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Original Article

# A study of influence of iron deficiency anemia on levels of HbA1c among non-diabetic patients in a tertiary care hospital

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ABSTRACT

Aim and Objectives:Glycated haemoglobin, HbA1c is the “gold standard” for monitoring glycemic control. Worldwide, 50% of anemia is attributed to iron deficiency. Several studies have shown an association between iron deficiency anaemia and higher HbA1c levels, but the results are conflicting, and the matter is under debate. To shed additional light on this, in the present study we aimed to analyse the effect of iron deficiency anemia (IDA) on HbA1c levels in nondiabetic adults. Material and Methods:In this descriptive study conducted from 1st December 2018 to 30th November 2019, laboratory data of 30 cases with anemia, normal fasting and postprandial plasma glucose levels and normal HbA1c and 30 healthy controls were analysed. The results were statistically analysed. Results:Mean Hb among cases was 9.27 ± 2.32 (3.6 gm/dL to 11.7 gm/dL) and that among controls was 13.79 ±1.35 (12 gm/dL to 16.4 gm/dL). The total mean FBG and PPBG were 96.17 ± 3.19 and 10 ± 8.63 respectively. The mean HbA1c was 6.5 ± 0.6 g%. The mean HbA1c for cases with Iron Deficiency Anemia was more than that for healthy controls (5.63 ± 0.07 and 5.37 ± 0.07 respectively) with p-value0.0001. The mean iron for cases and controls were 30 and 58respectivelywith p-value 0.0001. The mean MCV, MCH and MCHC was 84.33 ± 0.97, 27.29 ± 0.97 and 32.2 ± 0.27 respectively (p-value-0.0030,0.0000, 0.0000).Conclusion:HbA1c is not affected by the blood sugar levels alone. There are certain confounding factors when HbA1c is measured, especially that of iron deficiency. Our study concludes that iron deficiency was associated with higher proportions of HbA1c, which could cause problems in the diagnostic and therapeutic interpretation of the HbA1c concentrations. Hence, the iron status must be considered during the interpretation of the HbA1c.



## 1. Introduction

Glycated haemoglobin, HbA1c is the “gold standard” for monitoring glycemic control and has been used as a predictor of diabetic complications. Worldwide, 50% of anemia is attributed to iron deficiency. [1] It has been found previously that reduced iron stores have a link with increased HbA1c, leading to false-high values of HbA1c in non-diabetic individuals. Iron deficiency anemia (IDA) is one of the most prevalent type of malnutrition. Ferritin is the form in which iron is stored, and testing amount of ferritin reflects the iron status. Studies have shown that reduced iron levels are correlated with increased levels of HbA1c leading to false high levels of HbA1c in non-diabetic individuals.[2]Recently, a review described the controversies concerning the role of anaemia on HbA1c [3]. Several studies have suggested an association between iron deficiency anaemia and higher HbA1c levels, but the results are conflicting [4-6]and the matter is under debate. To shed additional light on this, in the present study we aimed to analyse the effect of iron deficiency anemia (IDA) on HbA1c levels in nondiabetic adults.

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## 2. MATERIALS AND METHODS

In this descriptive study conducted from 1st December 2018 to 30th November 2019, laboratory data of 30 cases and 30 controls were analysed for Fasting and postprandial plasma glucose levels, HbA1c along with complete blood count (CBC), Peripheral blood smears, Serum Iron, Serum Ferritin. The results were statistically analysed.

### Inclusion criteria

A.For cases:
•Age 18-60 years
•Both Gender included
•Presence of anemia as defined by WHO
Hb: <12.5 g/dl (adult males)
<12 g/dl (non-pregnant women)
•Microcytic, hypochromic picture in peripheral blood Smear
•Serum Iron.
•Serum Ferritin:
<9 ng/ml (in females)
<15 ng/ml (in males)
•Normal fasting and postprandial plasma glucose levels
•Normal liver function tests
Normal blood urea, serum creatinine levels

**B. For controls:**

- Age and Gender matched healthy individuals attending the hospital for health check-ups will be included in the study

**Exclusion criteria**

A. For cases:

- Pregnancy
- Haemolytic anemia
- Diabetics or Impaired glucose tolerance or fasting blood sugar >100mg/dl.
- Haemoglobinopathies
- Kidney and liver disease.
- Renal disease.
- Confirmed case of malignancies
- Patients with iron, folic acid, & B12 supplementation.

