

Glycated haemoglobin variations among non - diabetics with nutritional anaemia

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Abstract: *Background:* Anemia is highly prevalent globally, significantly affecting public health and potentially playing a role in the development of various chronic illnesses. Iron deficiency accounts for approximately one-third of anemia cases. However, the impact of Iron Deficiency Anemia (IDA) on HbA1c levels remains controversial, with studies reporting conflicting findings. *Objectives:* Our aims and objectives were to determine the effects of iron deficiency anemia on HbA1c in non diabetic individuals, and to study how variations in HbA1c may correlate to the degree of anemia. *Materials & Methods:* This cross-sectional study consisted of 150 non diabetics (50 iron-deficiency anemia [IDA] patients, 50 Vit B12 deficiency patients and 50 healthy controls). The study was performed at the pathology laboratory, Owaisi group of hospitals attached to Deccan college of Medical Sciences for a period of 15months from December 2023 to March 2025 and was approved by our Institutional Ethical Committee. Medical history was recorded and HbA1c, Haemoglobin (Hb), Fasting Plasma Glucose (FPG) were record. *Results:* The IDA patients in this study had a mean HbA1c (7.33) which was higher than the non anemics (4.99). This difference in the mean values is significant ($p<0.05$) statistically. *Conclusion:* The level of HbA1c was found to be directly proportional to the severity of anemia. Additionally, a statistical significance was noted between the healthy, mildly, moderately and severely anemics (again, $p<0.05$).

Keywords: HbA1c, Iron- deficiency anemia, Diabetes Mellitus, Non- diabetics, Haemoglobin, Fasting Plasma Glucose.

Introduction

The American Diabetes Association (ADA) recognizes a Glycated Hemoglobin (HbA1c) level of $\geq 6.5\%$ as a diagnostic criterion for Diabetes Mellitus (DM) [1]. HbA1c is defined as haemoglobin which is irreversibly glycated at one or both N-terminal valines of the beta chains [2-3]. HbA1c has been the most used and accepted test for monitoring the glycaemic control in individuals with diabetes. Once a haemoglobin molecule is glycated, it continues to remain in the red blood cell for the rest of its life-span (120 days).

Glycosylated Hb was defined as an area of an elution chromatogram containing hemoglobin glycosylation products. This elution peak was labeled as HbA1, in keeping with the existing

nomenclature (HbA, HbA2, HbF, etc. had been identified previously). Later it was recognized that the chromatographic HbA1 region is not homogeneous and consists of several component peaks, designated A1a, A1b and A1c, with HbA1c being the dominant one [4]. The HbA1c fraction also turned out to correlate best with mean serum glucose concentrations, ie, to be a better index of long-term glycemia. The glycation of haemoglobin occurs at a variable (non-linear rate) over time, during the lifespan of the red blood cell (RBC), which is of 120 days. The relative proportion of HbA1c depends on the mean glucose level over the previous 120 days. Laboratory normal range differs depending on whether HbA1 or HbA1c is measured and on the method used [5].

Situations where the average RBC lifespan is significantly low <120 days will usually give rise to low HbA1c results because 50% of glycation occurs in 90-120 days [6]. Conditions that prolong the erythrocyte lifespan or conditions related to decreased RBC turnover will lead to prolonged exposure of the cell to glucose, resulting in higher HbA1c [7]. Similarly, states that reduce the lifespan of RBCs or conditions where RBC exposure to glucose is shortened (i.e., from an increase in RBC turnover) will result in reduced HbA1c concentrations. Iron deficiency anemia and vitamin B12 deficiency anemia fall into the former scenario.

HbA1c concentrations are not only altered by blood glucose levels [8], but they also vary significantly in a few diseases and pathological states, such as hypoproliferative anemia and hemoglobinopathies [9-10]. Initially, HbA1c was a marker for monitoring glycemic control. However, recently HbA1c has also been recommended as a diagnostic indicator of diabetes [9]. Factors altering HbA1c concentrations are Hb variants [11], hemolytic anemia, renal failure [12], hypothyroid [13], alcoholism, and hyperbilirubinemia. In this study, we aimed to look for HbA1c variations in iron deficiency anemia and their consideration with HbA1c as a diagnostic tool. Given the limited research conducted on the Indian population, this study was undertaken to evaluate the influence of Iron Deficiency Anemia (IDA) on HbA1C levels in non-diabetic individuals, eliminating the confounding effect of glucose on HbA1C. Additionally, we examined how HbA1C levels vary with the severity of anemia, categorized as mild, moderate, and severe.

