

## CORRELATION OF GLYCATED HAEMOGLOBIN WITH SERUM IRON, FERRITIN AND TIBC IN IRON DEFICIENCY ANEMIA

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**Article Info:** Received 07 February 2020; Accepted 27 February 2020

**DOI:** <https://doi.org/10.32553/ijmbs.v4i2.1007>

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**Conflict of interest:** No conflict of interest.

### Abstract

Iron deficiency anemia is a common type of anemia — a condition in which blood lacks adequate healthy red blood cells. Red blood cells carry oxygen to the body's tissues. As the name implies, iron deficiency anemia is due to insufficient iron. HbA1c shows patient's glycemic status of last 3 months. Some previous studies have been reported that iron deficiency anemia may increase the level of HbA1c.

**AIM:** To assess the status of HbA1c in patients of iron deficiency anemia & correlate HbA1c level with Serum Iron, Ferritin level.

**MATERIALS AND METHODS:** It is a cross sectional comparative study conducted in 1 yrs of duration. A total number of 60 subjects between the ages of 18 to 60 years were enrolled for the present study. Detailed medical history and relevant clinical examination data and written consent were obtained from all subjects by explaining the study procedure.

**RESULTS:** Haematological parameter like Hb, MCV, MCH, HCT and RDW were showed significantly difference in iron deficiency case and control groups. Total haemoglobin, MCV, MCH, HCT were significantly reduced in IDA case group whereas there is increase RDW level in case group as compare to control ( $p < 0.001$ ).

**CONCLUSION:** There is negative correlation of HbA1c with Haemoglobin, Serum Iron and ferritin level whereas positive correlation is detected with TIBC. The correlation of HbA1c is found to be significant with serum iron and MCH.

**Keywords:** Hb, MCV, MCH, HCT and RDW

### Introduction

Glycated haemoglobin is produced by a ketoamine reaction between glucose and the N-terminal valine of both  $\beta$ -chains of the haemoglobin molecule. The major form of glycated haemoglobin is haemoglobin A1c (HbA1c) [1,2]. The measurement of glycated haemoglobin is the standard method for assessing the long-term glycaemic control. When plasma glucose is consistently elevated, the nonenzymatic glycation of haemoglobin increases; this alteration reflects the glycaemic history over the previous 2–3 months, since erythrocytes have an average lifespan of 120 days [3,4] HbA1c is widely used as a screening test for diabetes mellitus, and American Diabetes Association has recently endorsed HbA1c  $\geq 6.5\%$  as a diagnostic criterion for diabetes mellitus [5]. HbA1c is majorly affected by the blood glucose levels alone. However, certain studies have proven that the HbA1c levels are altered by various other coexisting factors such as hemolytic anemia, Iron deficiency anemia, hemoglobinopathies, pregnancy, and

vitamin B12 deficiency [6]. Along with diabetes, especially that of iron deficiency anemia, which is a major public health problem in developing countries like India.

In a study carried out by Brooks *et al.*, significantly observed elevated HbA1c values in iron deficiency anemia patients and decreased levels after iron supplement treatment [7]. Similar results were also found in studies carried out by Gram-Hansen *et al.* [8] and Coban *et al.* [9]

HbA1c is routinely used an important marker of glycemic control, and it is of utter importance to exclude factors which could spuriously elevate its levels. Hence, the present study was planned to detect HbA1c level in euglycemic iron-deficient anemia cases and compare with age & sex matched healthy control and correlation of glycated haemoglobin level along with serum iron and ferritin level in IDA patient.

## AIMS AND OBJECTIVES

To assess the status of HbA1c in patients of iron deficiency anemia & correlate HbA1c level with Serum Iron, Ferritin level.

## MATERIALS AND METHODS

It is a cross sectional comparative study conducted in 1 yrs of duration. A total number of 60 subjects between the ages of 18 to 60 years were enrolled for the present study. Detailed medical history and relevant clinical examination data and written consent were obtained from all subjects by explaining the study procedure.

Considering the average of HbA1c value of 4.5 and allowable error of 1 at  $p < 0.05$  and power of the test 80%, estimated sample size is 60 which include 30 cases (clinically diagnosed patients of iron deficiency anemia) & 30 age and sex matched controls belonging to age group of 18 to 60 years were selected.

The cases included in present study were selected from patients attending outpatient department (OPD) and indoor patient department (IPD) of internal medicine. Samples were assessed at the Central Clinical Laboratory (CCL), Department of Biochemistry.

### Inclusion criteria

Cases: Clinically diagnosed cases of IDA

### Exclusion criteria

Patients having history of Impaired glucose tolerance, Diabetes Mellitus, Haemoglobinopathy, Hemolytic anemia, Chronic renal diseases, Chronic alcoholism, Pregnancy, Blood transfusion within a period of 6 months.

The following parameters were evaluated

- i. Haematological parameters: HiCN and Electrical impedance method (10)
- ii. Serum iron: Ferrozin method (11)
- iii. TIBC: Ferrozin method (11,12)
- iv. Serum ferritin: ELISA Method (13)
- v. Blood glucose: GOD-POD Method (14)
- vi. HbA1C: immunoturbidometric method (15)

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

## OBSERVATIONS AND RESULTS

Mean blood glucose level (BSL-F and BSL-PP) showed no significant difference in glycemic status of both case and control groups.

