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

Madhura Navule Siddappa¹, Kowsalya Ramprasad²

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

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 ± 148) and HbA1c (mean = 9.2 ± 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

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.¹ 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).²

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.³ Thus serum ferritin levels would reflect body iron stores in normal individuals.⁴ 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.⁵,⁶ However iron is toxic when in excess and increased iron accumulation causes organ dysfunction through the production of reactive oxygen species.³ 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.⁷,⁸ So far, many studies have proved role of oxidative stress due to iron overload in causation of diabetes and its complications.⁹,¹² 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
assess the long-term metabolic control in patients with DM. HbA1c levels are well associated with the response to treatment and hence act as an important marker with which chances of developing complications in diabetics can be predicted. But, HbA1c may be affected by a variety of genetic, haematologic and illness-related factors. 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) ≥126 mg/dl (normal value 70–110 mg/dl) or 2 hour postprandial glucose (PPG) ≥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 ± 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±148] and HbA1c (mean=9.2±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
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.\(^\text{16}\) The prevalence of the disease is increasing rapidly all over the world with India having the largest number of diabetic patients in the world.\(^\text{17}\) Also studies from United Kingdom have proved three to four fold increase in prevalence of the disease in south Asians than in the Europeans.\(^\text{18}\)

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.\(^\text{19}\) 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.\(^\text{20}\)

Diabetic nephropathy is more likely to develop in type 2 DM patients with poor glycemic control.\(^\text{21}\) 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.\(^\text{22-24}\)

---

**Table 1: Clinical data of study groups 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
According to the American Diabetes Association (ADA) Guidelines 2007, HbA1c level of greater than 7% has an increased risk of progression to diabetic complications.25 Although Glycosylated haemoglobin (HbA1c) test is a method for estimating the degree of hyperglycemia over a period of 2 to 3 month,26 HbA1c level is known to be affected by many factors like different types of anemia, different methods of estimation and serum iron levels.27-29 It’s 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.9, 30-33 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.34 Similar findings were also reported by Canturk et al,35 who proved the prevalence of hyper ferritinemia in poorly controlled diabetic cases.

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.34 Similar findings were also reported by Canturk et al,35 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.36 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.37

Association of iron overload and progression of type 2 DM to nephropathy has been demonstrated.38 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.39 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.39,40 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.

ACKNOWLEDGEMENT

We are grateful to all our nephrologists and laboratory technicians who helped in this study.

REFERENCES

1. Jawa A, Kcomt J and Fonseca VA. Diabetic nephropathy and retinopathy. Med Clin North Am 2004;88(4):1001-36, xi.
2. Skyler JS. Microvascular complications. Retinopathy and nephropathy. Endocrinol Metab Clin North Am 2001; 30(4):833-856.

3. Andrews NC. Disorders of iron metabolism. N Engl J Med 1999; 341(26):1986-1995.

4. Jacobs A and Worwood M. The biochemistry of ferritin and its clinical implications. Prog Hematol 1975; 9:1-24.

5. Dunn LL, Suryo Rahmanto Y and Richardson DR. Iron uptake and metabolism in the new millennium. Trends Cell Biol 2007; 17(2):93-100.

6. Kovacevic Z, Kalinowski DS, Lovejoy DB, Yu Y, Suryo Rahmanto Y, Sharpe PC, et al. The medicinal chemistry of novel iron chelators for the treatment of cancer. Curr Top Med Chem 2011; 11(5):483-499.

7. Ford ES and Cogswell ME. Diabetes and serum ferritin concentration among U.S. adults. Diabetes Care 1999; 22(12):1978-1983.

8. Liu Q, Sun L, Tan Y, Wang G, Lin X and Cai L. Role of iron deficiency and overload in the pathogenesis of diabetes and diabetic complications. Curr Med Chem 2009; 16(11):113-129.

9. Fernandez-Real JM, Lopez-Bermejo A and Ricart W. Cross-talk between iron metabolism and diabetes. Diabetes 2002; 51(8):2348-2354.

10. Lassman MN, Genel M, Wise JK, Hendler R and Felig P. Carbohydrate homeostasis and pancreatic islet cell function in thalassemia. Ann Intern Med 1974; 80(1):65-69.

11. Niederau C, Berger M, Strommel W, Starke A, Strohmeyer G, Ebert R, et al. Hyperinsulinaemia in non-cirrhotic haemochromatosis: impaired hepatic insulin degradation? Diabetologia 1984; 26(6):441-444.

12. Walter RM, Jr., Uriu-Hare JY, Olin KL, Oster MH, Anawalt BD, Critchfield JW, et al. Copper, zinc, manganese, and magnesium status and complications of diabetes mellitus. Diabetes Care 1991; 14(11):1050-1056.

13. International Expert Committee report on the role of the A1C assay in the diagnosis of diabetes. Diabetes Care 2009; 32(7):1327-1334.

14. Farmer A. Monitoring Diabetes 5th edition ed: Wiley Blackwell; 2017.

15. Gallagher EJ, Le Roith D and Bloomgarden Z. Review of hemoglobin A(1c) in the management of diabetes. J Diabetes 2009; 1(1):9-17.

16. Wild S, Roglic G, Green A, Sicree R and King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care 2004; 27(5):1047-1053.