**B. For controls:**

- Subjects with history of previous Iron deficiency anaemia and Diabetes mellitus.

The study protocol was approved by the Institutional Ethical Committee at the St John's Medical College and Hospital, Bangalore, India for the study.

**Laboratory Analysis:**

Complete blood count parameters were measured by Sysmex Automated Haematology Analyzer. The anaemic patients were selected, based on their haemoglobin levels and the blood smears(mostly microcytic hypochromic), which suggested iron deficiency anaemia and other haematological investigation (like MCV,MCH,MCHC).

The HbA1c levels were determined by BIO-RAD D100 IIB(HPLC) Chemistry Analyzer.

Serum Ferritin levels were determined by chemiluminescence assay and Serum Iron levels by Colorimetric method.

Hemoglobin levels were evaluated as defined by WHO which considers anemia, a value less than <12.5 g/dl (adult males) &<12 g/dl (non-pregnant women)

HbA1c, FBG & PPBG levels were evaluated as defined by ADA guidelines (2019) which consider diabetes a value >6.5%, >100 mg/dl &>140 mg/dl.

Iron less than <65 µg/dl (male) and <50 µg/dl (female). Ferritin less than <21.8 ng/mL(male) and <4.63 ng/mL considered as Iron Deficiency Anemia.

**Statistical methods:**

Descriptive statistical analysis has been carried out in the present study. Results on continuous measurements are presented on Median (25 percentile – 75 percentile) and results on categorical measurements are presented in Number (%). Significance is assessed at 5 % level of significance. Non-parametric Man-Whitney test has been used to find the significance of study parameters on continuous scale between two groups. Effect size has been computed. Pearson correlation is used to find the correlation between Hb, MCV, MCH, MCHC, serum iron and serum ferritin.

**Results:**

Laboratory data of 30 patients with anemia and Normal HbA1c levels (5 male and 25 female) and 30 apparently healthy controls (14 male and 16 female) were evaluated in the study. Both the groups were well matched for age and gender. (Table 1 & 2).

The range of hemoglobin among cases from 3.6 gm/dL to 11.7 gm/dL and in controls from 12 gm/dL to 16.4 gm/dL. Mean Hb among cases was 9.27 ± 2.32 and that among controls was 13.79 ± 2.95.

Table 3 shows the Mean and SD of HbA1c, Fasting Blood Glucose, Postprandial Blood Glucose, serum iron, serum ferritin, MCV, MCH, MCHC of the study groups. A significant increase were observed in HbA1c(p=0.01), and Serum Iron, MCV, MCH, MCHC (p<0.01) whereas, no significant difference was observed in levels of fasting Blood Glucose, Post prandial blood Glucose and Serum Ferritin.

In our study, we did not find any significant correlation between hemoglobin and HbA1c (r = -0.2182, P = 0.0940). Table 4 shows the correlation for red cell indices like MCV, MCH, MCHC, serum Ferritin, serum iron and HbA1c in IDA subjects. MCV, MCH and MCHC had a significant negative correlation against HbA1c (Pearson correlation coefficient r = -0.4683, p-value =0.002, r = -0.5221, p-value = 0.0000 & r = -0.4323, p-value =0.0006 respectively). No significant correlation was found between HbA1c and iron (r = -0.2463, P = 0.0578), and HbA1c and ferritin (r= -0.0779, P = 0.5540).

**Table 1: Comparison of cases and control according to age groups.**

| Age group | Cases | | Control | |
|---|---|---|---|---|
| | No | % | No | % |
| ≤ 30 | 2 | 6.67% | 11 | 36.67% |
| 31 – 40 | 8 | 26.67% | 6 | 20% |
| 41 – 50 | 10 | 33.33% | 5 | 16.67% |
| >50 | 10 | 33.33% | 8 | 26.67% |
| Total | 30 | 100% | 30 | 100% |
| Mean ±SD | 46.3± 10.46 | | 39.03±11.89 | |