**Table No 1 Shows:** Haematological parameter like Hb, MCV, MCH, HCT and RDW were showed significantly difference in iron deficiency case and control groups. Total haemoglobin, MCV, MCH, HCT were significantly reduced in IDA case group whereas there is increase RDW level in case group as compare to control ( $p < 0.001$ ).

Parameter	Case	Control	P value
BSL-F	88.9 ± 9.03	84.7 ± 7.6	0.06
BSL-PP	118.7 ± 11.27	120.4 ± 10.82	0.56
Serum Iron	30.9 ± 11.63	99.4 ± 22.04	<0.0001
TIBC	399.5 ± 28.86	301.9 ± 33.58	<0.0001
Ferritin	6.17 ± 3.34	63.27 ± 15.73	<0.0001
Hb (g/dl)	8.9 ± 1.5	13.81 ± 1.04	<0.0001
HCT (%)	26.64 ± 5.2	40.97 ± 3.4	<0.0001
MCV (fl)	70.03 ± 5.1	91.44 ± 3.87	<0.0001
MCH (pg)	23.51	30.88 ± 1.59	<0.0001
RDW 9%)	19.14 ± 3.92	13.42 ± 0.62	<0.0001
HbA1c	6.55 ± 0.77	5.27 ± 0.83	<0.0001

Serum iron and ferritin levels were significantly decreased in IDA patients compared to controls. TIBC levels were observed to be significantly higher in IDA patient group. HbA1c levels were significantly high in IDA compared to control.

Parameter	Pearson Correlation	P- Value
Hb	-0.3265	0.078
Serum Iron	-0.3728	0.042
Ferritin	-0.3136	0.091
TIBC	0.174	0.357
MCH	-0.4962	0.005

**Above table No 2 showed** the correlation of HbA1c level with total haemoglobin, serum iron, ferritin and TIBC were studied in present study. There is negative correlation of HbA1c with Haemoglobin, Serum Iron and ferritin level whereas positive correlation is detected with TIBC. The correlation of HbA1c is found to be significant with serum iron and MCH ( $p < 0.05$ )

## DISCUSSION

HbA1c is majorly affected by the blood glucose levels. However, certain studies showed 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 world and developing countries like India too. Present study was

conducted to assess the status of HbA1c in iron deficiency anemia with euglycemic status and correlation of with serum iron, ferritin, TIBC and haemoglobin with HbA1c in IDA group.

Present study was conducted on 60 subjects with IDA (n=30) and age and sex matched healthy controls (n=30) groups. Diagnosis of iron deficiency anemia requires laboratory- confirmed evidence of anemia, as well as low iron stores (S. Iron, Ferritin).(16) Complete haematological study was assessed to recognize the indices of iron deficiency anemia (reduced Hb, Hct, MCV, MCH, MCHC and raised RDW) along with blood smear examination. Iron status was evaluated to confirm iron deficiency (reduced Serum iron, ferritin, and increase TIBC in IDA.).(17,18)

Ferritin is a storage form of iron, and it reflects the true status of iron [19]. Hence, in present study, its correlation with HbA1c was assessed, but there is no significant correlation was found. As explained previously, in iron deficiency anemia, ferritin is decreased with increase in the red cell life span, and increased red cell life span is associated with increased HbA1c. However, one of the studies did not show any significant correlation of serum ferritin levels and life span of red blood cell [20], indicating the lack of significant correlation between HbA1c and ferritin in our study. Various studies have shown elevated ferritin in diabetic population, though its mechanism is still not well cleared. In a study by Raj and Rajan [21], ferritin showed positive correlation with HbA1c in diabetic individuals. In addition, Canturk *et al.* [22] 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 [23] did not find any significant correlation between HbA1c and ferritin in diabetic population. We could not explain the lack of correlation of serum ferritin levels with HbA1c in present study. Our study did not show any significant correlation between hemoglobin and HbA1c ( $r =$ ,  $P =$ ). When correlation for red cell indices and HbA1c in anemic subjects was studied, no significant correlation was found between HbA1c and MCV ( $r =$ ,  $P =$ ), and borderline significant association was found between HbA1c and MCH ( $r =$ ,  $P =$ ). Although association of elevated A1C with severity of iron deficiency anemia remains unexplained, its borderline association with red cell indices proves the role of erythrocyte morphology and lifespan in elevating A1C.

Though we tried to collect as much data as possible for inclusion and exclusion of subjects in our study, some data might have been missed. We could not conclude any effect of BMI on HbA1c levels due to the lack of

sufficient data. We could not get the follow up data of patients after iron therapy, which might have given a new dimension to our study.

#### CONCLUSION:

This study found a positive correlation between iron deficiency anemia and increased HbA1C levels and we found that HbA1c is better glycemic marker for IDA. There is negative correlation of HbA1c with Haemoglobin, Serum Iron and ferritin level whereas positive correlation is detected with TIBC. The correlation of HbA1c is found to be significant with serum iron and MCH.

