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SERUM FERRITIN AND HbA1c LEVELS IN TYPE 2 DIABETES MELLITUS

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ABSTRACT

Introduction: Complications due to diabetes are a major cause of disability, reduced quality of life and death. Recent studies have emphasized the role of serum ferritin in insulin resistance and the incidence of diabetes mellitus. However, the role of ferritin as a marker of iron overload in pancreatic damage and peripheral insulin resistance or its role as an inflammatory marker is not clear. The aim of this study is to establish a correlation between serum ferritin, FBS and HbA1c in type 2 diabetes mellitus and to evaluate the role of serum ferritin on the glycemic status in type 2 diabetes mellitus. **Methods:** This was a cross- sectional study of 100 cases, visiting medical outpatient department of a tertiary care teaching hospital. Diabetic patients were compared with age and sex matched normal healthy controls. Effect of serum ferritin on glycemic status, gender and age was noted. **Results:** Statistically significant increase of FPG, HbA1C and serum ferritin levels were observed in type 2 diabetes mellitus group than controls in both females and males while there was no statistically significant difference of hemoglobin between diabetic group and controls in females and males. There was a high (r= 0.62, r= 0.66) positive correlation between SF and HbA1c of females and males respectively in diabetic group P-value=< 0.01. **Conclusion:** Higher positive correlation of serum ferritin with HbA1c shows that hyperglycemia affects ferritin levels possibly due to inflammation or oxidative stress or a combination of the two.

KEYWORDS: Type 2 diabetes mellitus, Glycosylated haemoglobin, Ferritin.

INTRODUCTION

Diabetes is a metabolic disorder characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both[1]. Diabetes has become a major public health concern both in developing and developed countries worldwide affecting 382 million people aged between 40 and 59 years and 80% of them live in low and middle income countries. It is expected to rise to 592 million by the year 2035 [2].

Diabetes imposes a large economic burden on individuals and families, national health systems and countries. Health spending on diabetes accounted for 10.8% of total health expenditure worldwide [3].

People with type-2 diabetes mellitus develop characteristic microvascular complications such as retinopathy, nephropathy and neuropathy. There is also increased risk of macrovascular complications such as cardiovascular, cerebrovascular and peripheral vascular disease [4].

Complications due to diabetes are a major cause of disability, reduced quality of life and death. Approximately 5.1 million people aged between 20 and 79 years died from diabetes accounting for 8.4% of global all cause mortality in this age group [5]. In India 65.1 million in the age group of 20 and 79 have diabetes (8.56%) and expected to rise to 109 million by the year 2035[2].

The pathogenesis of type 2 diabetes mellitus (T2DM) is complex and involves the interaction of genetic and environmental factors. Individuals with T2DM show both insulin resistance and beta cell defects [6]. Type 2 diabetes is largely preventable. A number of risk factors, such as

overweight and obesity and physical inactivity are modifiable, and can also help reduce the complications that are associated with diabetes. But in most countries, the prevalence of overweight and obesity continues to increase [7].

Hyperglycemia in diabetes mellitus not only defines the disease but is the cause of its most characteristic symptoms and long-term complications. The complications of diabetes mellitus are influenced not only by the duration of the diabetes mellitus but also by the average level of blood glucose along with glycated haemoglobin [4].

Serum ferritin is an acute phase reactant, and is a marker of iron stores in the body [8] Iron is a transitional metal that can easily become oxidized and thus act as an oxidant [9]. Increased accumulation of iron affects insulin synthesis and secretion in the pancreas and liver.

Recent studies have shown that serum ferritin was proportional to serum glucose concentration, diastolic blood pressure, HDL cholesterol, and insulin resistance. In fact, the higher the ferritin levels, the higher the incidence of type-2 diabetes [10, 11]. Amongst the various markers of glycemic control, glycated hemoglobin has now been established as the most reliable. However, ferritin's role as a marker of iron overload in pancreatic damage and peripheral insulin resistance or its role as an inflammatory marker is not clear. Hence this study aims to examine the relationship between serum ferritin and HbA1c levels in type-2 diabetes mellitus [12].