Material and Methods

This was an analytical (descriptive), cross-sectional study conducted by the Pathology Laboratory, Owaisi Hospital and Research Centre, associated with the Deccan College of Medical Sciences between December 2023 to March 2025 and was approved by our Institutional Ethical Committee. In all, 100 non diabetics aged >18 years (with 50 healthy controls) were a part of our study. Individuals were diagnosed with Iron Deficiency Anemia (IDA) if they had hemoglobin levels below 13 g/dL in males and 12 g/dL in females, hematocrit values under 40% in males and 36% in females, a

mean corpuscular volume (MCV) less than 80 fl, mean corpuscular hemoglobin (MCH) below 26 pg/cell, mean corpuscular hemoglobin concentration (MCHC) under 32 g/dL, and a peripheral smear indicating a microcytic hypochromic pattern.

In all, 50 healthy, non anemic individuals were enrolled as controls and all the laboratory parameters were analysed for them as well. Medical history was recorded to exclude Diabetes Mellitus, the patients had to have no history of diabetes whatsoever and either FPG or RBS, performed close to the date of complete blood picture (CBP). Individuals with a history of kidney diseases, chronic alcoholics, pregnant women and people with haemolytic anemia were excluded from both the study and control group. By considering the Hb levels, anaemic patients were categorized as mild anaemia (12- 12.9 gm/dl for males and 11-11.9 gm/dl for females), moderate anaemia (9-11.9 gm/dl for males and 8-10.9 gm/dl for females), and severe anaemia (<9 gm/dl for males and <8 gm/dl for females) [14].

Measurements: The levels of absolute HbA1C were derived from its measured levels by using the formula [15].

$$\text{Absolute HbA1C (gm/dl)} = \frac{\text{HbA1C (\%)} \times \text{Hb (gm/dl)}}{100}$$

Statistical Analysis: The data was analysed using Python Software and presented as mean S.D for the continuous variables. Unpaired t-test was used for comparing group means. Correlation between two variables was calculated using Pearson's coefficient. A p-value greater than 0.05 was considered significant statistically.

Results

In this study, nondiabetics with IDA had a mean HbA1C% (7.33 ± 0.48). It was significantly higher (p<0.05) than the non-anaemic group (4.33 ± 0.20). Absolute HbA1C (gm/dl) in the patients with IDA was 0.53±0.06 and in the people without anemia was 0.50 ± 0.03. It was also statistically significant (p<0.05). An unpaired t-test was used to make statistical analysis (Table-1).

Parameters	IDA	Non Anaemic	T-test	P-value
HbA1C %	6.86±0.64	37.77 ± 2.4	-85.68	< 0.0001
Absolute HbA1C (gm/dl)	0.53± 0.06	0.50 ± 0.03	3.07	0.0029

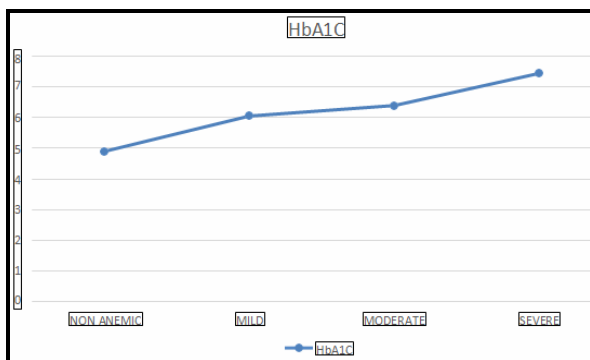
Parameters	IDA	Non Anaemic	T-test	P-value
Hemoglobin [Hb] in gm/dl	9.69 ± 1.88	13.26 ± 0.68	-12.83	1.98 × 10 ⁻¹⁹
Hematocrit in %	28.29 ± 6.59	43.32 ± 3.55	-14.40	8.45 × 10 ⁻²⁴
Mean Corpuscular Volume in fl	71.63 ± 9.93	88.58 ± 4.37	-11.23	2.26 × 10 ⁻¹⁷
Mean Corpuscular Hemoglobin in pg/cell	24.95 ± 3.08	30.31 ± 1.88	-10.65	2.65 × 10 ⁻¹⁷
Mean Corpuscular Hemoglobin concentration in %	29.85 ± 1.44	34.12 ± 0.95	-17.69	7.41 × 10 ⁻³¹

The negative t-test value indicates that the mean HbA1c % in the non-anaemic group is much higher than in the IDA group (Table-2).

In patients who had IDA and people who were non anaemic, the difference in the values of mean Hb, Hct, MCV, MCH and MCHC was significant in statistical terms (p<0.05)

All values remain highly significant (p < 0.05), confirming that there is a statistically significant difference between the IDA and non-anaemic groups. Then, we also interpreted the levels of HbA1C in various degree of anaemia and have come to a conclusion that its levels increase as the severity of anaemia increases. This was found to be statistically significant too [p<0.05] Variation of HbA1C corresponding to various levels of anaemia (Fig-1).

