9.75	48.56 ± 6.75	>0.05
BMI (kg/m <sup>2</sup> )	28.22 ± 2.68	32.16 ± 1.88	<0.01
FBG (mg/dl)	98.6 ± 2.1	198 ± 46.1	<0.001
PPBG (mg/dl)	118.12 ± 2.1	286.12± 2.65	<0.01
Hb(%)	14.98 ± 1.4	15.12 ± 1.1	<0.01
HbA1c (%)	5.01 ± 0.45	9.48 ± 2.65	<0.01
Serum ferritin (ng/ml)	182.12 ± 26.14	359.11 ± 87.18	<0.01

**Table 2: Mean and Standard deviation of serum Ferritin in different levels of HbA1c among diabetic cases**

HbA1c (%) (Range)	No	Mean ±SD	
		HbA1c (%)	Ferritin (ng/dl)
6.00-7.50	13	7.14 ± 0.48	89.45± 2.14
7.51 - 9.00	9	7.29 ± 0.54	144.12 ± 14.12
9.01 - 10.5	12	8.97 ± 0.88	181.15 ± 21.01
> 10.5	16	11.21 ± 1.14	214.14± 5.85

**Table 3: Correlation between HbA1c and serum ferritin levels in Diabetic cases**

HbA1c Range	Serum ferritin	
	r value	p Valve
6.00-7.50	0.61	<0.05
7.51 - 9.00	0.58	<0.05
9.01 - 10.5	0.88	<0.01
> 10.5	0.74	<0.01
<b>Overall</b>	<b>0.98</b>	<b>&lt;0.01</b>

## Discussion

Chronic hyperglycemia is a major etiological factor which triggers both micro and macro vascular complications in diabetes mellitus. Persistent hyperglycemia in uncontrolled diabetics may cause low grade inflammation which result in increase of inflammatory markers which predispose to detrimental consequences in diabetes mellitus. The biochemical process of advanced glycation appears to be enhanced in diabetes as a result of hyperglycemia, oxidative stress and lipid peroxidation. In result of this, a different group of chemical moieties are generated which result in development and progression of vascular complications via activation of intracellular signaling pathways, generation of proinflammatory and prosclerotic cytokines.<sup>6,7</sup> In the present study, the most common age group of diabetic cases was 41-50 years followed by 51-60 years which is similar to the findings of Mc Nair et al and Yoon.<sup>8,9</sup>

Serum ferritin is considered as a marker of iron status in the body and an inflammatory marker. Oxidative stress can increase ferritin synthesis to avoid further oxidative damages as ferritin can bind free redox-active iron. In the present study, the diabetic cases had increased waist hip ratio indicating an increase in BMI ( $\text{kg}/\text{m}^2$ ). In our study it was observed that 74% of diabetics were with central obesity with BMI greater than  $25\text{kg}/\text{m}^2$ . This findings were consistent with the findings of Wrede Ce et al.<sup>[10]</sup> In the present study, the mean HbA1c% in diabetic cases was  $9.48 \pm 2.65$  % and in the control group was  $5.01 \pm 0.45$ % and the difference was statistically significant with p value  $<0.01$ . Most of the studies earlier had reported that HbA1c is a good indicator of glycaemic control and highest values are found in cases with poorest control.<sup>[11]</sup> An elevated level of HbA1c is associated with increased development and progression of micro vascular complications in patients with type 2 diabetes mellitus.

In the present study, serum ferritin levels are increased in diabetic cases than the controls. The mean serum ferritin concentration among diabetic cases was  $359.11 \pm 87.18$  ng/ml with  $182.12 \pm 26.14$  ng/ml in control group. Data of our study was almost similar to the findings of Ford et al, Sumesh r et al in their studies and also was statistically significant with p value  $<0.01$ .<sup>12,13</sup> All the linear regression coefficients between ferritin concentration, FBS, Hb1ac showed positive correlation and statistically significant. The present study

indicates that serum ferritin was lowest in control group without diabetes and moderately raised in individuals with slightly elevated HbA1c levels and highest in cases with poorest glycaemic control with highly elevated HbA1c%. Most of the studies globally have indicated that serum ferritin can be employed as a marker for not only glucose homeostasis but also insulin resistance in both type 2 diabetics and control subjects. In a study by Jiang et al, CRP was also adjusted along with serum ferritin in diabetic cases because ferritin reflects both the storage of iron as well as acute inflammation.<sup>14,15</sup> Due to high levels of blood glucose in the cases of the study, there is an increased glucotoxicity which can contribute to oxidative stress and increased inflammation resulting consequently in higher serum ferritin levels. A significant positive correlation is seen between cases with high FBS, high PPBS, increased HbA1c% and serum ferritin cases of the study. The prevalence of hyperferritenemia is 74.2% in the diabetic cases in the present study which is similar to the findings in the study of Meghna Borah et al.<sup>16</sup>

To conclude this study clearly explains that there is a significant increase in the serum ferritin in cases of diabetes mellitus compared to normal controls and hyperferritenemia may be one of the causes for decreased insulin production and development of insulin resistance in diabetic individuals. It was also observed that poorly controlled diabetic cases with elevated HbA1c% had significant hyper ferritenemia. So it is suggestive that increased iron levels play a significant role in development of complications like microangiopathy, retinopathy in poorly controlled diabetics. Hence reliable and highly sensitive methods to detect free/catalytic iron that participates in tissue damage due to oxidative stress should be developed. Most of the studies reported that the levels of serum ferritin decreased after proper control of blood sugar levels which might be considered as one of the methods of diabetes indices control among diabetes subjects.<sup>[17]</sup> Hence the study of iron and related parameters may be a useful offshoot of the conventional studies on diabetes and its complications. Hence the need for measures which can reduce the iron overload like phlebotomy and iron chelation therapy may be considered as a measure in prevention of complications in cases with poor glycemic control. Hence monitoring the prevalence of serum ferritin is beneficial in long run among the diabetics.

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