MATERIALS AND METHODS

This was a cross- sectional study of 100 cases, visiting medical outpatients department of Dr. PSIMS and RF, Gannavaram. The study was approved by the institutional ethical committee. 50 diabetic patients were compared with 50 age and sex matched normal healthy controls. The cases were randomly recruited. After taking a signed informed consent, all cases were subjected to a detailed questionnaire which included age of onset, duration of diabetes, treatment modalities, family history of diabetes and associated risk factors.

The selection criteria was based on patients having haemoglobin level of more than or equal to 10g/dl, known cases of type-2 diabetes mellitus (M/F) in the age group of 35-65 years and cases with duration of diabetes of 2-10 years were included in the study.

Diabetics with history or evidence of conditions leading to iron loss like gastro-intestinal blood loss, history of blood transfusion or donation in the last one year, subjects with type 1 diabetes mellitus, gestational diabetes mellitus, hemochromatosis, thalassemia, hemosiderosis, on iron supplementation, thiazide diuretics, antioxidants drugs, steroids, subjects with chronic infections, neoplastic condition, renal disease, liver disease, alcoholics, smokers, critically ill patients admitted in intensive care unit were all excluded from the study.

The serum ferritin, HbA1c, FPG, and hemoglobin percentage were estimated in 50 normal healthy individuals (control group) and 50 cases of type-2 diabetes mellitus (diabetic group). Both controls and cases were grouped based on their gender. There were 24 and 26 numbers of female and male controls and 25 numbers each of female and male cases.

The subjects were asked to fast for a period of at least 8hrs. 5ml of venous blood was drawn from each person. Samples were used for the measurement of the plasma glucose by glucose oxidase method, HbA1c by latex agglutination inhibition assay by Randox Daytona autoanalyzer and serum ferritin by chemilumescence method by Lumax Monobind analyzer. Blood hemoglobin was estimated using spectrophotometer by cyanmethaemoglobin method. Hemolysed samples were discarded and all reagents used were brought to at room temperature before analysis.

STATISTICAL ANALYSIS

Statistical analysis was done using SPSS software (Graph pad Prism 6). Statistical analysis of the following parameters, serum ferritin(SF), glycated hemoglobin (HbA1c), fasting plasma glucose (FPG) and hemoglobin percentage (Hb %) were done by t – test using graph pad (prism 6). Interpretation was based on comparison between controls and diabetic group and according to age and gender. Unpaired t-test was used for quantitative variables and Pearson's correlation coefficient for comparison of other variables. A P-value of <0.05 was considered significant and >0.05 as non-significant.

RESULTS

The mean age of the controls in years are 40.41 ± 5.50 and 40.73 ±8.16 in females and males respectively. The mean age of the cases in years are 41.2 ± 8.06 and 43.32 ± 8.35 in females and males respectively. There was no statistical significant difference of age between the two groups in either gender.

The mean FPG, HbA1c and serum ferritin levels were increased in diabetic group compared to controls while hemoglobin levels were decreased in diabetic group. There is statistically significant difference between FPG, HbA1c and serum ferritin of diabetic and controls and no statistically significant difference of hemoglobin levels between the two groups [Table 1].

Table 1. Comparison of parameters in control & diabetic groups

Parameters	Controls	Diabetic	P value
FPG (mg/dl)	85.48 ± 12.08	182.26 ± 54.42	<0.05
HbA1c (%)	4.46 ± 0.60	7.15 ± 0.98	<0.05
Serum Ferritin (µg/l)	16.71 ± 6.27	134.37 ± 65.79	<0.05
Hb (g/dl)	11.79 ± 0.93	11.49 ± 1.18	>0.05

The mean fasting plasma glucose levels, HbA1c levels, serum ferritin levels were more in diabetic group compared to controls in both females and males, while hemoglobin levels was more or less equal in both in diabetic and control groups [Table 2].

