

## Original Research Article

# A study on influence of iron deficiency anaemia over HbA1c levels

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### ABSTRACT

**Background:** Haemoglobin A1c (HbA1c) is a glycosylated form of haemoglobin reflects average plasma glucose over the previous 8 to 12 weeks. HbA1c can be affected by multiple non-glycaemic parameters. Iron deficiency anaemia (IDA) is one among them which is the most common type of anaemia in India. However, reports on the effects of iron deficiency anaemia on HbA1c levels are inconsistent. Hence we conducted a study to find out the influence of iron deficiency anaemia over HbA1c levels.

**Methods:** 120 patients confirmed to have iron deficiency anaemia were enrolled in this study. HbA1c levels were measured at baseline and 3 months after treatment, and these values were compared with those in the control population.

**Results:** The mean baseline HbA1c level in anaemic patients (4.62%) was significantly lower than that in the control group (5.45%,  $P < 0.001$ ). A significant increase was observed in the patients HbA1c levels at 3 months after treatment (5.82%,  $P < 0.001$ ). There was a significant correlation observed between haemoglobin and HbA1c level (Coefficient of correlation=0.26,  $P < 0.01$ ) in the study group before correction.

**Conclusions:** In contrast to the observations of previous studies, ours showed that HbA1c levels increased with treatment of iron deficiency anaemia. This could be attributable to nutritional deficiency, racial-ethnic variations and/or certain unknown variables. Further studies are warranted.

**Keywords:** Diabetes, Glycosylated haemoglobin, Haemoglobin A1c, HbA1c, IDA, Iron deficiency anaemia

## INTRODUCTION

Haemoglobin A1c (HbA1c) is a glycosylated form of haemoglobin (Hb) that is formed when the NH<sub>2</sub>-terminal valine residue of the  $\beta$  chain of globin is glycosylated.<sup>1</sup> Over 50 years ago, Glycosylated Haemoglobin was identified as abnormal haemoglobin in diabetic patients. After that discovery, more studies were conducted to correlate HbA1c with glucose measurements.<sup>2</sup> As Glycosylated Haemoglobin (HbA1c) reflects average plasma glucose over the previous eight to twelve weeks, it is used for assessing glycaemic control in diabetic patients.<sup>3</sup> Since

2010 it is being used as a diagnostic test for diabetes and more recently as a screening test for persons at high risk of diabetes.<sup>4</sup> According to American Diabetes Association guidelines, HbA1c level should be maintained below 6.5% in all diabetic patients in order to prevent complications.<sup>5</sup> HbA1c levels are not only affected by blood glucose levels alone. They are also altered with age, haemoglobin traits and conditions that change the red cell turnover like anaemia.<sup>4</sup>

Iron deficiency anaemia (IDA) is one of the most common types of anaemia found worldwide.<sup>6</sup> The WHO (World Health Organization) estimates that 2.1 billion

people globally have iron deficiency anaemia which is approximately 30% of the world population at the time.<sup>6-8</sup> The prevalence of iron deficiency is higher in low and high-income countries. In these countries, diabetes is also a rapidly increasing issue.<sup>9</sup> India is one among them. Hence it is imperative to find out the influence of iron deficiency anaemia over HbA1c levels which is one of the commonest investigation done routinely in diabetic patients. There were multiple studies in the past investigating the relationship between iron deficiency anaemia and HbA1c and some of the theories postulated by them were a) alteration in the quaternary structure of the haemoglobin molecule in iron deficiency anaemia result in increased level of glycosylation, b) lifespan of the red blood cells present in the circulation may be prolonged during anaemic state resulting in higher HbA1c levels in iron deficiency anaemia patients and after treatment with iron there will be increased bone marrow red cell production and release of new immature red cells resulting in lower HbA1c levels, c) there was a balance between haemoglobin concentration and HbA1c levels in normal individuals and if the serum glucose was maintained constant, a fall in haemoglobin concentration could cause an increase in the glycated fraction, e) usage of different assay methods may alter the HbA1c levels.<sup>10-13</sup>

Even though more theories were postulated till date, the results were conflicting and the mechanism of how iron deficiency anaemia affects HbA1c remains elusive. Hence we were prompted to conduct a study on influence of iron deficiency anaemia over HbA1c levels.

