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A Co-relative study of HbA1c and Serum Ferritin in Type 2 Diabetes

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Abstract
Background: *Type 2 Diabetes is a chronic metabolic disorder associated with increased mobidity and mortality. Raised serum ferritin levels reflects the increased body iron stores and is often associated with insulin resistance and increased incidence of Type 2 diabetes.*
Objective: *The study aims at estimation and comparison of FBG ,PPBG,HbA1c and serum ferritin in Type 2 diabetics and controls and establish a relationship between FBG,PPBG,HbA1c with serum ferritin.*
Material and methods*: A total of 100 subjects were included in the study 50 controls and 50 patients with Type 2 diabetes.*
Results: *The mean levels of FBG,PPBG,HbA1c and serum ferritin were significantly high in patients of Type 2 diabetes compared to controls(p <0.01). There was positive co-relation between HbA1c and serum ferritin in Type 2 diabetes.*
Conclusion: *The study showed a positive co-relation between HbA1c and serum ferritin.*
Keywords: Type 2 diabetes ,FBG(Fasting blood glucose) ,PPBG(Postprandial blood glucose) HbA1c,serum ferritin.

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I. Introduction

Diabetes mellitus is one of the most common problems caused by a combination of insulin resistance and impaired insulin secretion by pancreatic β cells.[1] Type 2 diabetes has a rising attitude globally. The worldwide spread of diabetes among general population is estimated to increase to 300 million in 2025.[2,3]

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. [4] Diabetes mellitus 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 . [5] People with 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.[6]

The pathogenesis of Type 2 diabetes involves the interaction of genetic and environmental factors. Individuals with Type 2 diabetes show both insulin resistance and beta cell defects. [8] The complications of diabetes mellitus are influenced by the duration of the diabetes mellitus and the average level of blood glucose as indicated by HbA1c. [6]

Glycated hemoglobin (HbA1c) is a stable, irreversible product of non-enzymatic glycosylation of the hemoglobin β-chain by serum glucose. HbA1c is used as an indicator for the state of glycemic control, progression of the disease and development of complications in diabetic patients. [7]

Serum ferritin is an acute phase reactant and a marker of iron stores in the body. [9,12]Iron is a transitional metal that can easily become oxidized and thus act as an oxidant. [10] Increased accumulation of iron affects insulin synthesis and secretion in the pancreas and liver. [10] Elevated iron stores may increase the risk of developing diabetes. [11] Both iron and glucose affect each others' metabolism. [10] Moreover it is recognized that iron influences glucose metabolism even in the absence of significant iron overload. [11]

Recent studies have shown that serum ferritin was proportional to serum glucose concentration, diastolic blood pressure, HDL cholesterol, and insulin resistance. In fact, higher the ferritin levels, higher the incidence of Type 2 diabetes. [13,14] Studies have demonstrated that increased body iron stores are associated with the development of glucose intolerance, gestational diabetes, Type 2 diabetes and insulin resistance syndrome. [15-19] Patients with increased serum ferritin levels co-relate with complications of Type 2 diabetes like retinopathy, nephropathy and vascular dysfunction. [20-22]

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 was carried out to examine the relationship between serum ferritin and Type 2 diabetes and to establish a correlation between serum ferritin and FBG, PPBG as well as with glycated hemoglobin (HbA1c%).

II. Material and methods

This prospective comparative study was carried out on patients of Department of Biochemistry at Dr. Shankarrao Chavan Government Medical College and Hospital, Vishnupuri, Nanded, Maharashtra from March 2021 to November 2021. A total 100 adult subjects (both male and females) of aged ≥ 40, years were for in this study.

Study Design: Prospective observational study

Study Location: of Department of Biochemistry at Dr. Shankarrao Chavan Government Medical College and Hospital, Vishnupuri, Nanded, Maharashtra

Sample size calculation: The sample size was estimated on the basis of a single proportion design. The target population from which we randomly selected our sample was considered 20,000. We assumed that the confidence interval of 10% and confidencelevel of 95%. The sample size actually obtained for this study was 188 patients for each group. We planned to include 400 patients (Group I- Control 200 subjects, Group II- Cases 200 patients) .

Subjects & selection method: The study population was drawn from consecutive diabetic patients who presented to Dr. Shankarrao Chavan Government Medical College and Hospital for routine monthly checkupand blood investigations.

The subjects were divided into two groups :

Group 1 (Control) includes 200 age and gender matched healthy controls selected randomly visiting OPD for routine checkup.

Group 2(Cases) includes 200 diagnosed cases of Type 2 diabetes treated in our college on OPD basis.

Inclusion criteria:

Cases: Diagnosed patients of Type 2 diabetes within the age group of 40 to 65 yrs

Controls: Healthy subjects in the age group of 40 to 65 yrs with normal blood glucose levels.