Table 2. FPG, HbA1c, Serum Ferritin and Hb in control and diabetic groups

Diabetic / Control by gender	FPG (mg/dl)	HbA1c (%)	SF (µg/l)	Hb (g/dl)
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Female (controls)	82 ± 12.53	4.36 ± 0.65	14.79 ± 6.09	11.3 ± 0.81
Female (Diabetic)	157.4 ± 52.22	6.75 ± 0.95	96.95 ± 46.66	10.86 ± 1.09
Male (controls)	88.69 ± 10.92	4.56 ± 0.55	18.49 ± 6.02	12.25 ± 0.82
Male (Diabetic)	207.12 ± 45.09	7.56 ± 0.87	171.80 ± 61.18	12.13 ± 0.91

Males had higher serum ferritin levels compared to females both in diabetic and control groups. A small variation of mean HbA1c levels was observed between males and females in both diabetic and controls. Mean fasting plasma glucose levels were higher in males than females. A small variation in Hemoglobin levels was seen on comparison of males and females [Table 2].

Statistically significant increase of FPG, HbA1C and serum ferritin levels were observed in cases of type 2 diabetes mellitus than controls in both females and males while there was no statistically significant difference of hemoglobin between diabetic and controls in females and males. [Table 3].

Table 3. p - value (comparison between control & diabetic groups)

Gender	HbA1c	SF	FPG	Hb
Male	<0.05	<0.05	<0.05	>0.05
Female	<0.05	<0.05	<0.05	>0.05

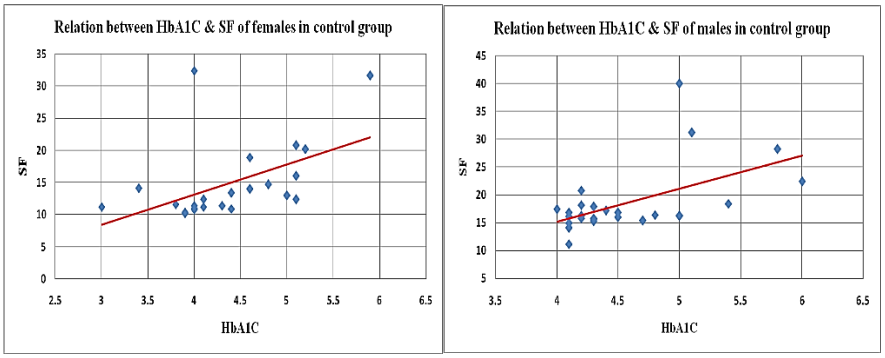
Comparison of serum ferritin was done with HbA1c, FPG and age in both genders.

There was a moderate (r= 0.50, r = 0.54) positive correlation between SF and HbA1c of females and males respectively in controls, p-value =< 0.05 in both [Figure 1]. There was a high (r= 0.62, r= 0.66) positive correlation between SF and HbA1c of females and males respectively in diabetic group, p-value=<0.01 in both [Figure 1].

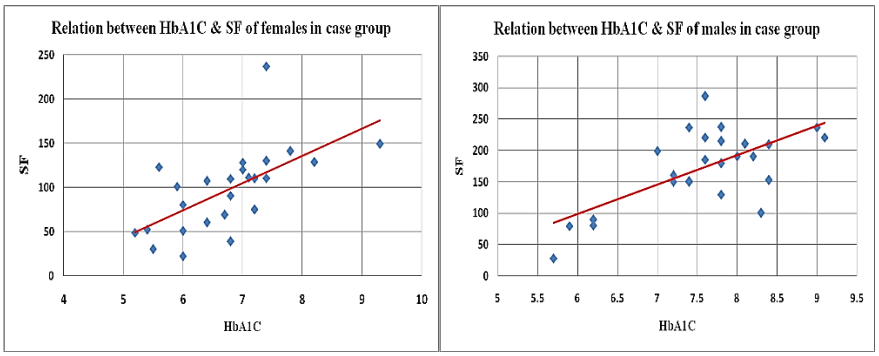
There was a low (r = 0.37, r = 0.43) positive correlation between SF and FPG of females and males respectively in controls p-value=>0.05 in both [Figure-2].There was a moderate (r=0.51 r= 0.46) positive correlation between SF and FPG of females and males respectively in diabetic group, p-value=<0.01[Figure 2]. There was a low (r = 0.2, r= 0.19) positive correlation between SF and age of females and males respectively in controls, p-value =>0.05 in both [Figure 3]. There was a low (r = 0.28, r= 0.10) positive correlation between SF and age of females and males respectively in diabetic group, p-value =>0.05 in both [Figure 3]

