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**Original Research Article**

# Study of Serum Ferritin and its relation with Glycemic control in Type 2 Diabetes Mellitus

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## Abstract

**Introduction:** Type 2 diabetes mellitus is a common metabolic disorder. It has been hypothesized that, oxidative stress plays a major role in the etiology of the diabetic process, as well as in pathogenesis of various diabetic complications. Appreciating the role of catalytic Iron released by high serum Ferritin levels, in causation of oxidative stress and insulin resistance, we carried out this study to understand the relationship between the serum Ferritin and type 2 diabetes mellitus and we also studied glycated haemoglobin to know the correlation between glycemic control and serum Ferritin.
**Objectives:** To compare the levels of serum Ferritin in patients with type 2 diabetes mellitus and non-diabetic healthy individuals. To know if any correlation exists between serum Ferritin and Glycated Hemoglobin in type 2 diabetes mellitus.
**Methods:** The study was conducted at Rajarajeswari Medical College and Hospital, Bengaluru, Karnataka from January 2012 to December 2012. The subjects of our study were fifty clinically diagnosed cases of type 2 diabetes mellitus and fifty non-diabetic healthy volunteers in the age group of 30 – 70 years who have satisfied the inclusion and exclusion criteria. Serum Ferritin levels and Glycated Hemoglobin were estimated by microplate immunoenzymo metric assay and ion exchange resin method respectively.
**Results:** Serum Ferritin levels were significantly high in all cases compared to controls. There was moderate co-relation between serum Ferritin and Glycated Haemoblobin.
**Conclusion:** The findings of the study project that, estimation of serum Ferritin may be useful in screening diabetics with poor glycemic control who are at high risk of developing complications. Thus serum Ferritin estimations in type 2 diabetes mellitus can be one of the adjuvant aids in investigations which guides in taking timely medical intervention and preventing further progression of disease.
**Keywords:** Type 2 Diabetes mellitus, serum Ferritin, Glycated hemoglobin.

## 1. Introduction

Diabetes mellitus is the most common endocrine disease. It is described as a group of metabolic disorders of multiple etiologies characterized by chronic hyperglycemia associated with disturbances of carbohydrate, fat and protein metabolism due to absolute or relative deficiency of insulin secretion and / or action [1]. This disorder is associated with significant long term sequels, particularly damage or dysfunction of various organs especially kidneys, eyes, nerves, heart and blood vessels [2].

It has been hypothesized that, oxidative stress plays a major role in the etiology of the diabetic process, as well as in pathogenesis of various diabetic complications. Appreciating the role of catalytic Iron released by high serum Ferritin levels in causation of oxidative stress and insulin resistance, we carried out this study to understand the relationship between the serum Ferritin and type 2 diabetes mellitus and we also studied glycated haemoglobin to know the correlation between glycemic control and serum Ferritin.

## 2. Methodology

Fifty clinically diagnosed cases of type 2 diabetes mellitus and fifty non-diabetic healthy volunteers in the age group of 30-70 years attending General Medicine OPD, Rajarajeswari Medical College and

Hospital, Bengaluru between January 2012 and December 2012 were cases and controls respectively. Study subjects with history of multiple transfusions, liver disease and pancreatitis, pregnancy, anemia and history of any other medical or surgical illness and cases with secondary complications of diabetes mellitus like micro or macro vascular disorders, were excluded from the study.

After obtaining Institutional ethical clearance, informed consent was taken from all cases and controls. For blood investigations, 5ml of venous blood was collected under aseptic precautions from both the study groups in the below mentioned tubes. Clot activator tubes for estimation of serum Ferritin and Fluoride EDTA tubes for estimation of blood glucose and glycated hemoglobin.