17. Huizinga MM and Rothman RL. Addressing the diabetes pandemic: a comprehensive approach. Indian J Med Res 2006; 124(5):481-484.

18. Mather HM, Chaturvedi N and Kohely AM. Comparison of prevalence and risk factors for microalbuminuria in South Asians and Europeans with type 2 diabetes mellitus. Diabet Med 1998; 15(8):672-677.

19. Ayodele OE, Alebiosu CO and Salako BL. Diabetic nephropathy- a review of the natural history, burden, risk factors and treatment. Journal of the National Medical Association 2004; 96(11):1445-1454.

20. Gall MA, Hougaard P, Borch-Johnsen K and Parving HH. Risk factors for development of incipient and overt diabetic nephropathy in patients with non-insulin dependent diabetes mellitus: prospective, observational study. BMJ 1997;314(7083):783-788.

21. Mogensen CE. Microalbuminuria, blood pressure and diabetic renal disease: origin and development of ideas. Diabetologia 1999;42(3):263-285.

22. Mulec H, Blohme G, Grande B and Bjorck S. The effect of metabolic control on rate of decline in renal function in insulin-dependent diabetes mellitus with overt diabetic nephropathy. Nephrol Dial Transplant 1998;13(3):651-655.

23. Nathan DM, Genuith S, Lachin J, Cleary P, Crofford O, Davis M, et al. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. N Engl J Med 1993; 329(14):977-986.

24. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). UK Prospective Diabetes Study (UKPDS) Group. Lancet 1998; 352(9131):837-853.

25. Standards of Medical Care in Diabetes-2007. Diabetes Care 2007; 30(suppl 1):S4-S41.

26. King H, Aubert RE and Herman WH. Global burden of diabetes, 1995-2025: prevalence, numerical estimates, and projections. Diabetes Care 1998;21(9):1414-1431.

27. Coban E, Ozdogan M and Timuragaoğlu A. Effect of iron deficiency anemia on the levels of hemoglobin A1c in nondiabetic patients. Acta Haematol 2004;112(3):126-128.

28. El-Aguouza I, Abu Shalah A and Sirdah M. The effect of iron deficiency anemia on the levels of haemoglobin subtypes: possible consequences for clinical diagnosis. Clin Lab Haematol 2013; 24(5):285-289.

29. van Heyningen C and Dalton RG. Glycosylated haemoglobin in iron-deficiency anemia. Lancet 1985; 1(8433):874.

30. Dekker LH, Nicolaou M, van der AD, Busschers WB, Brewster LM, Snijder MB, et al. Sex differences in the association between serum ferritin and fasting glucose in type 2 diabetes among South Asian Surinamese, African Surinamese, and ethnic Dutch: the population-based SUNSET study. Diabetes Care 2013; 36(4):965-971.

31. Sun L, Zong G, Pan A, Ye X, Li H, Yu Z, et al. Elevated Plasma Ferritin Is Associated with Increased Incidence of Type 2 Diabetes in Middle-Aged and Elderly Chinese Adults. The Journal of Nutrition 2013;143(9):1459-1465.

32. Jiang R, Ma J, Ascherio A, Stampfer MJ, Willett WC and Hu FB. Dietary iron intake and blood donations in relation to risk of type 2 diabetes in men: a prospective cohort study. Am J Clin Nutr 2004;79(1):70-75.

33. Sheu WH, Chen YT, Lee WJ, Wang CW and Lin LY. A relationship between serum ferritin and the insulin resistance syndrome is present in non-diabetic women but not in non-diabetic men. Clin Endocrinol (Oxf) 2003; 58(3):380-385.

34. Kim NH, Oh JH, Choi KM, Kim YH, Baik SH, Choi DS, et al. Serum ferritin in healthy subjects and type 2 diabetic patients. Yonsei Med J 2000;41(3):387-392.

35. Canturk Z, Cetinbaslan B, Tarkurn I and Canturk NZ. Serum ferritin levels in poorly- and well-controlled diabetes mellitus. Endocr Res 2003; 29(3):299-306.

36. Rajpathak S, Ma J, Manson J, Willett WC and Hu FB. Iron intake and the risk of type 2 diabetes in women: a prospective cohort study. Diabetes Care 2006; 29(6):1370-1376.

37. Fernandez-Real JM, Lopez-Bermejo A and Ricart W. Iron stores, and the risk of type 2 diabetes in men: a prospective cohort study. Am J Clin Nutr 2004;79(1):70-75.

38. Sheets WH, Chen YT, Lee WJ, Wang CW and Lin LY. A relationship between serum ferritin and the insulin resistance syndrome is present in non-diabetic women but not in non-diabetic men. Clin Endocrinol (Oxf) 2003; 58(3):380-385.

39. Kim NH, Oh JH, Choi KM, Kim YH, Baik SH, Choi DS, et al. Serum ferritin in healthy subjects and type 2 diabetic patients. Yonsei Med J 2000;41(3):387-392.

40. Raj S and Rajan G. Correlation between elevated serum ferritin and HbA1c in type 2 diabetes mellitus. Int J Res Med Sci 2013;1(1):12-15.
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

Source of Support: Nil, Conflict of Interest: None declared.