

Study Of HbA1c In Iron Deficiency Anaemia.

Mr. Gangaram Laxman Bhadarge

*Tutor Dept. of Biochemistry Jawaharlal Nehru Medical College, DattaMeghe Institute of
Medical Sciences Sawangi (Meghe) Wardha-442001*

Dr. Prajakta Warjekar

Assistant Professor Dept. of Biochemistry DattaMeghe Medical College-441110

Dr. Pradip Jain

Professor and HOD Dept. of Biochemistry DattaMeghe Medical College-441110

Dr. Prakash Kute

*Associate Professor Dept. of Biochemistry Jawaharlal Nehru Medical College,
DattaMeghe Institute of Medical Sciences Sawangi (Meghe) Wardha-442001*

Dr. V. Shegaonkar

*Professor Dept. of Medicine Jawaharlal Nehru Medical College, DattaMeghe Institute of
Medical Sciences Sawangi (Meghe) Wardha-442001*

Address for Correspondence

Dr. Pradip Jain

*Professor and HOD Dept. of Biochemistry
DattaMeghe Medical College-441110*

Mob No. 9822566869

ABSTRACT

INTRODUCTION:

Iron deficiency anaemia increased when iron intake concentrations, iron reserves, and iron loss by the body was inadequate to completely sustain erythrocyte development. Anemia with iron deficiency rarely causes death, but the effect on human health is severe. This disorder is quickly diagnosed and treated in developed countries, but it is often ignored by doctors. On the other hand, in underdeveloped nations, it is a health issue that affects large portions of the population. Hemoglobin A1C (HbA1c) represents the glycemic status of the patient over the previous three months. Previous studies have reported that, independent of glycemia, iron deficiency can increase HbA1C concentrations.

Aim: This study is aimed to analyze the effect of iron deficiency anemia on HbA1c levels in anaemic population and compare with healthy individuals.

MATERIAL AND METHODS: This study included 50 patients of iron deficiency anemia and 50 healthy subjects as the control group. This is a cross-sectional investigation wherein cases were out-patients. Patients went to the Directly Observed Treatment Short-course focus in the Dept. of General Medicine, DattaMeghe Medical college, SMHRC, Nagpur in collaboration with JNMC, ABVRH (DattaMeghe Institute of Medical Sciences Deemed To Be University), Sawangi, Wardha, Maharashtra.

RESULT: HbA1c results suggested that IDA was associated with higher concentrations of HbA1c. The mean HbA1c of cases was 6.81 % while it was 5.04 % in healthy control. Application of statistical test shows that the difference of HbA1c between case and control group was statistically significant. CONCLUSION: In this study, we observed a positive association between IDA and elevated HbA1C level in non diabetic

population. HbA1C rises with severity of anaemia. In iron depletion, this spurious elevation of HbA1C contributes to over or under diagnosis of diabetes at the cutoff point (6.5 percent).

KEY WORDS: HbA1c, Sr.Ferritin, CBC

INTRODUCTION:

One fourth of the world's populations is affected by anaemia, and the primary cause is iron deficiency. Chronic fatigue, reduced cognitive ability, and decreased well-being are associated with anaemia. Patients with iron deficiency anaemia of unknown aetiology are sometimes referred to a gastroenterologist since the disease is of gastrointestinal origin in the majority of instances. Proper treatment increases the quality of life, alleviates iron deficiency symptoms, and decreases the need for blood transfusions. Treatment options include oral and intravenous iron therapy, but in some gastrointestinal disorders, such as inflammatory bowel disease, celiac disease, and autoimmune gastritis, the effectiveness of oral iron is restricted.¹