## METHODS

The present study was designed as a prospective interventional study conducted in the Department of General Medicine, ESIC Medical College and PGIMS, Chennai over a period of eighteen months from September 2014 to February 2016 after obtaining Institutional ethical committee clearance. Both in and out-patients coming to the medicine department were enrolled for the study. Informed written consent was obtained from the patients.

120 patients of both sex (male and female) aged between 18 and 60 years fulfilling the inclusion and exclusion criteria were included in the study. A detailed history was recorded with a complete clinical examination. Patients with a history of acute blood loss, haemolytic anaemia, hemoglobinopathies, kidney disease, pregnancy, established diabetes, impaired fasting glucose, or impaired glucose tolerance, known case of malignancy were excluded. Samples were collected to estimate complete blood count, peripheral smear and reticulocyte index.

Based on haemoglobin values, patients were termed as anaemic with cut off of <13 gm/dl in men and <12 mg/dl in women according to WHO criteria.<sup>14</sup> Those with predominantly microcytic indices (MCV <80 fl) and

hypochromic indices (MCH<26 pg/cell) with peripheral smear of microcytic hypochromic picture were considered to have iron deficiency anaemia, which was then also confirmed by low serum ferritin levels (<30 ng/dl) and low serum iron levels (<65 µg/dl in males and <50 µg/dl in female). Since haemolytic anaemias, hemoglobinopathies, and uraemia can be present in asymptomatic individuals, confirmed iron deficiency anaemia patients were screened to rule out these disorders. Those with a reticulocyte index greater than 2.5 in the absence of overt bleeding were considered to have haemolytic anaemia and were excluded from the study. Haemoglobin electrophoresis was performed to rule out hemoglobinopathies. Urine for pregnancy test was done to rule out pregnancy. Kidney function test was done to rule out renal failure. Those with fasting blood sugar of more than 100 mg/dl were excluded. HbA1c level was measured in all patients. Capillary electrophoresis method was used for estimating HbA1c which separates A<sub>1c</sub> from other Hb fractions via charge difference at high voltage using electro-osmotic flow.

All patients included in our study were treated with oral ferrous sulphate (containing 65 mg element iron) with adequate doses as per the severity of anaemia.<sup>14</sup> After 3 months of treatment, complete blood count, reticulocyte index, peripheral smear and HbA1c levels were measured again.

Age and gender-matched 120 healthy participants were enrolled to serve as controls after they provided written consent. All the laboratory parameters analysed for patients were analysed for the control group as well. However, in the case of the control group, the readings were recorded just once, at the time of enrolment. The exclusion criteria for the control group were the same as for study group.

The data were presented as mean±SD for continuous variables. A Student's t-test was applied for comparison of group means. Pearson's coefficient of correlation was calculated to determine the correlation between two variables. P-value of less than 0.05 was considered statistically significant.

## RESULTS

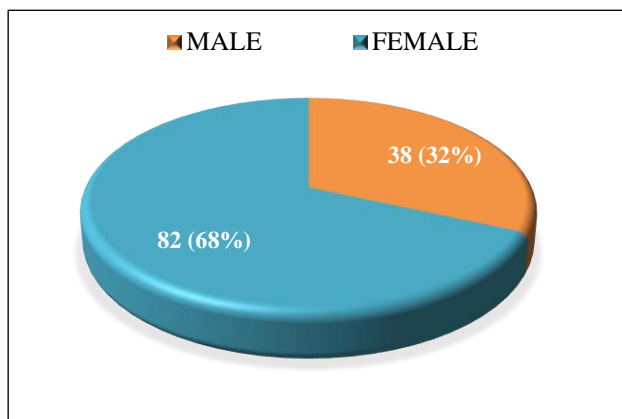
Of 120 patients in the study group, 82 were female (68%) and 38 were male (32%). This suggests that iron deficiency anaemia is more common in females (Figure 1). In the control group, 64 (53.3%) were female and 56 (46.7%) were male.