Exclusion criteria:

The patients with following diseases were excluded from the study-

1.Patients with anemia, males with Hb < 13 g/dl and females with Hb <12 g/dl
2.Patients on iron therapy in the past 3 months
3.Chronic kidney disease
4.Chronic Liver disease
5.Thyroid dysfunction
6.Acute infections, fever
7.Malignancies
8.Corticosteroid therapy
9.Pregnant and lactating females

Under all aseptic and antiseptic conditions 5 ml of blood sample was collected from each subject from a suitable peripheral vein (preferably antecubital vein) by venipuncture using a sterile disposable syringe and divided into a sterile empty vial and an EDTA vial. EDTA vials are used for estimation of glycated hemoglobin. The rest of the sample was then allowed to stand for some time and then centrifuged for separation of serum. This serum was used for estimation of the other parameters.

Procedure methodology

After written informed consent was obtained, a well-designed questionnaire was used to collect the data of the recruited patients retrospectively. The questionnaire included socio-demographic characteristics such as age, gender, nationality, height, weight, and consanguineous marriage, physical activity and lifestyle habits like smoking and alcohol , type of DM, its duration, and clinical and biochemistry laboratory investigations such as fasting blood glucose, glycated hemoglobin (HbA1c), and serum ferritin.

All parameters were quantified on samples collected in the fasting state. The following tests were done in the above subjects

1) Fasting Plasma Glucose and Post –prandial Plasma Glucose by GOD-POD method
2) HbA1c by cation –exchange resin method.
3) Ferritin by Immunoinhibition method.

Statistical analysis

Statistical analysis was done using SPSS software (Graph pad Prism 6). Statistical analysis of the following parameters, FBG (fasting plasma glucose) ,PPBG(Post prandial blood glucose), glycated hemoglobin (HbA1c), and serum ferritin(SF)were done by t – test using graph pad (prism 6). The type 2 diabetics were divided into 5 subgroups based on HbA1c levels and were compared by One-way ANOVA. 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.

IV. Results

This study group comprised of 400 subjects (200 cases and 200 controls). The parameters in the **table 1** were compared between Type 2 diabetics and controls. BMI was found to be significantly high in the cases as compared to controls. The FBG, PPBG , HbA1c and serum ferritin were significantly high in the Type 2 diabetics compared to controls (Table 1). The mean value of HbA1c in Type 2 diabetics (9.31 ± 2.19)was significantly high compared to controls (5.06 ± 0.24) .The mean serum ferritin levels in Type 2 diabetics was 165.20 ±81.09 compared to 43.02 ±17.81 in the controls which was statistically significant ($p < 0.01$) (Table 1).

Table1: Comparison of anthropometric and chemical parameters in controls and Type 2 Diabetes.

	Parameters	Group-1 Controls	Group-2 Type 2 Diabetes	p value
1	BMI (kg/m^2)	20.67 ± 2.36	24.69 ± 2.14	<0.01
2	FBG(mg/dl)	80.21 ± 11.45	153 ±72.02	<0.01
3	PPBG(mg/dl)	120.43 ± 12.32	276.28 ± 97.72	<0.01
4	$HbA1_C$ %	5.06 ± 0.24	9.31 ± 2.19	<0.01
5	Sr.Ferritin (ng/ml)	43.02 ±17.81	165.20 ±81.09	<0.01

The Type 2 diabetics were further divied into 5 groups based on HbA1c % levels (Table 2).Most of the patients showed HbA1c between 8.1% to 10%. It was found that highest serum ferritin levels (229.52 ± 8.18 ng/ml) were found in cases with HbA1c ≥10.1% (11.12 ±0.34 %). Whereas the cases with good glycemic control with HbA1c % within 6-7 % had lowest serum ferritin levels (90.23 ± 40.43 ng/ml). The variables were compared in the 5 subgroups by One-way ANOVA. The HbA1c % showed a strong positive correlation (r= 0.94) with serum ferritin which was highly significant ($p<0.01$) also (Table 2)(Fig 1).

Table2: Correlation between HbA1c and serum ferritin levels in Type 2 Diabetes

$HbA1_C$ Range %	Mean $HbA1_C$	No of patients in each group	Mean Ferritin (ng/ml)	p value	r value
6.0 -7.0	6.56 ±0.21	23	90.23 ± 40.43	<0.01	0.94
7.1-8.0	7.24 ± 0.53	34	102.47 ±10.73		
8.1 – 9.0	8.51 ± 0.40	58	119.14 ± 19.82		
9.1 -10.0	9.12 ± 0.27	65	167.28 ± 29.15		
≥10.1	11.12 ±0.34	20	229.52 ± 8.18		