For the development of heme and haemoglobin, erythrocytes and their precursors require great quantities of iron. Iron is central to the structure and function of hemoglobin. Mono- or diferric transferrin, occurring at high plasma concentrations, is the most immediate source of iron for erythroblast. Iron deficiency anaemia is typically caused by low iron saturation of available transferrin. Iron is loaded onto diferric transferrin from three sources: the gut (diet), macrophages (recycled iron), and liver (stored iron ferritin). In general, iron reserves are degraded or destroyed before the host develops anaemia. Consequently, dietary and erythrocyte-recycled iron must satisfy the requirements for erythrocyte growth. If iron losses persisted, the newly formed erythrocytes would have decreased haemoglobin, allowing the amount of iron supplied by the same number of senescent erythrocytes to decrease.²

Alternatively, anaemia is characterized as a reduced absolute number of circulating RBCs or a condition in which the number of RBCs (and consequently their oxygen carrying capacity) is inadequate to meet physiological needs.³ Anemia may also be diagnosed using RBC count, mean corpuscular volume, blood reticulocyte count, but most commonly diagnosed by a low Hb concentration or a low hematocrit. Approximately one-third of the world's population (32.9 percent) are estimated to suffer from anaemia in the blood film study.⁴

The iron-supplemented population classification lists the following levels as anaemic: levels of Hgb (g / dl) below 11 g / dl in the first trimester; levels of 10.5 g / dl in the second trimester; and levels of 11 g / dl in the third. This is the norm in India that must be embraced. The ICMR classification for severity of anemia was also accepted by the expert community.⁵

Classification:⁶

Grading of anemia, according to the National Cancer Institute, is as follows:

1. Mild: Hemoglobin 10.0 g/dL to lower limit of normal
2. Moderate: Hemoglobin 8.0 to 10.0 g/dL
3. Severe: Hemoglobin 6.5 to 7.9 g/dL
4. Life-threatening: Hemoglobin less than 6.5 g/dL.

HbA1c: Hemoglobin A (HbA) (95-98 percent), haemoglobin A2 (HbA2) (2-3 percent), and fetal haemoglobin (HbF) (1 percent) are the forms of haemoglobin contained in adults. Also, subtypes of HbA that can be characterised by electrophoresis are HbA0, HbA1a1, HbA1a2, HbA1b, and HbA1c. HbA1c is 70-90 percent of HbA1, which is HbA1's glycosylated form. HbA1c results from the glycosylation of praline's haemoglobin β chain N-terminal which represents the concentration of plasma glucose. The concentration of HbA1c is mainly influenced by both haemoglobin and plasma glucose.⁷

The most prevalent fraction of HbA1 is HbA1c, and it is formed by terminal valine glycation in the haemoglobin β -chain. It represents the glycemic status of the patient over the previous 3 months. HbA1c is commonly used as a diabetes mellitus screening procedure, and HbA1c has recently been approved by the American Diabetes Association as a diagnostic criterion for diabetes mellitus. A research conducted by Sinha et al. has explained its alteration in other conditions, such as hemolytic anaemia, hemoglobinopathies, pregnancy, and vitamin B12 deficiency. While iron deficiency is the most common nutritional deficiency, there have been contradictory reports on the clinical significance of iron deficiency at HbA1c levels. HbA1

values were measured at 355 in a report conducted by Brooks et al. Before and after treatment with iron, non-diabetic patients with iron deficiency anaemia. Elevated HbA1c values were significantly observed in patients with iron deficiency anaemia and decreased levels after treatment with iron.⁸

Hemoglobin A1c (HbA1c) is the gold standard for diabetes diagnosis, but the HbA1c level, including anaemia, is affected by many clinical conditions. And, iron deficiency anaemia (IDA) is among the most common causes of anaemia worldwide. Since these factors can lead to an incorrect increase or decrease in HbA1c leading to misinterpretation of the disease condition, in the diagnosis of DM and monitoring of long-term blood glucose levels in anaemia. Therefore, the importance of the Hb value in serial HbA1c monitoring and the Hb cut-off value below which HbA1c cannot be regarded as an important diagnosis.