The mean(±SD) age of the study and control group were 34.1 (±8.39) and 32.1 (±8.34) year respectively (Table 1). The mean (±SD) fasting blood sugar in the study and control group were 88.53 (±5.23) and 88.51 (±5.31) mg/dl while that in postprandial blood sugar was 112.35 (±13.73) and 114.65 (±12.52) mg/dl respectively (Table 2).

The mean( $\pm$ SD) haemoglobin level in the study group before and after correction were 6.8 ( $\pm$ 1.08) and 12.7 ( $\pm$ 0.44) g/dl respectively (Table 3). The mean( $\pm$ SD) haemoglobin level in the control group was (13.4 $\pm$ 0.35) g/dl (Table 2).

The mean ( $\pm$ SD) MCV(fl), MCH (pg/cell), serum iron ( $\mu$ g/dl),serum ferritin (g/l) levels in the study group before and after correction were 64.46 ( $\pm$ 6.67), 19.62 ( $\pm$ 3.02), 21.26 ( $\pm$ 4.69), 6.87 ( $\pm$ 1.5) and 91.35 ( $\pm$ 2.81), 28.28 ( $\pm$ 0.83), 117.15 ( $\pm$ 12.66), 237.24 ( $\pm$ 25.27) and similarly for control group was 91.31 ( $\pm$ 2.85), 28.28 ( $\pm$ 0.84), 117.17 ( $\pm$ 13.09), 232.26 ( $\pm$ 28.39) respectively (Table 2 and 3).

These data provided evidence that haemoglobin, MCV, MCH, serum iron and serum ferritin were indeed lower in the study group (before correction) than in healthy controls, and the observed difference was statistically significant ( $P<0.001$ ). There were a significant increase in the haemoglobin, MCV, MCH, serum iron and serum ferritin levels of the study group after correction for iron deficiency anaemia with  $P$  value  $<0.001$  (Table 2 and 3).



**Figure 1: Sex distribution in the study group.**

**Table 1: Age distribution between study and control group.**

Age (Years)	Study group (n=120)	Control group (n=120)
< 20	5 (4.2%)	12 (10%)
21-30	40 (33.3%)	51 (42.5%)
31-40	41 (34.2%)	28 (23.3%)
>40	34 (28.3%)	29 (24.2%)
Mean	34.1	32.06
SD	8.39	8.34
P Value	0.064 Not significant	

The mean ( $\pm$ SD) HbA1c (%) levels in the study group before and after correction were 4.62 ( $\pm$ 0.30) and 5.82 ( $\pm$ 0.32) respectively, while that in control group was 5.45 ( $\pm$ 0.281). This show that the HbA1c levels in the study group before correction were significantly lower than in controls; however, there was a significant increase in

HbA1c levels in the study group after correction of iron deficiency anaemia with  $P$  value  $<0.001$  (Table 2 and 3).

**Table 2: Comparison of haematological/biochemical variables between study (before correction) and control group.**

	Study group (n=120)	Control group (n=120)	P value
Haemoglobin (g/dl)	6.8 $\pm$ 1.08	13.4 $\pm$ 0.35	$<0.001$
MCV (fl)	64.46 $\pm$ 6.67	91.31 $\pm$ 2.85	$<0.001$
MCH (pg/cell)	19.62 $\pm$ 3.02	28.28 $\pm$ 0.84	$<0.001$
FBS (mg/dl)	88.53 $\pm$ 5.23	88.51 $\pm$ 5.31	0.98
PPBS (mg/dl)	112.35 $\pm$ 13.73	114.65 $\pm$ 12.52	0.185
Serum iron ( $\mu$ g/ml)	21.26 $\pm$ 4.69	117.17 $\pm$ 13.09	$<0.001$
Serum ferritin (g/l)	6.87 $\pm$ 1.5	232.26 $\pm$ 28.39	$<0.001$
HbA1c (%)	4.62 $\pm$ 0.30	5.45 $\pm$ 0.28	$<0.001$