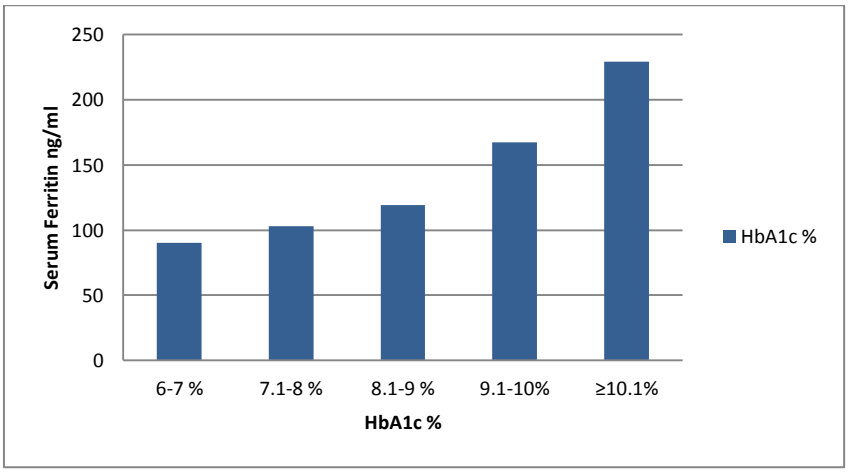

Figure 1: HbA1c % versus serum ferritin (ng/ml) in Type 2 diabetes

In the study, it was also observed that there was strong positive correlation between serum ferritin and FBG (r = 0.72) and PPBG (r = 0.58)which was also highly significant (p<0.01).

IV. Discussion

Type 2 diabetes is a chronic metabolic disorder with short term and long term complications leading to morbidity and mortality.Our study showed a highy significant difference in BMI between the cases and controls. Similar findings were obtained by Borah M et al . [23]

In our study the mean FBG levels were 80.21 ± 11.45 in the controls and 153 ±72.02 in the cases The FBG a level was significantly high (p<0.01) in Type 2 diabetics showing a positive correlation with serum ferritin . Similar findings were found by Borah M et al[23], Pardeep Agarwal et al, [24] Raj S et al, [25]Sultan et al, [26] and Padmaja et al. [27]

In our study the mean PPBG levels were 120.43 ± 12.32 in the controls and 276.28 ± 97.72 in the cases The PPBG level was significantly high (p<0.01) in Type 2 diabetics showing a positive correlation with serum ferritin . Similar findings were found by Mukesh et al[28] and Patil et al. [29] But study by Raj et al [25] found no correlation between serum ferritin and postprandial sugar levels. In Type 2 diabetes increase in FBG and PPBG is due to increased insulin resistance and decreased production of insulin and usually a combination of both.

In the present study we found that the mean levels of HbA1c% in controls and Type 2 diabetics were 5.06 ± 0.24% and 9.31 ± 2.19 % respectively. The difference was highly significant (p<0.01)These finding correlated with Borah M et al, [23] Pardeep Agarwal et al, [24] Arora et al, [30] Trivelli LA et al [31] and Koenig RJ et al. [32] HbA1c reflects the average blood glucose over past 3 months. The poorly controlled Type 2 diabetics show increased HbA1c due to increased glycation of hemoglobin.

The serum ferritin levels in the Type 2 diabetics was found to be higher than that in the control group and it was statistically highly significant (p<0.01) Similar findings were obtained by Canturk Z et al,[22] Sharifi et al , [38] Ford et al, [39] Tuomainen et al, [40] Eschwege et al , [41] Kim et al[42] andSmotra S et al. [43] They concluded that serum ferritin can be employed as a marker of not only glucose homeostasis but also insulin resistance both in Type 2 diabetics and control subjects. Elevated ferritin levels were 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. [43]

It was also observed in the present study that serum ferritin levels show a positive correlation with HbA1c%, that is statistically highly significant (p<0.001). The Pearson's correlation coefficient "r" found to be 0.94 establishes the strong positive correlation between the two parameters. Serum ferritin levels also had a positive correlation with FBG and PPBG(r= > 0.5). Similar findings were obtained by Canturk, [22] Eschwege E, [39] Ford et al [41] and Smotra S et al. [43]

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 proteins, it is an essential element in all cell metabolism and growth, but it is toxic when unleashed. [33]

Superoxide and hydrogen peroxide produce highly reactive hydroxyl radical in which iron salts play a catalytic role in a reaction. This reaction is commonly referred to as the metal catalyzed Haber-Weiss reaction . [34]

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^{\cdot} + OH^{-}$$

The role of iron in the pathogenesis of Type 2 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 phelobotomy or iron chelation therapy. [35] 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 thereby incrases the production of free radicals in the body. Thus, these glycochelates formed in vivo, could also be involved in the vascular complications of diabetes. [36]

Different theories regarding the role of ferritin in Type 2 diabetes 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. [13,37] Others determined serum 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. [38]

A prospective case control study conducted by Thilip Kumar G et al. [17] reported that patients with Type 2 diabetes had significantly higher serum ferritin level when compared to healthy controls but there is no correlation between serum ferritin with mean blood glucose and HbA1c 2. A study by Jose- Manuel Fernande[37]

reported a correlation between serum ferritin with basal plasma glucose and no correlation with HbA1c in diabetics and normal controls.

V. Conclusion

Increased ferritin levels possibly due to inflammation or oxidative stress or a combination of the two mechanisms play an important role in pathogenesis of Type 2 diabetes. Higher positive correlation of serum ferritin with HbA1c shows poor metabolic control. Increased levels of serum ferritin in Type 2 diabetes as compared to controls indicates that serum ferritin may be used as a marker of severity of Type 2 diabetes but further studies on large scale are required.

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