Serum Ferritin (Reference range: Males: 16-220 ng/ml and Females 10-124 ng/ml) was determined in serum by a microplate immunoenzymometric assay (ELISA) in Euphoria 4.0 Fully automated ELISA Processer. Up on mixing monoclonal biotinylated antibody and serum containing the native antigen, reaction results between the native antigen and the antibody, forming an antibody-antigen complex. Simultaneously the biotin attached to the antibody binds to the streptavidin coated on the microwells resulting in immobilization of the complex. After a suitable incubation period, the antibody-antigen bound fraction is separated from unbound antigen by decantation or aspiration. Another antibody(directed at a different epitope) labeled with an enzyme is added. Another interaction occurs to form an enzyme labeled antibody-antigen-biotinylated-antibody complex on the surface of the wells. Excess enzyme is washed off via a wash step. A suitable substrate is added to produce color measurable with the use of microplate spectrophotometer. The enzyme activity on the well is directly proportional to the native antigen concentration.

**2.1 Glycosylated Hemoglobin ($HbA_{1C}$) was estimated by Ion exchange resin method:**

Whole blood was mixed with lysing reagent to prepare a hemolysate, then mixed with a weakly binding cation-exchange resin. The non-glycosylated Hemoglobin binds to the resin leaving Glycosylated Hb free in the supernatant. The Glycosylated Hb percentage is determined by measuring the absorbance of the Glycosylated Hb fraction and of the total Hb.

**2.2 Expected range**

Non Diabetic: < 4.0-6.0%, Good Control: <7%, Fair Control: 7.0-8.0%, Poor Control: >8.0%

FBS and PPBS was determined by glucose-oxidase peroxidase method in fully automated Mindry BS 300 analyzer.

**2.3 Statistical Method**

Descriptive statistical analysis has been carried out. The data was entered in excel spreadsheet and statistically analyzed using Chi – Square t – test and Fisher extract.

## 3. Results

The mean age of the diabetics was 52.84 ± 8.46 years and of controls was 53.34 + 9.38 years. In both the study groups males and females were 44 % and 56 % respectively. The maximum numbers of patients were in the age group 41 – 60 years. The mean FBS was 155 mg/dl in cases and 86.78 mg/dl in controls. The mean PPBS was 254 mg/dl and 121 mg/dl in cases and controls respectively. The mean Glycated hemoglobin was 7.24 % in cases and 5.21 % in controls. The mean serum Ferritin was 199.48 ng/ml in cases and 54.18 in controls. The mean serum Ferritin in diabetic patients under control (≤ 7mg%) was 129.3 ng/ml and 223.023 ng/ml in uncontrolled(≥ 7mg% ) patients.

**Table 1: Age Distribution in study group**

| Age in years | Cases | | Controls | |
|---|---|---|---|---|
| | No | % | No | % |
| 30-40 | 6 | 12.0 | 5 | 10.0 |
| 41-50 | 18 | 36.0 | 17 | 34.0 |
| 51-60 | 15 | 30.0 | 14 | 28.0 |
| 61-70 | 11 | 22.0 | 14 | 28.0 |
| Total | 50 | 100.0 | 50 | 100.0 |
| Mean ± SD | 52.84±8.46 | | 53.71±9.09 | |

Samples are age matched with P=0.621

**Figure 1: Gender Distribution in study group**

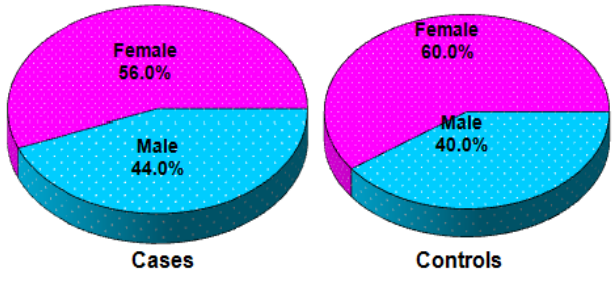


**Table 2: Comparison of Glucose parameters in study group**

| Glucose parameters | Cases | Controls | P value |
|---|---|---|---|
| FBS | 155.06±61.64 | 86.92±10.14 | <0.001** |
| PPBS | 254.16±100.30 | 121.35±11.52 | <0.001** |