There are currently up to 30 HbA1c measurement methods, which can be categorised into four groups, including HPLC ion-exchange, HPLC affinity, immunoassay, and enzymatic methods. No important difference in HbA1c measured with ion-exchange HPLC and two immunoassay methods was found by Haliassos et al. Rai et al. found no significant effect of from the type of method used to measure the HbA1c level.⁷

Ferritin: Serum ferritin can be normal or elevated, indicating iron reserves. This is a supply and demand epidemic, not total body iron deficiency. In the sense of ESA-driven bone marrow stimulation, the patient has quantities of iron in the body that may be common for a person who is not anaemic and not an ESA, but the rate at which iron is released from stores and the rate at which iron is supplied to the erythroid marrow by transferrin is insufficient to keep up with RBC production.⁹

The authors assessed serum ferritin in the general population, patients with iron deficiency, and individuals with iron overload to establish the relationship between serum ferritin levels and total body iron stores. They showed that serum ferritin was increased in iron overload patients and decreased in iron deficiency disease patients.¹⁰⁻¹¹

Ferritin is a type of iron storage and represents the true iron status in anaemia of iron deficiency, ferritin decreases with an increase in the life span of red cells, and increased life span of red cells is correlated with increased HbA1c.⁶

MATERIAL AND METHODS:

Study Area

This study included 50 patients of iron deficiency anemia and 50 healthy subjects as the control group. This is a cross-sectional investigation wherein cases were out-patients. Patients went to the Directly Observed Treatment Short-course focus in the Dept. of General Medicine, DattaMeghe Medical college, SMHRC, Nagpur in collaboration with JNMC, ABVRH (DattaMeghe Institute of Medical Sciences Deemed To Be University), Sawangi, Wardha, Maharashtra.

Inclusion Criteria :

1. Patients without diabetes.
2. Patient belonging to rural vidharbharegions area.
3. > 25 yrs. of age.
4. Giving consent for study.

Exclusion Criteria

1. Diabetic Patients were excluded from this study.
2. HIV Patients.
3. Tuberculosis patients.
4. Cancer Patients were excluded

Sample Collection :

Total 5 ml of venous blood was collected and distributed in EDTA and Plain vial for serum under aseptic condition, the serum sample were used for the estimation of Ferritin and the EDTA sample were used for the estimation of CBC and HbA1c.

Biochemical Analysis

- 1.HbA1c was estimated by HPLC method 12. and Estimation of serum ferritin and insulin was
- 2.done by using automated Chemiluminescence Immunoassay system (CLIA).13
- 3.CBC were Performed on Beckman Coulter counter.

Results:

Table 1: Comparison of CBC in IDA Patients and Non-IDA Individuals

| Parameters | IDA | Non-IDA | P-Value |
|------------|--------------|--------------|------------|
| RBC | 3.26 ± 0.76 | 4.88 ± 0.42 | P<0.0001 |
| Hgb | 8.96 ± 1.84 | 15.2 ± 2.1 | P<0.0001 |
| HCT | 29.34 ± 5.87 | 49.19 ± 3.73 | P<0.0001 |
| MCV | 86.5 ± 7.86 | 90.12 ± 4.68 | P = 0.0062 |
| MCH | 28.69 ± 4.04 | 28.73 ± 1.44 | P = 0.9476 |
| MCHC | 31.77 ± 2.19 | 32.75 ± 0.99 | P = 0.0048 |
| RDW | 18.08 ± 3.44 | 13.32 ± 1.46 | P<0.0001 |

Table 2: Comparison of HbA1c and Serum Ferritin Level in IDA Patients and Non-IDA Individuals

| Parameters | IDA | Non-IDA | P-Value |
|------------|-------------|-------------|----------|
| HbA1c | 6.81 ± 1.37 | 5.04 ± 1.21 | P<0.0001 |
| S.Ferritin | 12.26±4.71 | 92.04±8.65 | P<0.0001 |

Discussion :

HbA1c :Our results suggested that IDA was associated with higher concentrations of HbA1c. The mean HbA1c of cases was 6.81 % while it was 5.04 % in healthy control. Application of statistical test shows that the difference of HbA1c between case and control group was statistically significant.