DISCUSSION

Type 2 diabetes mellitus (DM) is a chronic metabolic disorder and its prevalence has been increasing steadily all over the world. People living with type 2 DM are more vulnerable to

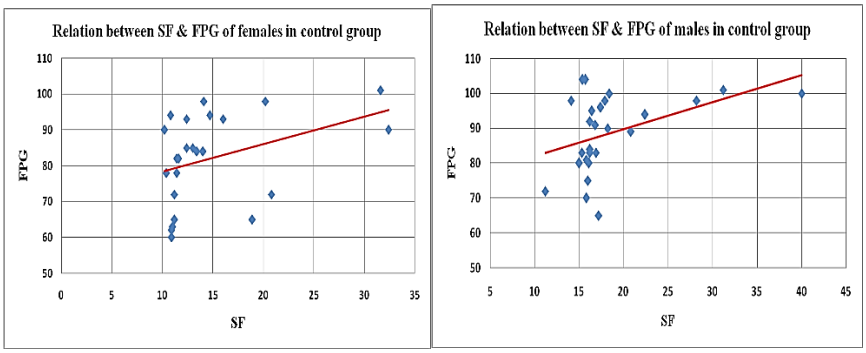


i) r= 0.50 ii) r = 0.54

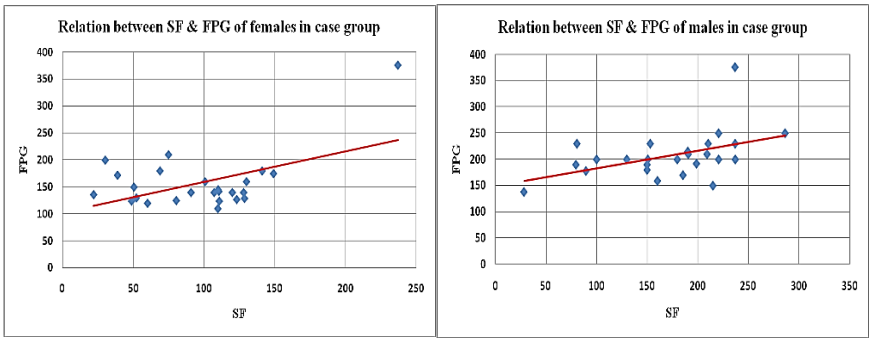


iii) r= 0.62 iv) r= 0.66

Figure 1. Relation between SF & HbA1c of females and males in control and diabetic groups (cases)

