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CORRELATION BETWEEN SERUM FERRITIN AND GLYCATED HEMOGLOBIN IN PATIENTS OF TYPE 2 DIABETES MELLITUS

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Abstract

Background: Type 2 diabetes mellitus is a metabolic disease with irreversible organ damage. Although fasting plasma glucose and glycosylated hemoglobin are used for screening to identify individuals with type 2 DM, in certain scenarios there may be spurious results. Iron and ferritin have been implicated in the pathogenesis of type 2 DM. **Materials And Methods:** The study was a case control, observational study performed in a tertiary hospital in coastal Karnataka. It included 60 cases of type 2 diabetes mellitus fulfilling the inclusion criteria and 60 age, gender matched controls. Demographic details, comprehensive history, physical examination and relevant laboratory tests including fasting plasma glucose, serum ferritin and glycosylated hemoglobin were performed on both cases and controls. **Results:** There is a significant correlation between serum ferritin levels, duration of diabetes and glycosylated hemoglobin levels on performing Karl Pearson correlation, with t-test p value 0.000 ($p<0.05$) between cases and controls. Serum ferritin value of 187ng/mL can be used as cut off value to identify cases and controls with sensitivity of 98.3% and specificity of 100% (AUC- 0.978). **Conclusions:** There is a significant positive linear association between serum ferritin levels, fasting plasma glucose, duration of diabetes and glycated hemoglobin and a cut off value of 187 ng/mL could potentially be used as a screening test apart from fasting glucose and glycosylated hemoglobin for identifying patients with type 2 diabetes mellitus

Keywords: Type 2 diabetes mellitus, serum ferritin, glycosylated hemoglobin, hyperferritinemia, hyperglycemia

Introduction

Diabetes Mellitus is a complex metabolic disease marked by persistent hyperglycemia leading to irreversible organ damage resulting in significant morbidity and mortality. Global diabetes prevalence was about 8.8% of the world population in 2017, which is expected to further increase to 9.9% by the year 2045. In India(2010) 65.1 million people have diabetes(8.56%) which is expected to rise to 109 million by the year 2035.(1) Iron has been

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identified to a play a role in the pathogenesis of type 2 diabetes mellitus through one of the following mechanisms: hepatic dysfunction, insulin resistance through activation of JNK (c-Jun N- terminal kinase) signaling pathway by reactive oxygen species generated by iron catalyzed reactions and insulin deficiency secondary to B-cell apoptosis induced by oxidative stress due to catalytic iron. (2) Serum ferritin is an acute phase reactant and is a marker of iron stores in the body. (3) Higher levels of ferritin may contribute to the development of both micro-vascular and macro-vascular diabetic complications.(4,5)

Glycosylated hemoglobin (HbA1c) is formed due to irreversible glycosylation of the hemoglobin by glucose. Glycated haemoglobin is the most reliable marker of glycemic control. However, several conditions can result in spurious values of HbA1C namely different ethnicity, age related increase, hemoglobinopathies, gestational diabetes mellitus, recent blood transfusion, alcohol intake, chronic opioid usage, splenectomy, use of certain drugs like ribavarin, aspirin, dapsone, uraemia and severe hyperbilirubinaemia. (6)

Studies have shown that ferritin levels are proportional to glucose levels, diastolic BP, HDL cholesterol levels and also to insulin resistance. (7,8) Hence the above study was carried out to establish the association between ferritin and glycated haemoglobin in patients with Type 2 DM

AIM: To establish an association between serum ferritin and HbA1c; and to evaluate the role of serum ferritin on glycemic status in Type 2 diabetic patients.