Fig-1: HbA1C variation with severity of Anaemia



ANOVA= 78.52, p =. 1.26 × 10⁻²⁵ (Highly significant)

No anemia to mild anemia, t-test=-8.93, p=3.14 × 10⁻¹¹ (Highly significant) Mild anaemia to moderate anaemia, t-test=-3.11, p=0.0046 (Significant) Moderate to severe anaemia, t-test=-6.47, p=3.44 × 10⁻⁵ (Highly significant)

Discussion

HbA1C is the most commonly occurring form of haemoglobin A and serves as an indicator of glycaemic control of an individual over the past three months. The American Diabetes Association (ADA) has not only established it as the primary marker for managing blood sugar levels but has also incorporated it into diagnostic criteria. While HbA1C primarily reflects blood glucose levels, various non-diabetic conditions, such as anaemia, can also influence its concentration. Notably, approximately one-third of anaemia cases are due to iron deficiency. Our findings revealed that haemoglobin (Hb), haematocrit (Hct), and red blood cell indices were lower in anaemic individuals, accompanied by a microcytic hypochromic blood picture, confirming iron deficiency anaemia (IDA). These observations align with those reported by Barbieri et al [16].

In this study, non-diabetic individuals with IDA exhibited significantly higher HbA1C levels compared to those without IDA, with the difference being statistically significant. This conclusion was reached after excluding

patients with other factors that could interfere with HbA1C levels. Our results are consistent with those of Christy et al [17], who also noted that HbA1C was significantly elevated in IDA patients but decreased following iron therapy. However, the mechanisms responsible for this increase in HbA1C levels in IDA remain unclear.

Several theories have been proposed to explain the rise in HbA1C in IDA: (a) modifications in the quaternary structure of haemoglobin may enhance globin chain glycation; (b) a reduction in total haemoglobin concentration might lead to a higher glycated fraction, as HbA1C is measured as a percentage of total haemoglobin A; and (c) in IDA, impaired red blood cell production results in a longer lifespan of circulating erythrocytes, consequently leading to increased HbA1C levels. This necessitated the need for calculation of a correction factor.

A common approach is to establish a linear regression model between HbA1c % and Hb (g/dL) in anaemic individuals. The slope of the regression equation gives us the correction factor, which can be used to adjust HbA1c values based on the haemoglobin level. The correction factor for falsely elevated HbA1c values in anaemic patients, based on my dataset, is -0.265 per 1 g/dL decrease in haemoglobin. The formula to be applied is;

$$\text{Corrected HbA1c} = \text{Measured HbA1c} - 0.265 \times (12 - \text{Hb})$$

This means that for every 1 g/dL drop in haemoglobin, the HbA1c value is overestimated by ~0.265%. Our results were comparable to those of Shanti B et al [18], and Silva JF et al [19], who concluded that a state of anaemia might lead to an increase of HbA1C levels in individuals [18-19].

Study	Year	Number Screened	Significance
Shanti B et al. [18]	2013	100	P<0.05
Silva JF et al. [19]	2016	122	P<0.05
Present study	2025	100	P<0.05

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Ford ES et al. [20] found no notable variation in mean HbA1c levels based on IDA status or before and after iron therapy. Similarly, Saudek CD et al. [21] argued that red blood cell lifespan was unlikely to play a major role in HbA1c fluctuations during IDA treatment. They suggested that discrepancies in HbA1c levels before and after iron supplementation were primarily due to differences in laboratory measurement techniques.

Some researchers like Sinha N et al. [22], Ford ES et al. [20] even contradicted our results and concluded in their studies that HbA1C levels were decreased in IDA.

Study	Year	Number Screened	Significance
Sinha N et al. [22]	2012	100	p>0.05
Ford ES et al., [20]	2011	8296	p>0.05

Limitation: This study, like all the other studies mentioned above, had some limitations. We had a very small sample size and due to it having a cross-sectional design, we couldn't follow up the subjects. Since we conducted this study only in people without DM, the effect might be even more pronounced in people with DM, exaggerated their poor metabolic control.

Conclusion

This study identified a positive association between IDA and elevated HbA1c levels in a non-diabetic population, with HbA1c rising as anaemia severity increases. The misleading elevation of HbA1c due to iron deficiency can result in misclassification of diabetes, particularly around the 6.5% diagnostic threshold. Therefore, our findings emphasize the crucial need to rule out IDA through iron studies and correct it before making any diagnostic or treatment decisions based solely on HbA1c levels.

Conflicts of interest: There are no conflicts of interest.

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