**Table 3: Comparison of HbA1c in study group**

| HbA1c | Cases | | Controls | |
|---|---|---|---|---|
| | No | % | No | % |
| <7 | 19 | 38.0 | 50 | 100.0 |
| 7-8 | 20 | 40.0 | 0 | 0.0 |
| >8 | 11 | 22.0 | 0 | 0.0 |
| Total | 50 | 100.0 | 50 | 100.0 |
| Mean ± SD | 7.24±0.99 | | 5.21±0.28 | |

P=<0.001**

Non Diabetic: < 4.0-6.0%, Good Control: <7%, Fair Control: 7.0-8.0%, Poor Control: >8.0%

**Figure 2: Comparison of Serum Ferritin levels in study group**

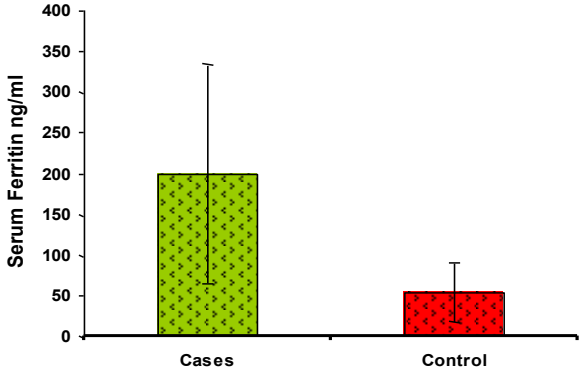


**Table 4: Comparison of Serum Ferritin levels in study group**

| Serum Ferritin ng/ml | Cases | Controls |
|---|---|---|
| <16 for Male; <10 for female | 0 | 0 |
| 16-220 for male & 10-124 for female | 24 (48.0%) | 50 (100.0%) |
| >220 for male & >124 for female | 26 (52.0%) | 0 |
| Total | 50 (100.0%) | 50 |

P<0.001**

(Reference range: Males: 16-220 ng/ml and Females 10-124 ng/ml)

**Table 5: Pearson correlation of Serum Ferritin ng/ml with FBS (mg/dl), PPBS (mg/dl), and HbA1c%**

| Pair | Cases | | Controls | |
|---|---|---|---|---|
| | r value | p value | r value | p value |
| Serum Ferritin ng/ml vs FBS (mg/dl), | 0.167 | 0.248 | 0.071 | 0.626 |
| Serum Ferritin ng/ml vs PPBS (mg/dl), | 0.272 | 0.056+ | 0.115 | 0.430 |
| Serum Ferritin ng/ml vs HbA1c% | 0.290 | 0.041* | 0.108 | 0.459 |

**Table 6: Serum Ferritin Level in Cases & Controls**

| S. Ferritin mg/ml | Cases | | | | Controls | | | |
|---|---|---|---|---|---|---|---|---|
| | Male | | Female | | Male | | Female | |
| | No | S. F. Level | No | S. F. Level | No | S. F. Level | No | S. F. Level |
| 10 – 124 | 4 | 90.25 | 12 | 81.81 | 21 | 67 | 28 | 38.64 |
| 125 – 220 | 8 | 156.12 | 6 | 160.14 | 1 | 220 | | |
| 201 – 300 | 5 | 244.6 | 8 | 254.62 | | | | |
| > 301 | 5 | 447.2 | 2 | 482.5 | | | | |
| Total | 22 | | 28 | | 22 | | 28 | |

## 4. Discussion

This study is a cross sectional study of fifty diabetic and fifty non-diabetic subjects. In our study females subjects were more than male and majority of them were in 41-60 years with average age of 52 years. The major aspect of management of type 2 diabetes mellitus is the requirement of sensitive serum markers to identify the development of the disease and its complications, so that early diagnosis and medical interventions can delay or diminish the morbidity associated with type 2 diabetes mellitus. This study was carried out to explore the possibility of utility of serum Ferritin as a marker of the oxidative stress process in diabetics. This valuable information would be helpful in proper medical intervention.

The fact that the iron is easily oxidized and reduced, which is essential for its normal metabolic functions, makes the iron potentially hazardous to participate in the generation of powerful oxidant species such as hydroxyl radicals[3].

