

# Assessment of serum Ferritin level and its correlation with HbA1c in Diabetic Nephropathy



Madhura Navule Siddappa<sup>1</sup>, Kowsalya Ramprasad<sup>2</sup>

<sup>1</sup>Assistant Professor, <sup>2</sup>Associate Professor, Department of Biochemistry, Institute of Nephrourology, Victoria Hospital Campus, Bangalore.

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## ABSTRACT

**Background:** Serum ferritin levels reflecting the body iron stores, is known to be elevated in type 2 Diabetes Mellitus. However its association with diabetic complications including Diabetic nephropathy (DN), and overall glycemic control needs to be validated.

**Aims and Objectives:** The aim of this study was to find the Serum Ferritin level abnormalities in DM patients with nephropathy in comparison with DM patients without nephropathy and to find correlation of Serum Ferritin (SF) levels with levels of Glycated Hemoglobin (HbA1c) in patients with diabetic nephropathy. **Materials and Methods:** This is a retrospective study, which included eighty five registered patients with Type 2 DM (44 Type II DM without nephropathy cases and 41 cases of Type II DM with nephropathy). SF and HbA1c was estimated in all cases across both the groups and were compared with age and sex matched controls and analysed. **Results:** Serum Ferritin levels were higher in diabetics with nephropathy compared to diabetics without nephropathy ( $p < 0.0001$ ). SF levels were higher in diabetic groups compared to control group ( $p < 0.001$ ). The correlation between HbA1c and SF was assessed among all cases of DM with nephropathy group using pearson correlation test and it showed a significantly positive correlation ( $r = 0.431$ ) with a SF (mean =  $938 \pm 148$ ) and HbA1c (mean =  $9.2 \pm 2.02$ ). **Conclusion:** Serum ferritin levels positively correlate with HbA1c levels in Type II DM cases with nephropathy, which suggests that serum Ferritin levels can be used as a surrogate marker of glycemic control in Type II DM with nephropathy.

**Key words:** Serum ferritin; Glycated hemoglobin; Diabetic nephropathy; Iron overload

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## INTRODUCTION

Diabetic nephropathy is a macrovascular complication in patients with Diabetes Mellitus (DM) characterized by persistent albuminuria, elevated blood pressure and a high risk of cardiovascular morbidity and mortality.<sup>1</sup> This major life threatening complication develops in approximately 20% to 40% of Type I DM and <20% of Type II DM patients (type 2 DM).<sup>2</sup>

The total amount of body iron is around 3-4 grams, of which only 1-2 mg of iron is absorbed and circulated in the blood, while the rest is stored in the body as Ferritin.<sup>3</sup> Thus serum ferritin levels would reflect body iron stores in normal individuals.<sup>4</sup> Iron is an essential element for

life, required for many cellular processes like oxidation-reduction reactions, cellular proliferation, DNA synthesis, oxygen transport, and cell growth.<sup>5,6</sup> However iron is toxic when in excess and increased iron accumulation causes organ dysfunction through the production of reactive oxygen species.<sup>3</sup> Elevated iron stores may induce diabetes through a variety of mechanisms, including oxidative damage to beta cells of pancreas, affecting insulin synthesis in the liver, interference with functioning of insulin and by increasing insulin resistance.<sup>7,8</sup> So far, many studies have proved role of oxidative stress due to iron overload in causation of diabetes and its complications.<sup>9-12</sup> HbA1c or Glycated Haemoglobin, provides information about overall control of glucose in the previous 6-8 weeks, is considered the best available biochemical parameter to

### Address for Correspondence:

Dr. Kowsalya R, Associate Professor, Department of Biochemistry, Institute of Nephrourology, Victoria Hospital Campus, Bangalore.

**Phone:** +91-9008172078. **E-mail:** r.kowsalya@gmail.com

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assess the long-term metabolic control in patients with DM.<sup>13</sup> HbA1c levels are well associated with the response to treatment and hence act an important marker with which chances of developing complications in diabetics can be predicted.<sup>14</sup> But, HbA1c may be affected by a variety of genetic, haematologic and illness-related factors.<sup>15</sup> Thus, it is important to have a marker alternate to HbA1c to assess the severity of the disease in type 2 DM. In the present study we focus on the assessment of serum ferritin levels in type 2 diabetic patients complicated with nephropathy, and to find the correlation between ferritin levels and Hba1c in these patients.

