# HbA1c Status in Type II Diabetes Mellitus with and without Iron Deficiency Anemia

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*Authors' contributions*

*This work was carried out in collaboration among all authors. Author ASC deigned the study, managed literature searches, performed the statistical analysis, wrote the protocol and wrote the first draft of manuscript. Authors ANS and SBT managed the analysis of the study and revised the draft of the manuscript. All authors read and approved the final manuscript.*

*Original Research Article*

## ABSTRACT

**Background:** Hemoglobin A1C (HbA1c) reflects patient's glycemic status over the previous 3 months. Previous studies have reported that iron deficiency may elevate HbA1c concentrations, independent of glycemia.
**Aim:** To assess the status of HbA1c in clinically diagnosed cases of type II Diabetes mellitus (DM) with and without iron deficiency anemia (IDA).
**Study Design:** Case control study in rural hospital of Talegaon Dabhade, Pune.
**Methodology:** The study includes 36 clinically diagnosed cases of type II DM with IDA and 36 controls which are age & sex matched having type II DM without IDA. Hematological parameters, fasting and post prandial blood glucose& HbA1c level were assessed in all subjects. Serum ferritin levels were assessed only in cases. Comparison between the parameters of cases and controls was done using appropriate statistical analysis.
**Results:** Levels of HbA1c are increased in cases [clinically diagnosed patients of diabetes mellitus with iron deficiency anemia that is IDA] as compared to controls [clinically diagnosed patients of diabetes mellitus without IDA] irrespective of glycemic status.
**Conclusion:** This study found a positive correlation between iron deficiency anemia and increased HbA1c levels, Hence IDA is to be taken in consideration while interpreting HbA1c in diagnosis and monitoring of Diabetes mellitus.

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## 1. INTRODUCTION

Lifestyle related diseases like diabetes have emerged as a major public health problem due to rapid urbanization and industrialization [1,2]. Diabetes mellitus is one of the most prevalent endocrine disorder in the world [3,4]. It is fast gaining the status of a potential epidemic in India with more than 62 million diabetic individuals currently diagnosed with the disease [5]. Diabetes mellitus is a heterogeneous group of diseases characterized by chronic elevation of glucose in the blood. It is a metabolic disease due to absolute or relative insulin deficiency causing hyperglycemia. Chronic exposure to high blood glucose is a leading cause of nephropathy, retinopathy, neuropathy and other types of tissue damage.

Hyperglycemia in diabetes is responsible for glycation of proteins and this correlates with blood glucose level. Hyperglycemia is associated with rise in glycated hemoglobin [HbA1c] concentration. HbA1c is the predominant form of hemoglobin found in HbA1 fractions [6,7]. It is produced by ketamine reaction between glucose and N-terminal valine of both β chains of the hemoglobin molecule. It reflects the patient's glycemic status over previous 3 months as compared to sugar which reflects status over 24 hrs [8].

The American Diabetic Association [ADA] and the American College Of Endocrinology [ACE] recommended HbA1c levels as diagnostic criteria for diabetes mellitus. Physicians have adopted HbA1c levels as convenient way to screen for diabetes as well as to monitor the therapy [9]. The HbA1c is abnormally elevated in patients with uncontrolled diabetes mellitus and it correlates positively with the metabolic control [10]. According to the American Diabetes Association [ADA] Guidelines published in 2007, HbA1c levels should be maintained below 7% in all diabetic patients [11]. The values which are greater than 7% indicate an increased chance of progression to the diabetic complications especially the micro vascular like nephropathy, neuropathy and retinopathy [12].

Recently HbA1c testing has been included within the diagnostic criteria recommended for diagnosis of diabetes by American Diabetes Association. HbA1c level of 6.5% is recommended as the cut off point for diagnosis of diabetes mellitus [13].

Previously it was believed that HbA1c is primarily affected by the blood glucose levels. However, certain studies have proved that the HbA1c levels are altered by various other coexisting factors, along with diabetes, especially that of iron deficiency anemia which is a major public health problem in developing countries like India.

Anemia is the most common blood disorder, and a common finding in patients with diabetes [14]. The prevalence of anemia is estimated about 10% to 30% in patients with diabetes [15,16]. Approximately one third of patients with anemia exhibit iron deficiency [15,17,18]. Raised HbA1c level is seen in iron deficiency anemia [8,10].

In diabetes mellitus many times it is seen that HbA1c concentration does not correlate with blood glucose level. This may be due to deranged iron status. Hence in case of raised HbA1c in diabetics, iron status of the patient has to be considered for treatment of DM more so when there is no correlation between blood sugar level and HbA1c.