Materials And Methods

The study was a case control study conducted in a tertiary care hospital in Coastal Karnataka. The study included individuals with type 2 diabetes mellitus in the age group 35-70 years of both genders admitted in medicine wards as cases. Patients with anaemia (Haemoglobin <10gm/dl), acute and chronic infections, chronic liver disease, chronic kidney disease, hemoglobinopathies, those on corticosteroid therapy and overt thyroid dysfunction were excluded from the study. The controls were selected from healthy individuals aged between 35-70 years recruited from bystanders. The sample size was calculated with 95% confidence interval and 80% power. The study included 60 cases and 60 controls. The study was performed after obtaining institutional ethical committee clearance (IEC 09-19/438)

The patients who are diagnosed to have diabetes mellitus, admitted in the inpatient department were subjected to a detailed clinical history. The patients who fulfil the inclusion criteria and are willing to participate in the study, were enrolled in the study. Enrolment of eligible participants was done only after obtaining an informed consent from the participant. Confidentiality of the patients "details was maintained at all levels. Complete demographic details, and relevant clinical and laboratory parameters were collected. After obtaining informed consent, 5ml of fasting venous blood was collected with dry disposable syringe under aseptic conditions in sterile, dry vial for biochemical analysis. Fasting Plasma Glucose (FBS) was assessed by glucose oxidase method. For HbA1c, sample of whole blood was taken in EDTA vial and analysed by HbA1c kit using Ion- exchange resin method. Serum Ferritin was assessed by DRG Ferritin Kit using principle of ELISA (Enzyme-Linked Immune Sorbent Assay). FBS, serum ferritin and HbA1C of age and gender matched non-diabetics was assessed for controls.

The collected data was coded and entered into Statistical Package for Social Sciences (SPSS). Detailed statistical analysis was done, and values were expressed in mean (with standard deviation). Correlation between the variables was analysed using Pearson"s

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correlation coefficient. Statistical Package SPSS version 23.0 was used for analysis and p value <0.05 was considered as statistically significant. Results were summarized using appropriate tables and figures.

Results

The baseline characteristics of the cases and controls included in the study have been tabulated in Table 1. Majority of study participants were males (73.3%). Half the study participants (cases and controls) belonged to the age group 46-55 years (50%). Among the cases 4 of them had diabetes mellitus for <5 years duration, 4 had >15 years duration, 21 had between 6-10 years, while majority i.e., 31 had between 11-15 years duration. 40 out of 60 cases were on treatment with only OHA, 14 were on treatment with only insulin (in-hospital setting) while 6 of the cases required both OHA and insulin. The cases were admitted with a primary diagnosis of peripheral neuropathy (n=17) followed by uncontrolled sugars without ketoacidosis (n=13), metabolic encephalopathy (n=9), for cataract surgery (n=8) and renal calculi (n=6), accelerated hypertension (n=4) and intervertebral disc prolapse(n=3).

Table 1: Baseline Characteristics of Cases and Controls

Characteristic		**Group**			
		Cases		**Control**	
		N	**%**	**N**	**%**
AGE	**35 - 45**	14	23.3%	14	23.3%
	46 - 55	30	50.0%	30	50.0%
	Above 55	16	26.7%	16	26.7%
	Total	60	100.0%	60	100.0%
GENDER	**F**	16	26.7%	16	26.7%
	M	44	73.3%	44	73.3%
	Total	60	100.0%	60	100.0%
FERRITIN	**<200**	4	6.7%	60	100.0%
	200 -400	36	60.0%	0	0.0%
	> 400	20	33.3%	0	0.0%
	Total	60	100.0%	60	100.0%
HBA1C	**< 6.5**	0	0.0%	60	100.0%
	6.5 - 8.5	20	33.3%	0	0.0%
	8.5 - 10.5	33	55.0%	0	0.0%
	> 10.5	7	11.7%	0	0.0%
	Total	60	100.0%	60	100.0%
FASTING PLASMA GLUCOSE	**< 100**	0	0.0%	60	100.0%
	100 - 200	40	66.7%	0	0.0%
	> 200	20	33.3%	0	0.0%
	Total	60	100.0%	60	100.0%

Table 2: Comparison of Mean (#SD) of cases and controls

Characteristic		**Cases (N=60)**	**Controls (N=60)**

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Age	**Mean (± SD)**	49.93 (±7.4)	49.93(±7.4)
Serum Ferritin levels		366.1(±90.63)	67.8(±11.63)
Fasting Plasma Glucose	**mg/dl**	184.9(±23.98)	84.7(±6.23)
HbA1C		9.1(±1.11)	4.99(± 0.34)

Table 2 depicts the mean (#SD) of cases and controls. Mean age of the two groups are comparable. Mean values of serum ferritin, fasting plasma glucose and HbA1C are higher in the cases than controls.