## MATERIALS AND METHODS

This is a retrospective case-control study, which included eighty five registered patients with type 2 DM at a tertiary care Nephrourology center, Bangalore, India. Patients attending routine Nephrourology OPD clinics between January 2017 to August 2017 were included as cases. We had 3 study groups. Group I (n=44) included Type II DM without nephropathy and group II (n=41) included Type II DM with overt nephropathy (having macroalbuminuria >300mg/24 hr or albumin creatinine ratio(ACR >30 mg/gm). Group III consisted of age and sex matched non diabetic controls (n=30). Serum Ferritin and HbA1c were estimated in all cases across all 3 groups and were analysed.

### Inclusion criteria

Eighty five patients with Type IIDM attending the OPD were recruited into the study among registered patients. Type 2 DM was diagnosed using American Diabetes Association criteria, fasting serum glucose (FSG)  $\geq 126$  mg/dl (normal value 70–110 mg/dl) or 2 hour postprandial glucose (PPG)  $\geq 200$  mg/dl (normal value <140 mg/dl). Patients had been divided into 2 groups according to diabetic nephropathy diagnostic criteria. Group I (n=41) included type II DM without nephropathy and other complication and group II included type 2 DM with overt nephropathy (having macroalbuminuria >300mg/24 hr or albumin creatinine ratio (ACR >30 mg/gm). Thirty non-diabetic healthy participants with ages ranged between 40 and 65 years old, were allocated for the control group. The control group consisted of individuals who had been referred to the laboratory center for routine checkup with no history of any medical disorder.

### Exclusion criteria

Type 1 diabetes mellitus, chronic disorders like Overt thyroid dysfunction, Chronic kidney disease, Chronic liver disease, Other states associated with altered serum ferritin levels like: Hemochromatosis, Chronic alcoholics, Chronic

inflammatory conditions like SLE/rheumatoid arthritis, Hepatitis, History of repeated blood transfusions, Iron deficiency anemia.

## LABORATORY ANALYSIS OF SAMPLE

All the parameters were assayed in the Biochemistry laboratory using Abbott CI 4100, chemistry and immunoassay analyser. Urea and creatinine were assayed using enzymatic method, urea by Urease method and creatinine by Jaffe s method. HbA1c is quantified by measuring the amount of HbA1c analyte captured on the matrix cell, using a conjugate of Anti-HbA1c and Alkaline Phosphatase as the signal-generating molecule, and the substrate, 4-Methylumbelliferyl Phosphate. Ferritin assay is done by Chemiluminescent Microparticle Immunoassay (CMIA) technique.

### Statistical analysis

Statistical analysis was performed using SPSS software. Continuous variables were expressed as the mean  $\pm$  standard deviation. Unpaired independent student t test was used to find out significance of SF values between Groups I, II, and III. The correlation between HbA1c and SF within cases of group I and II was done using Pearson correlation test.

## RESULTS

Clinical data of the 3 groups (Type II DM without nephropathy, Type II DM with nephropathy, Controls) are summarized in Table 1.

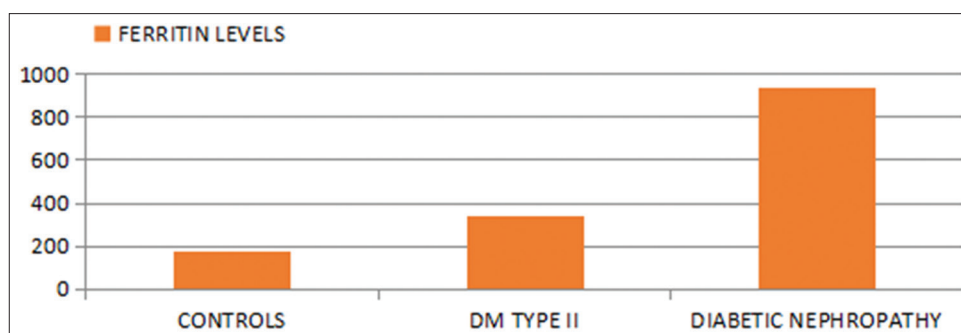
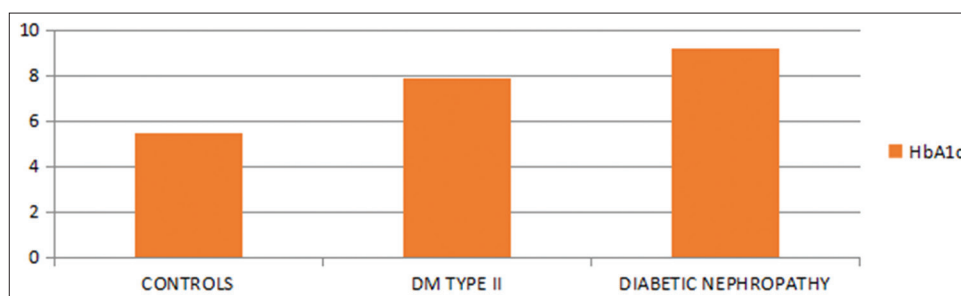
On comparison of all three groups, ferritin levels were higher in diabetics with nephropathy compared to diabetics without nephropathy ( $p < 0.0001$ ). However ferritin levels were higher in diabetic population also compared to control group ( $p < 0.001$ ) [Figure 1 ].