In the study conducted by Vishal Kalasker et al , the mean HbA1c of cases was 5.91±0.47 % while it was mean HbA1c of cases was 5.91±0.47 % while it was 6.54±0.39 % in healthy control. Application of statistical test shows that the difference of HbA1c between case and control group was statistically control group. 14

In the study conducted by Balasubramanian Shanthi et al , the mean HbA1c of cases was 7.6 ± 0.5 % while it was 5.5 ± 0.8 % in healthy control. Application of statistical test shows that the difference of HbA1c between case and control group was statistically significant indicating statistically higher HbA1c in cases group.15

In the IDA group, the correlation between RBC, red cell indices and HbA1c was measured and the outcome was not statistically significant. Similarly, a study conducted in India in 2014 showed no substantial correlation, but a significant borderline association was observed in IDA diabetic patients between MCH and HbA1c ($P = 0.05$). Among the hematological parameters, the mean difference between the two classes was statistically significant: RBC, Hgb, MCV, MCH. This result is similar to a study conducted in India 2016.

Iron deficient patients fall into three categories on the basis of haemoglobin: mild anaemia, moderate anaemia and extreme anaemia. (28.7 percent) of patients had mild, (46 percent) moderate and (25.3 percent) extreme anaemia, based on this classification. Extreme anemia was seen in (76 percent) patients, and mild in (24 percent) patients, in a similar study done in India. The current research revealed no significant association in IDA diabetic patients between sex, age and HbA1c, which is in line with the related study conducted in In.16-17

Our results suggested that IDA was associated with decrease concentrations of serum ferritin. The mean S.Ferritin of cases was 12.26 % while it 92.04% in healthy control. Application of statistical test shows that the difference of S.Ferritin between case and control group was statistically significant.18-19

Ferritin: It is a type of iron storage, and it represents the true status of iron. Serum ferritin as well as serum iron levels were indirectly proportional to HbA1C in our sample. As explained earlier, ferritin is reduced in IDA with an improvement in the life span of red cells associated with increased HbA1C. This goes hand in hand with other research findings from Shanthi B et al., and Raj S et al.20

CONCLUSION:

In this study, we observed a positive association between IDA and elevated HbA1C level in non diabetic population. HbA1C rises with severity of anaemia. In iron depletion, this spurious elevation of HbA1C contributes to over or under diagnosis of diabetes at the cutoff point (6.5 percent). This research therefore emphasizes the absolute importance of removing IDA from iron studies and correcting it before any diagnostic or therapeutic decision is taken based solely on the level of HbA1C.

REFERENCES:

1. Jimenez K, Kulnigg-Dabsch S, Gasche C. Management of Iron Deficiency Anemia. *GastroenterolHepatol(N Y)*. 2015;11(4):241-250.
2. Miller JL. Iron Deficiency Anemia: A Common and Curable Disease. *Cold Spring HarbperspectMed*. 2013;3(7):A011866. Published 2013 Jul 1.
3. Chaparro CM, Suchdev PS. Anemia Epidemiology, Pathophysiology, and Etiology in Low- and Middle-Income Countries. *Ann N Y Acad Sci*. 2019;1450(1):15-31.
4. Rakshale N, Sebastian T, Hatwar K, Bhoyar P, Bhute K, Bhusari P, et al. Knowledge regarding sickle cell anaemia among newly married couples: A qualitative study. *Intern J Cur Res Rev* 2020;12(19):128-133.
5. Good Clinical Practice Recommendations for Iron Deficiency Anemia in Pregnancy (IDA) in Pregnancy in India. *J ObstetgynaecolIndia*. 2011;61(5):569-571.
6. H. Celikhisar and G. D. Ilkhan, "Determining The Etiological Factors in Pleural Fluid by Crp, Albumin And Procalcitonin Levels", *jmrhs*, vol. 2020. 3,(2), pp. 880-885. DOI:<https://doi.org/10.15520/jmrhs.v3i2.157>.
7. Tas F, Eralp Y, Basaran M, Sakar B, Alici S, Argon A, Bulutlar G, Camlica H, Aydiner A, Topuz E. Anemia in oncology practice: relation to diseases and their therapies. *Am J ClinOncol*. 2002;25(4):371-9.
8. Guo W, Zhou Q, Jia Y, Xu J. Increased Levels of Glycated Hemoglobin A1c and Iron Deficiency Anemia: A Review. *Med SciMonit*. 2019;25:8371-8378. Published 2019 Nov 7.
9. Christy AL, Manjrekar PA, Babu RP, Hegde A, Rukmini MS. Influence of Iron Deficiency Anemia on Hemoglobin A1c Levels in Diabetic Individuals With Controlled Plasma Glucose Levels. *Iran Biomed J*. 2014;18(2):88-93.