Our study found a highly significant correlation between ferritin, HbA1C and fasting plasma glucose among cases as well as controls. The significance established between HBA1C and ferritin concludes that poorly controlled diabetes has hyperferritinemia. On performing Pearson correlation with the variables duration of diabetes, HbA1C and serum ferritin levels, with a level of significance of 0.05, the following scatter diagram was obtained (Fig 1). With the coordinates of the ROC curve, maximum sensitivity (98.3%) and specificity (100%) was seen at a ferritin cut-off value of 187 with AUC is 0.978 as represented with Fig 2. Pearson correlation coefficient (r) for cases is depicted in table 3 and for controls in table 4. On performing t test between cases and control p value for serum ferritin, fasting plasma glucose and HbA1C was 0.00.

Table 3: Karl Pearson correlation among cases.

Correlation among cases		**Duration of Diabetes**	**Ferritin**	**HbA1C**
Ferritin	Pearson Correlation	0.972		
	P	0.00		
	N	60		
HbA1C	Pearson Correlation	0.971	0.993	
	P	0.00	0.00	
	N	60	60	
Fasting Plasma Glucose	Pearson Correlation	0.976	0.992	0.992
	P	0.00	0.00	0.00
	N	60	60	60

Table 4: Karl Pearson Correlation among controls

Correlation among controls		**Ferritin**	**HbA1C**
HbA1C	Pearson Correlation	0.294	
	P	0.022	
	N	60	
Fasting	Pearson Correlation	0.252	0.290

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Plasma Glucose	P	0.052	0.025
	N	60	60

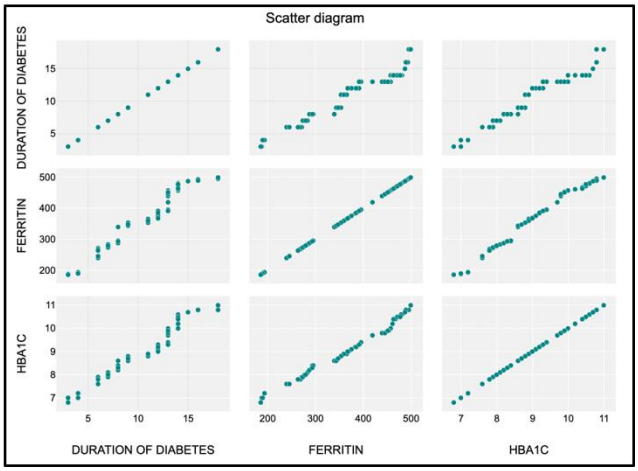

Figure 1: Correlation of HbA1C, Serum Ferritin levels and duration of diabetes.

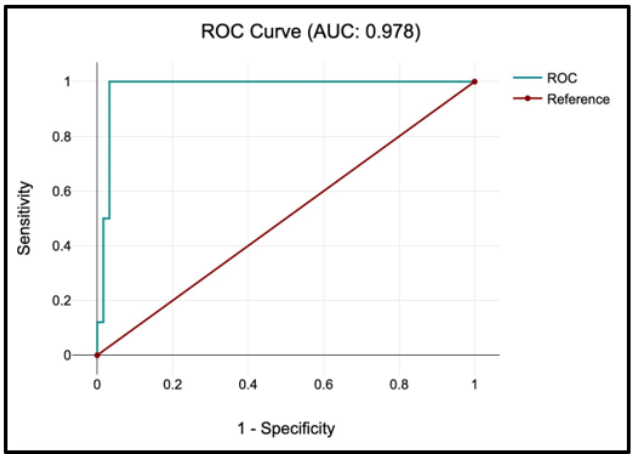

Figure 2: ROC Curve for serum ferritin with cut off value of 187 (AUC:0.978)

Discussion

Diabetes mellitus, a disease of abnormal carbohydrate metabolism, that has an increasing worldwide prevalence and this has significantly added to the socio-economic burden of the country. Although the pathogenesis of type 2 diabetes mellitus is multifactorial, insulin resistance is the hallmark of the disease. The role of ferritin in the etiopathogenesis of diabetes and its complications is not completely proven. However, there have been attempts to identify if serum ferritin could be a potential marker in diabetes mellitus. Therefore, our study was aimed at evaluating the role of ferritin on glycemic control among inpatients with T2DM admitted to the tertiary care hospital.