HbA1c levels were higher in diabetic nephropathy than in uncomplicated diabetic patients, which indicate that diabetic patients complicated with nephropathy have poor glycemic control compared to diabetics without nephropathy [Figure 2].

Correlation between serum ferritin and HbA1c was assessed in diabetic nephropathy patients. The correlation between glycated haemoglobin and serum ferritin was done by Pearson correlation test and it showed a significantly positive correlation ( $r=0.431$ ) with serum ferritin [mean= $938 \pm 148$ ] and HbA1c (mean= $9.2 \pm 2.02$ ) and ( $p$  value = $0.017$ ) which is significant at  $p < 0.05$  (Table- 2). This suggests that ferritin levels are higher in patients with

**Table 1: Clinical data of study groupss and controls**

Clinical data	Type II DM mean±sd	Type II DM with nephropathy mean±sd	Controls mean±sd
Number	44	41	40
Gender m/f	29/15	21/10	20/20
Age	53±13	56±10	50±11
Ferritin ng/ml	339±143	938±148	180±42
HbA1c g%	7.9±1.4	9.2±2.02	5.5±1.2
Urea mg/dl	34±9.8	139±59	30±8.9
Creatinine mg/dl	1.1±0.2	7.5±4.7	1.2±0.5

**Figure 1:** Comparison of ferritin levels between diabetes mellitus and controls**Figure 2:** Comparison of HbA1c between diabetic and controls

poor glycemic control in diabetic nephropathy patients. This further suggests that serum ferritin levels are directly related to severity of diabetes [Figure 3].

Correlation between serum ferritin and HbA1c was assessed in diabetics without nephropathy. The correlation between glycated haemoglobin and serum ferritin was done by Pearson correlation test and it showed a low positive correlation ( $r=0.256$ ) which is not significant at  $p < 0.05$  (Table- 3).

## DISCUSSION

Diabetes Mellitus is a predominant public health concern, affecting millions of people worldwide with one of the major causes of mortality and morbidity.<sup>16</sup> The prevalence of the disease is increasing rapidly all over the world with India having the largest number of diabetic patients in the world.<sup>17</sup> Also studies from United Kingdom have proved three to four fold increase in prevalence of the disease in south Asians than in the Europeans.<sup>18</sup>

Diabetic nephropathy is known to be the most common long term complication of type 2 DM and the leading cause of end-stage renal disease (ESRD) worldwide. Also it is estimated that nearly 20% of type 2 diabetic patients will develop ESRD during their lifetime due to Diabetic nephropathy.<sup>19</sup> In the present study among the 85 registered patients of type 2 DM, forty one had nephropathy and forty four were diabetic without any complication. There is male predominance in our study with respect to type 2 DM and DN. Few studies have also demonstrated the association of male gender, with the development of nephropathy in type 2 DM.<sup>20</sup>

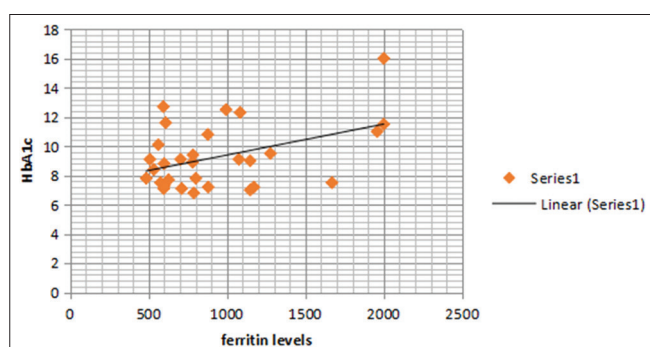
Diabetic nephropathy is more likely to develop in type 2 DM patients with poor glycemic control.<sup>21</sup> In the present study, type 2 DM patients with nephropathy have poor glycemic control compared to them without nephropathy as evidenced by higher levels of HbA1c in DN group. This result also is supported by studies, which proved that the risk of development of nephropathy in type 2 DM can be reduced by improving the glycemic control.<sup>22-24</sup>