In this study, we assessed the status of HbA1c in patients of type II diabetes mellitus with and without iron deficiency anemia.

## 2. MATERIALS AND METHODS

This study was carried out in the Department of Biochemistry in collaboration with Department of Medicine and Department of Pathology from January 2015 to December 2016.

### 2.1 Study Design

The study involves enrolment of 72 subjects between the ages of 30 to 55 years. The study includes 36 cases [clinically diagnosed patients of diabetes mellitus with iron deficiency anemia that is IDA] & 36 controls [clinically diagnosed patients of diabetes mellitus without IDA] which were age and sex matched.

The cases and controls included in present study were selected from patients attending outpatient department [OPD] and indoor patient department [IPD] of internal medicine at Dr. Bhausaheb Sardesai Rural Hospital Talegaon Dabhade.

Samples were assessed at the Central Clinical Laboratory [CCL], Department of Biochemistry.

## 2.2 Inclusion Criteria

Cases: Clinically diagnosed cases of diabetes mellitus with IDA between the age of 30-55 yrs.

## 2.3 Exclusion Criteria

Non diabetic patients, patients with complications of diabetes mellitus, hemolytic anaemia, chronic alcoholism, pregnancy, blood transfusion within a period of 6 months were excluded.

## 2.4 Collection of Blood Sample

2 mills of fasting as well as 2 hr post prandial blood sample were collected from Median cubital vein in fluoride bulb for plasma glucose estimation [BSL-F& BSL-PP].

2 mills venous sample was collected in EDTA bulb for estimation of complete blood count [CBC] and Glycated haemoglobin [HbA1c].

3 mills venous sample of case group was collected in plain bulb for estimation of serum ferritin.

## 2.5 Methods

The following parameters were evaluated

  i.   Haematological parameters: HiCN and Electrical impedance method [19]
  ii.  Serum ferritin: ELISA method [20]
  iii. Blood glucose: GOD-POD method [21]
  iv.  HbA1C: immunoturbidometric method [22]

Haematological parameters were analysed on Sysmex kx-21 autoanalyzer. All biochemical investigations were carried out on 'Erba 360 Fully automated biochemistry analyzer'.

## 2.6 Laboratory Criteria for Iron Deficiency Anemia [19,20,21]

PBS: Normocytic / Microcytic & Hypochromic picture
Hb : ↓ed [<13 g/dl in male, <12 g/dl in female]
MCV: ↓ed [85 ±9 fl]
MCH: ↓ed [29.5 ± 2.5 pg]
MCHC: ↓ed [33 ± 2 g/dl]
RDW: ↑ed [cv-11.6-14.6%, sd- 39-46fl]
Serum Ferritin: ↓ed [20-250 μg/l]

## 2.7 Statistical Analysis

The analysis was done using SPSS 19 software and statistical analysis was done using student unpaired t test. P-value less than 0.05 [P < 0.05] was considered to be statistically significant. P-value of less than 0.001 [P < 0.001] was considered to be statistically highly significant.

## 3. RESULTS AND DISCUSSION

Referring to [Table 1] no significant change was seen in the mean BSL-F and BSL-PP in both the groups. It indicated that the glycemic status of both case and control groups were comparable.

Mean HbA1c values were increased in cases as compared to controls which is statistically significant [P<0.05].

[Table 2] showed MCV, MCH and MCHC levels were highly significantly decreased [p<0.001] in cases [type II diabetes with IDA] compared to controls [type II diabetes without IDA]. RDW levels were highly significantly increased [p<0.001] in cases compared to control. This confirms the presence of IDA in cases.

Referring to [Table 3] out of total cases 27 showed decreased serum Ferritin levels and 9 cases showed normal serum ferritin level.

Diabetic complications cause death or permanent organ damage in thousands of patients each year worldwide. Therefore, the diagnosis and monitoring of the patients is very important and must be done correctly It is the basic marker for determining long-term glucose levels in diabetic patients [23]. The ADA has proposed the use of HbA1c for the follow-up and diagnosis of DM if the test is assayed in specific centers that provides standard and reference methods for the analysis. However, problems surrounding the standardization of this method persist [24]. Both the differences in the methods used and patients' physiological and pathological conditions can affect the results. Variables that determine the level of HbA1c include the mean serum glucose level within the last 2-3 months, the erythrocyte life span, the erythrocyte plasma membrane permeability to glucose, the tissue oxygen concentration, iron status and the integrates of the heme and globin structures [25].

Hence increased HbA1c concentration in an individual is considered to be due to diabetes. In

such cases other variables should be ruled out before confirming the diagnosis of DM.