Because iron participates in the formation of reactive oxygen species, organisms take care in the handling of iron and indeed, iron sequestration in transport and storage proteins (Ferritin). Iron is present in all most all cells of the body. About 75% of total iron is hemoglobin (Hb), 5% is in myoglobin and 15% in Ferritin[4]. Ferritin is ubiquitous intra cellular protein that stores iron and releases it in controlled fashion. It acts as buffer against iron deficiency and iron overload. The relationship between iron metabolism and type 2 DM may be bi-directional. Iron is a potent pro-oxidant that increases cell oxidative stress causing inhibition of insulin internalization and actions, resulting in hyperinsulinemia and insulin resistance. In hepatocytes and pancreas, iron accumulation may interfere with the insulin extracting capacity of the liver[5] and affect insulin synthesis and secretion in pancreas. Free iron exerts a positive feedback on Ferritin synthesis and oxidative stress increases the release of iron from Ferritin[6].

The abnormalities in Ferritin metabolism following glycation in hyperglycemic state might be a primary cause of hyperferritinaemia in type 2 DM (Glycated Ferritin has longer half-life). Glycation of transferrin decreases its ability to bind ferrous ion and by increasing the pool of free iron and stimulates Ferritin synthesis. Thus, type 2 DM is associated with abnormalities of Ferritin metabolism resulting in parallel increase of serum Ferritin levels [3][7].

In the present study, we observed significant ($P$<0.001) increase in serum Ferritin levels in diabetes mellitus cases compared to non-diabetic controls. Serum ferritin levels of all the controls were within the reference range. 26 cases (52%) had high serum ferritin levels where as 24 cases (48%) had serum ferritin level within the normal reference range but towards the higher side of the reference. This observation may be due to the better glycemic control in 48% of the cases (refer table No. 3 & 6). 22.7% of males and 7% of females had very high levels of serum ferritin. This finding may be due to the difference in the reference range among males and females (Reference range: Males: 16-220 ng/ml and Females 10-124 ng/ml). Fernandez *et al* and others [8-10] also have found increased serum Ferritin levels in the serum of diabetic subjects compared to controls. Jehn *et al*, argue that the modest elevations in Ferritin levels observed in diabetes may be a consequence rather than the cause of impending insulin resistance and that elevated Ferritin may not reflect elevated body iron stores or an intracellular labile iron pool that participates in oxidant injury[11-13].

We have also studied the HbA1c levels to know the correlation between glycemic control and serum Ferritin. The long term hyperglycemia status favours glycation reactions leading to formation of advanced glycated end products (AGE). This causes tissue damage by cross linking of collagen. Therefore complications in type 2 DM are dependent on glycemic control [6]. We observed highly significant difference in serum FBS and PPBS levels among cases and controls and also found that significant difference in Glycated Hemoglobin levels among cases and controls. Very high levels of serum ferritin were observed in cases with poor glycemic control. Similar results were observed by Smothra *et al* and others [14-16]. Studies have shown that short term improvement in glycemic control is followed by variable decreases in serum Ferritin concentration[17] and treating diabetic patients with desferoxamine[18]. Dymock MWJC *et al* support that phlebotomy is followed by drop in serum glucose due to improvement in both beta cell secretion and peripheral insulin action in type 2 diabetes mellitus and serum Ferritin values significantly decreased. Studies have also shown that that increased serum Ferritin levels are associated with increased serum insulin levels reflecting insulin resistance, poor glycemic control and complications of type 2 diabetes[19].

Our study shows that there is moderate correlation between increased serum Ferritin in diabetics compared to controls. Contrary to our study, F.Sharifi observed no correlation between serum Ferritin and blood glucose control in diabetes[20].

Hyperferritinemia may be one of the causes for the development of insulin resistance (diabetes mellitus) and its complications. However the study has to be carried out in large scale in south Indian populations.

## 5. Conclusion

The findings of this study project that estimation of serum Ferritin may be useful in screening diabetics with poor glycemic control who are at high risk of developing complications. Thus serum Ferritin estimations in type 2 DM, can be one of the adjuvant aids in investigations which guides in taking timely medical intervention and preventing further progression of disease.

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