#### REFERENCES

1. De Rosa MC, Sanna MT, Messina I, Castagno-la M, Galtieri A, Tellone E, et al. Glycated human haemoglobin (HbA1c): Functional characteristics and molecular modeling studies. *Biophys Chem.* 1998;72:323–35. [PubMed] [Google Scholar]
2. Peterson KP, Pavlovich JG, Goldstein D, Little R, England J, Peterson CM. What is haemoglobin A1c? An analysis of glycated haemoglobins by electrospray ionization mass spectrometry. *Clin Chem.* 1998;44:1951–58. [PubMed] [Google Scholar]
3. Shapiro R, McManus M, Garrick L, McDonald MJ, Bunn HF. The nonenzymatic glycolisation of human haemoglobin at multiple sites. *Metabolism.* 1979;28((suppl)):427–30. [PubMed] [Google Scholar]
4. Braunwald E, Fauci AS, Kasper DL, Hauser SL, Longo DL, Jameson JL. Diabetes mellitus. *Harrison's Principles of Internal Medicine.* (15) 2001;22:2105–09. [Google Scholar]
5. Standards of Medical Care in Diabetes—2011. *Diabetes Care.* 2011;34(1):S13. [Google Scholar]
6. Sinha N, Mishra TK, Sinha T, Gupta N. Effect of iron deficiency anemia on hemoglobin A1c levels. *Ann Lab Med.* 2012 Jan;32(1):17–22. [PMC free article] [PubMed] [Google Scholar]
7. Brooks AP, Metcalfe J, Day JL, Edwards MS. Iron deficiency and glycosylated haemoglobin A1. *Lancet.* 1980 Jul;316(8186) [PubMed] [Google Scholar]
8. Gram-Hansen P, Eriksen J, Mourits-Andersen T, Olesen L. Glycosylated haemoglobin (HbA1c) in iron- and vitamin B12 deficiency. *J Intern Med.* 1990 Feb;227(2):133–6. [PubMed] [Google Scholar]
9. Coban E, Ozdogan M, Timuragaoglu A. Effect of iron deficiency anemia on the levels of hemoglobin A1c

- in nondiabetic patients. *Acta Haematol.* 2004;112(3):126–8. [PubMed] [Google Scholar]
10. Vajpayee N, Susan S, Graham, Bem S. Basic examination of blood and bone marrow. In: McPherson RA, Pincus MR. *Henry's clinical diagnosis and management by laboratory methods.* Philadelphia: Elsevier Health Sciences; 2011 Sep 6.
  11. Tietz N.W., Norbert W, and Sheldon Berger. *Fundamentals of Clinical Chemistry.* Philadelphia: Saunders, 1970.
  12. Siedel J, Wahlefeld AW, Ziegenhorn J. A new iron ferro zine reagent without deproteinization. *ClinChem* 1984;30:975
  13. N. W. Tietz., *Textbook of Clinical Chemistry and Molecular Diagnostics*, 4th ed. Elsevier, p 1186-91
  14. N. W. Tietz., *Spectrophotometry.* Tietz *Textbook of Clinical Chemistry and Molecular Diagnostics*, 4th ed. Elsevier, p 64-5
  15. Sack DB, Carbohydrate in: BurtsCA, AshwoodER, editors, *Tietz Textbook of Clinical Chemistry.* 3rd edition. Philadelphia: W.B. Saunders Company; 1999. P790-6.
  16. 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):461–4.
  17. Glader B. Anemia: General considerations. In: Greer JP, Rodgers GM, Paraskevas F, Glader B, eds. *Wintrobe's clinical Haematology.* 11th ed. Philadelphia: Wolters Kluwer Company, 2004:947-78.
  18. Firkin F, Chesterman C, Penington D, Rush B. The red cell; Basic aspect of anemia. In: de Gruchy's *Clinical Hematology in Medical Practice.* 5th ed. Noia: Blackwell Science, Inc, 2006:17-36.
  19. John A. Iron Deficiency and Other Hypoproliferative Anemias. In: Longo D, Fauci A, Kasper D, Hauser S, Jameson J, Loscalzo J, editors, *Principles of Internal Medicine by Harrisons.* 17th ed. United States of America: McGraw-Hill; 2008. pp. 628–35. [Google Scholar]
  20. Weight LM, Byrne MJ, Jacobs P. Haemolytic effects of exercise. *Clin Sci (Lond)* 1991 Aug;81(2):147–52. [PubMed] [Google Scholar]
  21. Raj S, Rajan GV. Correlation between elevated serum ferritin and HbA1c in type 2 diabetes mellitus. *Int J Res Med Sci.* 2013;1(1):12–15. [Google Scholar]
  22. Canturk Z, Çetinarslan B, Tarkun I, Canturk NZ. Serum ferritin levels in poorly- and well-controlled diabetes mellitus. *Endocr Res.* 2003 Aug;29(3):299–306. [PubMed] [Google Scholar]
  23. Sharifi F, Sazandeh SH. Serum ferritin in type 2 diabetes mellitus and its relationship with HbA1c. *Acta Medica Iranica.* 2004;42(2):142–5. [Google Scholar]