In the present study all the cases and controls were known cases of type II DM clinically documented by physicians based on RFT and fundoscopy. The glycemic status of both cases and controls was under control and comparable. Diagnosis of iron deficiency anemia requires laboratory- confirmed evidence of anemia, as well as low iron stores [26]. Complete blood count was assessed to recognize the indices of iron deficiency anemia along with peripheral blood smear examination and serum ferritin level for confirmation of IDA in cases. This study showed significant decrease in Hb, Hct, MCV, MCH, MCHC and increased RDW and increased HbA1c concentration in cases compared to controls.

Study conducted by Silva J. et al [27] also observed a significant negative correlation between HbA1c with Hb, hematocrit and MCV. Hardikar et al [28] showed negative association with HbA1c and Hb; negative association were also found between MCV, MCH and MCHC with HbA1c.

In recent times much is talked about of serum ferritin, an acute phase reactant a marker of iron stores in the body and its association with diabetes mellitus.

In present study serum ferritin levels for confirmation of IDA in 36 cases revealed that 9 cases [25%] showed normal serum ferritin level and remaining 27 cases [75%] showed low level of serum ferritin. The probable correlation between ferritin and DM was considered first in 1993 by Kay et al. [29], after which other studies were focused on this subject. Ford et al [30] in the study in United States on 9486 diabetic adults determined high levels of ferritin in diabetics. Another study by Kwant [31] on the prevalence of C282Y mutation of hemochromatosis gene, determined the higher prevalence of this mutation in type 2 DM, that could be considered as an evidence for some relationship between these two disorders. Various studies have shown elevated ferritin in diabetic population, though its mechanism is still debatable, in some previous studies serum ferritin showed positive correlation with HbA1c in diabetic individuals [32]. In addition, Cantur KZ et al. [33] Found that serum ferritin was elevated as long as glycemic status

was not achieved, thus they found normal ferritin levels in diabetic individuals. Sharifi and Sazandeh [3] did not find any significant correlation between HbA1c and ferritin in diabetic population.

Study conducted by pramiladevi et al. [34] showed that as duration of DM increased there was increase in serum ferritin levels compared to recent onset. In this study 75% cases showed decreased serum ferritin levels may be due to associated IDA in the cases with recent onset of DM. 25% cases showed normal serum ferritin level inspite of IDA. This may be because of higher prevalence of mutation of hemochromatosis gene in type II DM.

The result of the present study also showed significant increase in HbA1c level in cases as compared to controls inspite of comparable glycemic status in both groups. This may be due to associated IDA in cases.

Hypoproliferative anemias such as iron-deficiency anemia prolong the lifespan of RBCs. In iron-deficiency anemia, increased malondialdehyde can enhance the glycation of Hb. Both these factors can lead to falsely elevated HbA1c levels in iron-deficiency anemia. It has recently been shown that the use of HbA1c to diagnose diabetes in a rural Indian population led to an overestimation of the prevalence of both diabetes and prediabetes [28]. The authors attribute this to the high prevalence of iron-deficiency anemia, leading to significantly elevated HbA1c levels in this population.

A study by Christy Alap et al [8] also showed that conditions such as iron deficiency anemia can significantly elevate HbA1c levels. a positive correlation between iron deficiency and increased HA1c levels. The results of this study are in agreement with other studies [35,36,37].

Iron-deficiency anemia is endemic in India. It is particularly common in adolescents as well as in women of the reproductive age group. It has been estimated that more than 50% of women 15–49 years of age and more than 85% of pregnant women in India have Hb levels below the lower limit of normal for their age and sex [38].

With iron-deficiency anemia and diabetes both being frequent in India, clinicians should be aware of this interaction while interpreting

**Table 1. Blood sugar levels [fasting and post prandial] and HbA1c in cases and controls**

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| Parameters | Cases [n=36] | Controls [n=36] | P value |
|---|---|---|---|
| BSL-Fasting | 98.86±11.75 | 101.72±7.96 | 0.88 |
| BSL-PP | 129.55±13.62 | 127.69±10.30 | 0.25 |
| HbA1c | 7.15+0.78 | 5.81+0.37 | 0.004* |

*$P < 0.05$*

**Table 2. Mean hemoglobin [Hb], MCV, MCH, MCHC and RDW in case and control groups**

| Parameters | Case [n=36] | Control [n=36] | P value |
|---|---|---|---|
| Hb[gm/dl] | 9.69±1.68 | 13.81±1.06 | 0.0001* |
| HCT [%] | 29.03±5.53 | 41.04±3.88 | 0.0001* |
| MCV [fl] | 71.66±6.02 | 91.69±4.54 | 0.0001* |
| MCH [pg] | 23.4±2.56 | 30.80±1.73 | 0.0001* |
| MCHC [g/dl] | 28.78±1.70 | 33.53±1.81 | 0.0001* |
| RDW [%] | 18.56±3.72 | 13.38±0.58 | 0.0001* |