10. D. F. Harvey, "Physician Burnout Quality of Life/ Wellness Resource Pilot Program", *jmrhs*, 2020, 3, (2), pp. 886-912. <https://doi.org/10.15520/jmrhs.v3i2.160>
11. Guo W, Zhou Q, Jia Y, Xu J. Increased Levels of Glycated Hemoglobin A1c and Iron Deficiency Anemia: A Review. *Med Sci Monit*. 2019;25:8371-8378. Published 2019 Nov 7.
12. Jay B. Wish. Assessing Iron Status: Beyond Serum Ferritin and Transferrin Saturation. *CJASN* Sep 2006, 1 (1) S4-S8.
13. Bhatt A, Vinayak N, Acharya N. Role of intravenous iron sucrose in comparison to oral ferrous sulfate for prophylaxis of anemia in pregnant women. *J Datta Meghe Inst Med Sci Univ* 2020;15(1):12-15.
14. Ambade VN, Sharma YV, Somani BL. Methods For Estimation Of Blood Glucose: A Comparative Evaluation. *Med J Armed Forces India*. 1998;54(2):131-133.
15. Campbell AK. Detection and Quantification Of Chemiluminescence, in *Chemiluminescence Principles and Applications in Biology and Medicine*. 1988 Ellis Horwood, 68-126.
16. Choudhary, D.S., Choudhary, D.S.D., Choudhary, D.H. And Gandhi, D.R. 2019. The Effect of Iron Deficiency Anemia on Glycated Hemoglobin (Hba1c) in Non Diabetic Adults. *International Journal of Medical and Biomedical Studies*. 3, 8 (Aug. 2019), 140-142.
17. Shanthi B, Revathy C, Manjula Devi AJ, Subhashree. Effect of Iron Deficiency on Glycation of Haemoglobin in Nondiabetics. *J Clin Diagn Res*. 2013;7(1):15-17.
18. Abidillah Mursyid, Waryana, Lastmi Wayansari, Wiworo Haryani (2017) Canteen Manager And Elementary Student Empowerment About Local Food To Combat Anemia *International Journal Of Scientific Research And Education*. 05,07 (July-17) 6726-33
19. Smith RJ, Koenig RJ, Binnerts A, Soeldner JS, Aoki TT. Regulation of Hemoglobin A1C Formation in Human Erythrocytes in Vitro: Effects of Physiologic Factors Other Than Glucose. *J Clin Invest*. 1982;69(5):1164-8.
20. Maheshwari, S., Jaan, A., Vyaasini, C. S., Yousuf, A., Arora, G., & Chowdhury, C. (2020). Laser and its Implications in Dentistry : A Review Article. *Journal of Current Medical Research and Opinion*, 3(08). <https://doi.org/10.15520/jcmro.v3i08.323>
21. Sheema UK, Rawekar A. Neurophysiological assessment and cognition in adult females with iron deficiency anaemia. *Eur J Mol Clin Med* 2020;7(2):1923-1925.
22. Shanthi B, Revathy C, Manjula Devi AJ, Subhashree Effect of Iron Deficiency on Glycation of Haemoglobin in Nondiabetics. *J Clin Diagn Res*. 2013 Jan; 7(1):15-7.