We conducted a case-control study which included a total of 120 participants (60 cases and 60 controls). Both the groups were matched with respect to age and gender. Participants in the age group of 35-70 years were included in the study. 50% of the participants were between the age of 46-55 years. The mean age of participants was 49.93#7.403 yrs. Majority of the study participants (73.3%) were males. Majority of the patients included in the study were admitted for peripheral neuropathy. It is well established

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that the development of complications of diabetes is influenced by
the duration of diabetes mellitus and the glycemic control. Notably, 51.7% of the patients included in the study had a duration of diabetes between 11-15yrs. The mean duration of diabetes in the study was 10.60#3.697 yrs. Majority of the patients (67%) were on OHA for treatment of diabetes. Patients who were only on dietary modifications were not included in the study.

Universally, HbA1c is the most accepted tool for glycemic monitoring in patients with diabetes mellitus. However, its measurement is affected by several factors. Hence, we intended to evaluate if serum ferritin had a correlation with glycemic control, to provide future recommendations for using ferritin for monitoring glycemic status of diabetic patients.
Several studies have shown that ferritin levels are higher in cases than controls. (9,10,11)
Similar results were obtained in our study wherein, the ferritin levels were found to be higher in cases than controls. All the controls had a ferritin value of less than 200. The mean ferritin among cases was 366.1#90.63 and in control group was 67.8#11.63.

There are several studies done to establish the association between serum ferritin and glycemic control in patients with type 2 diabetes mellitus. Sumesh Raj *et al.* in their study noted that ferritin, which reflects the body iron stores was found to be significantly higher in diabetics as compared to controls, and this significantly increased with the increasing duration of diabetes.(9) Similar results were found in the study by Michael S and Devi M, wherein the levels of ferritin was higher in cases than controls.(10) Tanveer Ahmed in his case-control study on T2DM patients also
found that ferritin was higher in cases than controls. Moderate correlation between serum ferritin and HbAa1c was also established in the study. (11)

A study done in Jordan by Al-fawaeir S *et al.* established a strong association of ferritin with HbA1c in both cases(diabetics) and controls. However, the association was not statistically significant in the control group. (12) In a case control study by Tariq S *et al.*, ferritin levels were significantly higher in diabetics compared to non-diabetics. The study also showed that the levels of ferritin increased with the increase in HBA1c, reflecting that poor glycemic control results in hyperferritinemia. (13) Data from a study done in a representative North Indian population also showed higher ferritin levels in T2DM patients as compared to controls.(14)

Association of ferritin with poor glycemic control in long standing diabetes has been established in many studies as outlined above reflecting the increased oxidative stress in these patients. Consistent with other studies, our study also found a significant correlation between ferritin and the duration of diabetes (r-0.972). Though literature supports the evidence of ferritin as a marker of poor glycemic status, some studies have failed to establish an association of ferritin with the glycemic control in diabetic patients. (15,16)

As ferritin is an inflammatory marker and an acute phase reactant, several factors can influence it. To avoid the influence of such confounding factors on ferritin levels through our initial screening we excluded patients with diseases that could possibly alter ferritin levels such as anemia, chronic infections, chronic liver disease, chronic kidney disease, patients on corticosteroid therapy and those with overt thyroid dysfunction.