*$P<0.001$ indicate highly significant*

**Table 3. Mean value of Serum Ferritin in cases**

| Parameters | 27 cases [75%] | 9 cases [25%] |
|---|---|---|
| Serum Ferritin [µg/l] | 10.2 | 117.44 |

inappropriately elevated HbA1c value in diabetics with glycemic control. IDA needs to be ruled out in such cases and if IDA is diagnosed, it should be treated which lowers HbA1c levels [38,39].

## 4. CONCLUSIONS

Iron deficiency anemia elevates HbA1c levels in type II Diabetes Mellitus, Hence IDA is to be taken in consideration while interpreting HbA1c in diagnosis and monitoring of Diabetes mellitus.

## CONSENT

All authors declare that written informed consent was obtained from the patient. Detailed medical history and relevant clinical examination data and written consent were obtained from all subjects by explaining the study procedure.

## ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and been performed in accordance with    the ethical standards. The study protocol was approved by the Institutional Ethics Committee of the institute.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

## REFERENCES

1. Rathod G, Rathod S, Parmar P, Parikh A. Study of knowledge, attitude and practice of general population of Waghodia towards Diabetes Mellitus. International Journal of Current Research and Review. 2014;6(1):63-68.
2. Rathod G, Parmar P, Rathod S, Parikh A. Prevalence of anemia in patients with Type 2 Diabetes Mellitus at Gandhinagar, Gujarat, India. IAIM. 2016;3(3):12-16.
3. Sharifi F, Sazandeh Sh. Serum ferritin in type II diabetes mellitus & its relationship with HbA1c.Acta medica Iranica. 2004;42(2):142-45.
4. Braunwald M, Fauci. Diabetes Mellitus. In: Harrison's principles of internal medicine.15th Ed. Mc Graw Hill. 2001;3341-4.
5. Kaveeshwar S, Jon C. The current state of diabetes mellitus in India. Australasian Medical Journal.AMJ. 2014;7(1):45-48.

6.   Telen M, Kaufman R**.** The mature erythrocyte function. In: Wintrobe`s Clinical Hematology, Greer JP, Foerster J (Eds), Lippincot Williams & Wilkins, Philadelphia, USA. 1998;193-227.

7.   Nadia H. Haemoglobin A1c and iron deficiency anemia our understanding through decades. Rom J Diabetes Nutr Metab Dis. 22(3);289-96

8.   Christy A, Manjrekar P, Babu R, Hegde A. Influence of Iron Deficiency Anemia on Hemoglobin A1C Levels in Diabetic Individuals with Controlled Plasma Glucose Levels. Iranian biomedical journal. 2014;18(2):88-93.

9.   Adeoye S, Abraham S, Erlikh I, Sarfraz S, Borda T, Yeung L. Anaemia and Haemoglobin A1c level: Is there a case for redefining reference ranges and therapeutic goals?. BJMP. 2014;7(1): 706.

10.  Kim C, Bullard K, Herman W, Beckles G. Association between iron deficiency and A1C Levels among adults without diabetes in the National Health and Nutrition Examination Survey. Diabetes care. 2010;33(4):780-5.

11.  Mayer T, Freedman Z. Protein glycosylation in diabetes mellitus: A review of laboratory measurements and of their clinical utility. ClinicaChimicaActa. 1983;127(2):147-84.

12.  Himanshushekhar, Ketan K, Ashmeetkaur, Pooja baJadeja. Effect of iron deficiency on glycated haemoglobin in nondiabetics. IJSN. 2014;5(3):477-47.

13.  American Diabetes Association. Standards of medical care in diabetes—2010. Diabetes care. 2010;33:11-61.

14.  Adejumo BI, Uchechukwu D, Chinwe O, Abduffatah A, Azukaego T, Tosan A. Incidence and risk of anemia in type 2diabetic patients in the absence of renal impairment. Health. 2012;4:304-08.

15.  Hong J, Ku C, Noh J, Ko K, Rhee B, Kim D. Association Between the Presence of Iron Deficiency Anemia and Hemoglobin A1c in Korean Adults: Korea National Health and Nutrition Examination Survey. Medicine. 2015;94(20):825.

16.  Hosseini MS, Rostami Z, Saadat A. Anemia and microvascular complications in patients with type 2 diabetes mellitus. Nephrourol. 2014;6:19976.