**Table 2: Gender wise distribution of cases and controls**

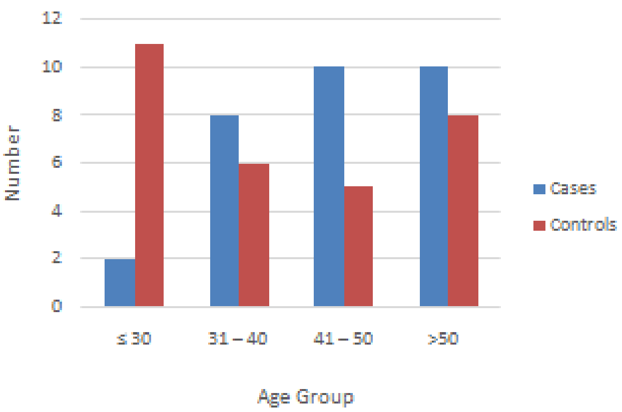
| Gender | Cases | | Controls | |
|---|---|---|---|---|
| | No | % | No | % |
| Male | 5 | 17 | 14 | 47 |
| Female | 25 | 83 | 16 | 53 |

**Table 3: Comparison of various parameters between cases and controls**

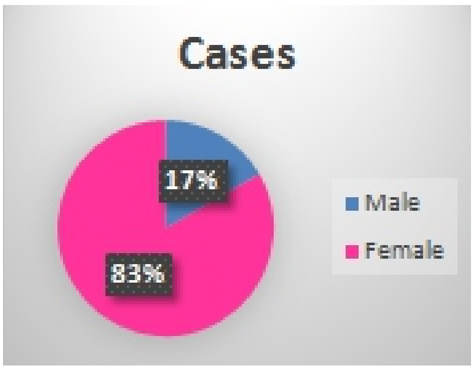
| Parameters | Cases (N=30) | Controls (N=30) | Total (N=60) | p-value |
|---|---|---|---|---|
| HbA1c | 5.63 ± 0.07 | 5.37 ± 0.07 | 5.50 ± 0.05 | 0.01 |
| FBG | 101.53 ± 3.37 | 89.2 ± 5.27 | 96.17 ± 3.19 | 0.05 |
| PPBG | 110.4 ± 8.72 | 100.4 ± 3.66 | 10 ± 8.63 | 0.25 |
| Iron | 30 (22 – 45) | 58 (42-85) | 43.5 (28.5 – 73) | 0.0001 |
| Ferritin | 25.4 (11.4 – 130.1) | 55.42 (36.5 – 105.1) | 45 (15.35 – 122.3) | 0.20 |
| MCV | 81.51 ± 1.70 | 87.14 ± 0.63 | 84.33 ± 0.97 | 0.0030 |
| MCH | 25.28 ± 0.69 | 29.30 ± 0.34 | 27.29 ± 0.46 | 0.0000 |
| MCHC | 30.86 ± 0.32 | 33.54 ± 0.26 | 32.2 ± 0.27 | 0.0000 |

**Table 4: Correlation between HbA1c and Hematological Parameters.**

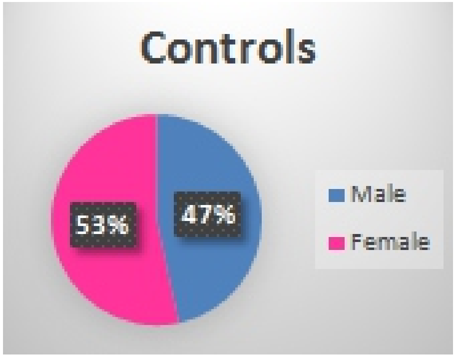
| HbA1c &Hematological parameters | Pearson correlation | p-value |
|---|---|---|
| Hb | -0.2182 | 0.0940 |
| MCV | -0.4683 | 0.0002 |
| MCH | -0.5221 | 0.0000 |
| MCHC | -0.4323 | 0.0006 |
| Iron | -0.2463 | 0.0578 |
| Ferritin | -0.0779 | 0.5540 |




**Graph 1: Comparison of cases and control according to age groups**




**References**




**Discussion:**

In the present study, HbA1c, Fasting Blood Glucose, Postprandial Blood Glucose, Serum iron, Serum ferritin, MCV, MCH, MCHC levels in 30 anemic patients were compared with 30 healthy age and gender matched controls. The study shows that there was significant increase in HbA1c levels with iron deficiency anemia when compared to controls.

The mean hemoglobin of the cases was 9.27(±2.32) gm/dl. About 23.3 % of the study population had severe anemia i.e. less than 8 gm/dl. The minimum hemoglobin observed in the study population was 3.6 gm/dl and the maximum was 11.7 gm/dl.