In our study we also tried to correlate ferritin with the fasting plasma glucose levels. Ferritin levels were found to be higher with the increasing fasting plasma glucose levels. A significant correlation was established using Pearson correlation test in both cases and controls (r- 0.992 in cases and 0.252 in controls). Analysing the levels of fasting plasma glucose in our study participants, 66.7% of the cases had a fasting plasma glucose value

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between 100-200 mg/dl and the remaining had fasting plasma glucose more than 200mg/dl. All the controls had a fasting plasma glucose value less than 100 mg/dl. The mean fasting plasma glucose among cases was 184.9#23.98
mg/dl, and among controls was 84.7#6.23 mg/dl.

Comparison of fasting plasma glucose levels in cases and controls was done and the p value of 0.000 was obtained (Highly significant). Our study found a significant correlation between fasting plasma glucose and HbA1C (r-0.992 in cases; r-0.290 in controls). A significant Karl Pearson correlation coefficient was noted between fasting plasma glucose levels and the duration of diabetes (r-0.976 and p value of 0.000). HbA1c reflects the average glycemia over the past three months and has a strong predictive value for diabetic complications. In our study, majority of the cases (55.0%) had HbA1c in the range of 8.5-10.5. Moreover, 11.7% of participants had poor glycemic control as reflected by a HbA1C value of >10.5. The mean HbA1C among cases was 9.1#1.11 and among controls was 5.0#0.34.

The results of our study also showed an increasing trend of HbA1C with the increasing duration of diabetes. Using Pearson correlation test, HbA1C showed a positive correlation (r=0.971) with the duration of diabetes mellitus. The Receivers Operating Characteristic (ROC) curve was used to analyse the sensitivity and specificity of ferritin at various cut-offs. Using the coordinates of the ROC curve, maximum sensitivity (98.3%) and specificity (100%) was seen at a ferritin value of 187, which is proposed as the cut-off value for assessing good glycemic control in diabetics. An outpatient-based case-control study by Pramiladevi *et al.* had proposed a ferritin cut-off value of 140 ng/ml in their study. (17) In our study ferritin value of 187ng/ml corresponded to HbA1c of 6.9% and fasting glucose value of 132mg/dl

In the current study, we found an increasing trend of serum ferritin with the worsening glycemic control (as represented by higher HbA1C). Using Karl Pearson correlation test, a Significant correlation was noted between ferritin and glycated hemoglobin in our study, in both the cases as well as the control group (r- 0.993in cases and r-0.294 in control).

The proposed cause of hyperferritinemia in poorly-controlled diabetes is the longer t1/2 of glycosylated ferritin. (18) Pancreatic inflammation in long standing diabetes and the possible development of subclinical hemochromatosis in these patients are other proposed mechanisms.

Although we did not follow up on patients to assess if the serum ferritin levels decreased with better glycemic control, the results of our study nonetheless supports the association of hyperferritinemia in uncontrolled diabetes. The causal role of ferritin in the pathogenesis of type 2 diabetes is not well established and more studies are needed in this regard.

Strengths of our study importantly is the emphasis on the role of ferritin as a marker of glycemic control in a representative South Indian population. As ferritin is an acute phase reactant, through our screening, we excluded conditions which could possibly alter ferritin levels, to eliminate such confounding factors. In our study, we established a cut-off value of ferritin for assessing good glycemic control. Our study provides future recommendations for using ferritin as a marker for early detection and prevention of diabetic complications.

Limitations of our study include lack of follow up due to the cross-sectional nature of the study design. Hence, we could not determine whether better glycemic control resulted in decrease in ferritin levels over a period. Small sample size of 60 cases and 60 controls, measuring other
markers such as iron, TIBC or transferrin saturation and failing to analyse the role of ferritin

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in patients with IFG and IGT were the other limitations noted.

Conclusion

There is a significant positive linear association between serum ferritin levels, fasting plasma glucose, ration of diabetes and glycated hemoglobin and a cut off value of 187 ng/mL could potentially be used as a screening test along with fasting glucose and glycosylated hemoglobin for identifying patients with type 2 diabetes mellitus. Although longitudinal studies are needed to establish causality between serum ferritin levels and pathogenesis of T2DM, implications drawn from this study provides insights into future recommendations for practice.

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