17.  Ciok J, Leibschang J. The report of UNICEF/WHO Joint Consultation on iron deficiency anemia,Geneva, February 3-5, 1999. Ginekol Pol 1999;70:573–77.

18.  Wu AC, Lesperance L, Bernstein H. Screening for iron deficiency. Pediatr Rev. 2002;23:171–78.

19.  Vajpayee N, Susan S, Graham, Bem S. Basic examination of blood and bone marrow. I: McPherson RA, Pincus MR. Henry's clinical diagnosis and management by laboratory methods.Philadelphia: Elsevier Health Sciences; 2011.

20.  Tietz NW. Textbook of Clinical Chemistry and Molecular Diagnostics, 4th ed. Elsevier; 1186-91.

21.  Tietz NW. Spectrophotometry. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics, 4th ed. Elsevier; 64-65.

22.  Sack DB, Carbohydrate in: Burts CA, Ashwood ER, editors, Tietz Textbook of Clinical Chemistry. 3rd edition. Philadelphia: W.B. Saunders Company. 1999; 790-6.

23.  Sacks DB, Arnold M, Bakris GL, Bruns DE, Horvath AR, Kirkman MS, et al. Guidelines and Recommendations for Laboratory Analysis in the Diagnosis and Management of Diabetes Mellitus. Diabetes Care. 2011;34:61-99.

24.  Goldstein D, Little, R, Lorenz, R, Malone, J, Nathan D, Peterson. C. Tests of glycemia in diabetes. Diabetes Care. 1995;18:896-990.

25.  Burtis A, Edward D, Ashwood MD. Tietz textbook of clinical chemistry. Third Edition. 2003:790-96.

26.  Eid WE, Potalla JV. Value of Hemoglobin A1c in Diagnosing Diabetes Mellitus within a Chronic Disease Management System Illustrated by the Receiver Operating Characteristic Curve. Endocrine Practice. 2010;16(1):14-20.

27.  Silva JF, Pimentel AL, Camargo JL. Effect of iron deficiency anaemia on HbA1c levels is dependent on the degree of anaemia. Available:http://www.sciencedirect.com/sci ence /article/pii/S0009912015004348

28.  Hardikar PS, Joshi SM, Bhat DS, Raut DA, Katre PA, Lubree HG et.al .Spuriously high prevalence of prediabetes diagnosed by HbA1c in young Indians partly explained by hematological factors and iron deficiency anemia. Diabetes Care. 2012;135:797-802.

29.  Kay M, Guay A, Simonson D. Non-insulin dependent diabetes mellitus and elevated

serum ferritin level. J Diabetes Complications. 1993;7(4):246-49.

30. Ford ES, Cogswel ME. Diabetes mellitus and serum ferritin concentration among U.S.A adults. Diabetes Care. 1999;22(12):1978-81.

31. Kwant J. Patients with type 2 diabetes have a high frequency of the C282 mutations of hemochromatosis gene. Clin Invest Med. 1998;21(6):251-57.

32. Raj S, Rajan GV; Correlation between elevated serum ferritin and HbA1c in type 2 diabetes mellitus. International Journal of Research in Medical Science, 2013;1(1):12-15.

33. Cantur K, Cetinarslay B, Tarkun I, Canturk N; Serum ferritin levels in poorly- and well controlled diabetes mellitus. Endocr Res. 2003;29(3):299-306.

34. Pramiladevi, Boke U, Kora S. Serum Ferritin Levels In Type II Diabetes Mellitus. Sch J App Med Sci. 2013;1(5):472-75.

35. Madhu S, Raj A, Gupta S, Giri S, Rusia U. Effect of iron deficiency anemia and iron supplementation on HbA1c levels -

Implications for diagnosis of prediabetes and diabetes mellitus in Asian Indians. Clin Chim Acta. 2017;468:225-29.

36. Naslı-Esfahani E, Larijani B, Amini P, Ghodssi-Ghassemabadi R, Razmandeh R. Effect of treatment of iron deficiency anemia on hemoglobin A1c in type 2 diabetic patients. Turk J Med Sci. 2017;47(5):1441-46.

37. Urrechaga E.Influenceof iron deficiency on Hb A1c levels in type 2 diabetic patients. Diabetes Metab Syndr. 2018;12(6):1051-55.

38. U. S. Preventive Services Task Force. Screening for iron deficiency anemia, including iron supplementations for children and pregnant women: recommendation statement Am Fam Physician. 2006;74(3):461–64.

39. Amira S, Rehab M, Amal H, Monerh H, Mona A, Turki A .Impact of Iron Deficiency Anemia Treatment on Type 2 Diabetic Complications. Biochem Mol Biol J. 2019;5(1):1-6.