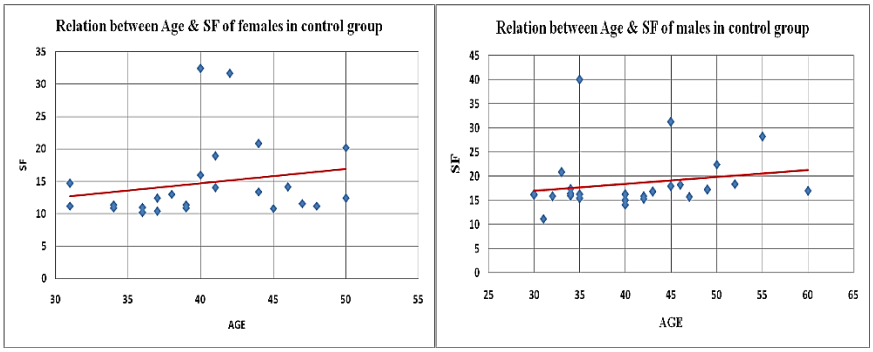


i) r = 0.37 ii) r = 0.43

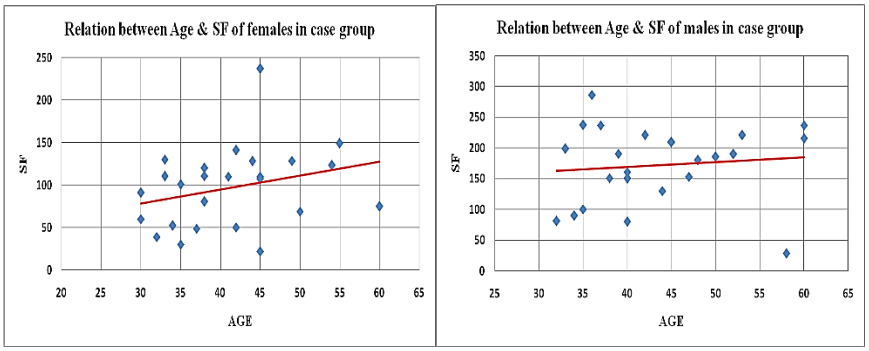


iii) r=0.51 iv) r= 0.46

Figure 2. Relation between SF & FPG of females and males in control and diabetic groups (cases)



i) r = 0.2 ii) r = 0.19



iii) r = 0.28 iv) r= 0.10

Figure 3. Relation between SF & Age of females and males in control and diabetic groups (cases)

various forms of both short- and long- term complications, which often lead to their premature death.

Oxidative stress has been implicated in the pathogenesis of the complications seen in type 2 DM [13]. "Oxidative stress", is defined as the oxidation of cellular components thereby introducing/producing harm to that cellular tissue. Oxidation reactions ensure that molecular oxygen is completely reduced to water, but the products of partial reduction of oxygen are highly reactive and create havoc in the living systems. Hence, they are also called reactive oxygen species (ROS) [14].

Oxygen normally accepts four electrons and is converted directly to water. However, partial reduction of oxygen can and does occur in biological systems. Thus, the sequential reduction of oxygen along the univalent pathway leads to the generation of superoxide anion, hydrogen peroxide, hydroxyl radical, and water [15, 16]. Superoxide and hydrogen peroxide appear to be the primary generated species. These species may then play a role in the generation of additional and more reactive oxidants, including the highly reactive hydroxyl radical (or a related highly oxidizing species) in which iron salts play a catalytic role in a reaction. This reaction is commonly referred to as the metal catalyzed Haber-Weiss reaction [15]:

$$Fe^{3+} + O_2 \longrightarrow Fe^{2+} + O_2$$

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+}\,OH + OH^-$$

$$O2^- + H_2O_2 \longrightarrow .OH + OH^-$$

Iron is the most abundant trace element in the body, and almost all iron occurs bound to proteins. Iron is a double-edged sword. In moderate quantities and leashed to protein, it is an essential element in all cell metabolism and growth, but it is toxic when unleashed [17].

Because of its ability to switch back and forth between ferrous and ferric oxidation states, iron is both a strong biological oxidant and reductant. Although the exact mechanism of iron-induced diabetes is uncertain, it is likely, to be mediated by three

key mechanisms: Insulin deficiency, insulin resistance and hepatic dysfunction [18].

The central importance of iron in the pathophysiology of disease is derived from the ease with which iron is reversibly oxidized and reduced. This property, while essential for its metabolic functions, makes iron potentially hazardous because of its ability to participate in the generation of powerful oxidant species such as hydroxyl radical [15].

Free radical damage is produced primarily by the hydroxyl radical (OH). Most of the OH generated in vivo comes from iron-dependent reduction of H_2O_2. When ferrous iron reduces H_2O to generate OH, it becomes ferric iron. Vitamin C (ascorbic acid) converts ferric iron back to ferrous iron, itself becoming oxidized ascorbic acid, thus allowing another cycle of .OH generation from renewed ferrous iron.

Another endogenous source of catalytic free iron is the iron released when the heme ring is opened by hemeoxygenase[9]. The intracellular generation of apoferritin is a cytoprotective antioxidant stratagem of endothelial cells [19, 20], since serum ferritin is elevated in T2DM.