**Table 2: Correlation between serum ferritin and HbA1c levels in diabetic nephropathy**

Diabetic nephropathy	Ferritin	HbA1c	Pearson correlation	p value
n= 41	938±148	7.9±1.4	r=0.431	0.017

**Table 3: Correlation between serum ferritin and HbA1c levels in diabetics without nephropathy**

Diabetic nephropathy	Ferritin	HbA1c	Pearson correlation	p value
n= 44	339±143	9.2±2.02	r=0.256	0.15

**Figure 3:** Scatter diagram showing correlation b/w ferritin levels and HbA1c in diabetic nephropathy

According to the American Diabetes Association (ADA) Guidelines 2007, HbA1c level of greater than 7% has an increased risk of progression to diabetic complications.<sup>25</sup> Although Glycosylated haemoglobin (HbA1c) test is a method for estimating the degree of hyperglycemia over a period of 2 to 3 month,<sup>26</sup> HbA1c level is known to be affected by many factors like different types of anemia, different methods of estimation and serum iron levels.<sup>27-29</sup> Its always better to have alternatives to HbA1c for measuring glycemic control in diabetics and ferritin can act as one such marker. So far many studies have proved that increased body iron stores reflected by serum ferritin levels, are increased in patients with type 2 DM.<sup>9, 30-33</sup>

In the present study, Serum Ferritin (SF) levels are found to be increased in both type 2DM and DN compared to control group. A study carried out in Korea University Hospital by Kim et al proved the higher levels of SF in type 2 DM, and concluded that SF can be employed as a marker of not only glucose homeostasis but also insulin resistance in Type II DM.<sup>34</sup> Similar findings were also reported by Canturk et al,<sup>35</sup> who proved the prevalence of hyper ferritinemia in poorly controlled diabetic cases.

Although the exact mechanism for association of elevated SF with Type IIDM is yet to be established, there are a number of prevailing theories. Iron overload where in

excessive deposition of iron in the liver may cause insulin resistance by interfering with the ability of insulin to suppress hepatic glucose production.<sup>36</sup> Oxidative stress can also lead to hyperglycemia through disturbed glucose metabolism. Conversely, insulin stimulates cellular iron uptake through increased transferrin receptor externalization. Insulin resistance coupled with poor glycemic control can also increase ferritin levels. Thus, insulin and iron levels can mutually affect each others effects leading to a vicious cycle of insulin resistance and diabetes mellitus.<sup>37</sup>

Association of iron overload and progression of type 2 DM to nephropathy has been demonstrated.<sup>38</sup> The significant role of iron overload in pathogenesis of nephropathy in type 2 DM patients has also been indicated by the observation that progression of DM to DN can be prevented either by an iron-deficient diet or iron chelators.<sup>39</sup> This is in accordance with our finding that ferritin levels were significantly increased in DN compared to type 2 DM without nephropathy in the present study.

Association between the iron overload and glycemic control in diabetic patients has also been demonstrated in several studies like Eschwege et al and Raj S et al.<sup>39,40</sup> A strong positive correlation between SF and HbA1 levels is found in these studies. Similar finding is observed in our study where positive correlation between SF and HbA1c in diabetic nephropathy patients and a low positive correlation between the same parameters in diabetic patients without nephropathy signifies the prognostic role of serum ferritin levels in diabetic patients.

## CONCLUSION

Serum ferritin levels positively correlate with HbA1c levels in both complicated and uncomplicated diabetics, which suggests that serum Ferritin levels can be a marker of glycemic control in Type II DM. Estimating serum Ferritin levels routinely in all Type IIDM patients with nephropathy and setting a cutoff value of serum Ferritin will act as a reliable surrogate marker for good glycemic control and will help prevent patients from progressing to overt nephropathy and other complications.

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**Authors Contribution:**

**MNS**- Collection of data, analysis of data, writing the manuscript; **KR**- Collection of data, and review of manuscript

**Work attributed to:**

Institute of Nephrourology, Victoria Hospital Campus, Bangalore.

**Orcid ID:**

Dr. Madhura Navule Siddappa - <https://orcid.org/0000-0002-9643-6305>

Dr. Kowsalya Ramprasad - <https://orcid.org/0000-0003-4131-2337>

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