The levels of HbA1c in cases (5.63±0.07) gm% was found to be higher when compared to the controls (5.37±0.07). This difference between the two groups was statistically significant(p- value 0.01). This is in agreement with the study done by Brooks et al. [7], Sluiteret al. [8], Mitchell et al. [9],Sing P [10] and S Kadhirvel [11] that revealed a relationship between IDA and HbA1c levels. The study explained the alteration in HbA1c levels in IDA on the basis of both modifications to the structure of hemoglobin and levels of HbA1c in old and new red blood cells. The mechanisms leading to increased glycated HbA1 levels were not clear, but it was proposed that, in iron deficiency, the quaternary structure of the hemoglobin molecule was altered, and that glycation of the globin chain occurred more readily in the relative absence of iron [7] The formation of glycated hemoglobin is an irreversible process and hence, the concentration of HbA1 inerythrocyte will increase linearly with the cell's age. For example, they found that in patients with normal blood glucose levels, but with very young red cells, as would be found after treatment of IDA, HbA1 concentration was reduced. However, if iron deficiency has persisted for a long time, the red cell production rate would fall, leading not only to anemia but also to a higher-than-normal average age of circulating erythrocytes and, therefore, increased HbA1c levels.[8]

The levels of Iron in cases (median- 30ng/mL; 25th-75th percentiles[P25-P75], 22.0 -45.0) was found to be lower when compared to the controls (median-58ng/mL; 25th-75th percentiles[P25-P75], 42.0 – 85.0) This difference between the two groups was statistically significant, p<0.001. Our study is in agreement with the study by Koga et al. According to this study there was an inverse association between the HbA1c and the serum iron, serum transferrin saturation and serum ferritin. Higher HbA1c levels were observed in iron deficiency anemia group than in the normal iron state group. This study concluded that in premenopausal women, regardless of anemia, iron

In our study, when we correlated HbA1c with Ferritin, there was no significantcorrelation observed. 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. Various studies have shown elevated ferritin in diabetic population, though its mechanism is still debatable. In a study by Raj and Rajan [13], ferritin showed positive correlation with HbA1c in diabetic individuals. In addition, Canturk et al [14] 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 [15] 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 this study.

The levels of Red cell indices in cases MCV(81.51±1.70), MCH (25.28±0.69), MCHC (30.86±0.32)was found to be higher when compared to the controls MCV(87.14±0.63), MCH(39.30±0.34), MCHC(30.86±0.32).This difference between the two groups was highly statistically significant, p Value-0.003,0.0000 & 0.0000 respectively.This is in good agreement with the study done by Hardikar et al [16] and Koga et al. [17]

Similar study done by Dr. Sukh Dev Choudhary et al, showed that iron deficiency was associated with higher proportions of HbA1c, which could cause problems in the diagnosis of uncontrolled diabetes mellitus in iron-deficient patients. [18]The exact mechanism through which iron deficiency anemia affects HbA1c levels still remains unclear. The explanations provided above are just hypothesis, requiring further studies to confirm and annotate the roles of these factors. As little work has been done in this field, furtherlarge-scale studies are required which may exemplifythis HbA1c enhancing effect and the mechanism of increased HbA glycation in iron deficiency properly. Till then it can be presumed that though, this variation of HbA1c does not appear to require screening for iron deficiency in determining the reliability of HbA1c in the diagnosis of diabetes and prediabetes in a given individual but it suggest that basically people with anemia who are close to the diagnostic threshold may require retesting or the use of another diagnostic method. Therefore, consideration should be given to performing glucose testing in patients who have low Hb concentrations and an HbA1c concentration just below the diagnostic threshold for diabetes and prediabetes or who have high Hb concentrations and an HbA1c concentration just above the diagnostic threshold for diabetes and prediabetes. It is therefore important to keep in mind iron deficiency when HbA1c levels do not correlate with clinical expectations. Also, HbA1c concentrations in diabetic patients with iron deficiency anemia should be interpreted with caution and the possibility of a factitiousincrease associated with iron deficiency.

## Conclusion:

HbA1c is not affected by the blood sugar levels alone. There are a few confounding factors when HbA1c is measured, especially thatof iron deficiency, which is the commonest of the nutritional deficiency diseases in India. Our study concludes that iron deficiency was associated with higher proportions of HbA1c, which could cause problems in the diagnostic and therapeutic interpretation of the HbA1c concentrations.Therefore, a concomitant measurement of hemoglobin, iron andHbA1c in anemic subjects might be required by clinicians toaccurately interpret glycemic status and to increase the reliability of the HbA1c measurements. Hence, the iron status must be considered during the interpretation of the HbA1c concentrations in Diabetes.

**Acknowledgement: NIL**

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