Under normal conditions a quantitative relationship exists between the level of plasma ferritin and the amount of storage iron [8]. In conditions of iron overload there is generally an increase in the expression of intracellular L-subunit rich ferritins, paralleled by an increase in these ferritins in the plasma [21].

Ferritin is considered a positive acute phase protein and is upregulated intracellularly in many cell types, and extracellularly, in the plasma as a result of an increase in cellular secretion. An important role for ferritin during the acute phase response is to restrict the availability of iron by sequestration into the cavity of the ferritin protein shell.

Oxygen radicals i.e., molecules containing unpaired electrons are generated in large amounts during infectious and inflammatory conditions [22]. They react with proteins, lipids and nucleic acids, resulting in degradation of the phagocytosed material in the confinements of the phagosome in the neutrophil and macrophage.

However, large amounts of these toxic metabolites leak to the fluids and tissues in the area of the inflammatory reaction and by reacting with cellular constituents can result in substantial damage [23]. Iron, due to its role in Fenton-type chemistry, can result in exacerbation of oxygen radical production.

In general, a reduction in the bio-availability of iron will offer protection against cell injury by hydroxyl radicals that are generated from neutrophil- and macrophage-derived superoxides [24]. Iron sequestration by cells in the zone of inflammation may therefore provide protection against the free radical assault [25]. This role of host cell protection against an increase in the free radical onslaught is consistent with observations that a reduction in ferritin sensitizes cells to pro-oxidant cytotoxicity, and that overexpression of ferritin reduces reactive oxidant species (ROS) in cells challenged by oxidants and by implication reduces the oxidative toxicity [8].

The role of iron in the pathogenesis of diabetes is suggested by an increased incidence of type 2 diabetes in diverse causes of iron overload and reversal or improvement in diabetes (glycemic control) with a reduction in iron load achieved using either phlebotomy or iron chelation therapy [18].The importance of protein glycation is well known in the pathogenesis of diabetic vascular complications. Transition metals also play a role in protein glycation induced by hyperglycemia. It has been shown that glycated proteins have a substantial affinity for the transition metals, and the bound metal retains redox activity and participates in catalytic oxidation. Thus, should similar glycochelates form in vivo, reactions mediated by the chelates could be involved in the vascular complications of diabetes [26].

Different theories regarding the role of ferritin in DM have been suggested. Ferritin has been referred as a marker for insulin resistance possibly due to iron deposition in the liver leading to hepatic insulin resistance and increased hepatic glucose production [10, 27]. Others has determined ferritin just as a marker of pancreatic inflammation, while pancreatic damage due to some degree of subclinical hemochromatosis has been considered in some cases of diabetes [12].

Two large epidemiological studies reported a strong association between elevated serum ferritin concentration and increased risk for diabetes [28, 29]. Recently this was also noted in two separate

studies by F. Sharifi et al in impaired glucose tolerance (IGT) and type2 DM [12]. In this study a statistically significant increase in FPG, HbA1c and serum ferritin levels were observed in type 2 DM compared to normal healthy controls. In the study by F. Sharifi et al [12] no significant difference was observed between normal controls and diabetics regarding age and haemoglobin, no significant correlation between serum ferritin, blood sugar or HbA1c (r= 0.23). A study by Jose- Manuel Fernandez [27] reported a correlation (r=0.44) between serum ferritin and basal serum glucose and no correlation with age and HbA1c.In this study, too no significant variation of haemoglobin levels were observed between diabetics and healthy controls. In contrast to the above studies a low to moderate positive correlation of serum ferritin with age and FPG and high positive correlation with HbA1c were noted in both genders.

CONCLUSION

Increased levels of serum ferritin in diabetic cases as compared to controls indicates that serum ferritin can be used as a marker of type2 DM. Higher positive correlation of serum ferritin with HbA1c shows that metabolic control or dysglycemia affects ferritin levels possibly due to inflammation or oxidative stress or a combination of the two as both mechanisms play an important role in pathogenesis of type 2 DM.

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CONFLICT OF INTEREST

Nil.

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