**Table 3: Comparison of haematological/biochemical variables of the study group before and after correction of iron deficiency anaemia.**

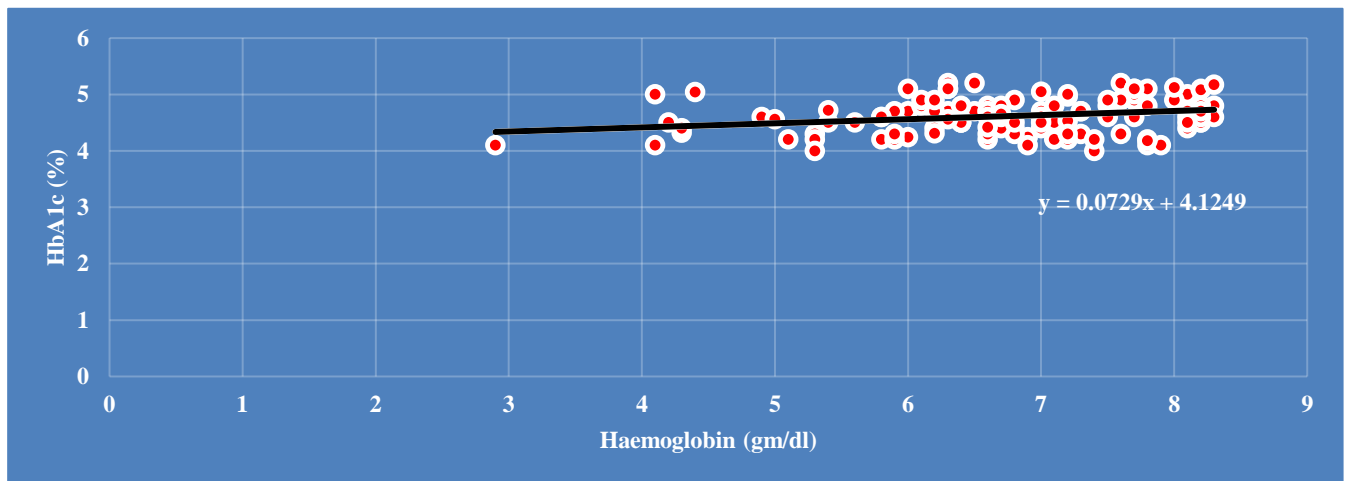
	Study group before correction	Study group after correction	P value
Haemoglobin (g/dl)	6.8 $\pm$ 1.08	12.7 $\pm$ 0.44	$<0.001$
MCV (fl)	64.46 $\pm$ 6.67	91.35 $\pm$ 2.81	$<0.001$
MCH (pg/cell)	19.62 $\pm$ 3.02	28.28 $\pm$ 0.83	$<0.001$
Serum iron ( $\mu$ g/ml)	21.26 $\pm$ 4.69	117.15 $\pm$ 12.66	$<0.001$
Serum ferritin (g/l)	6.87 $\pm$ 1.5	237.24 $\pm$ 25.27	$<0.001$
HbA1c (%)	4.62 $\pm$ 0.30	5.82 $\pm$ 0.32	$<0.001$

There was a significant correlation observed between haemoglobin and HbA1c level (Coefficient of correlation =0.26,  $p<0.01$ ) in the study group before correction (Figure 2). However, there was no positive correlation between haemoglobin and HbA1c levels (coefficient of correlation= -0.105;  $P=0.111$ ) in the study group after correction of iron deficiency anaemia (Figure 3).

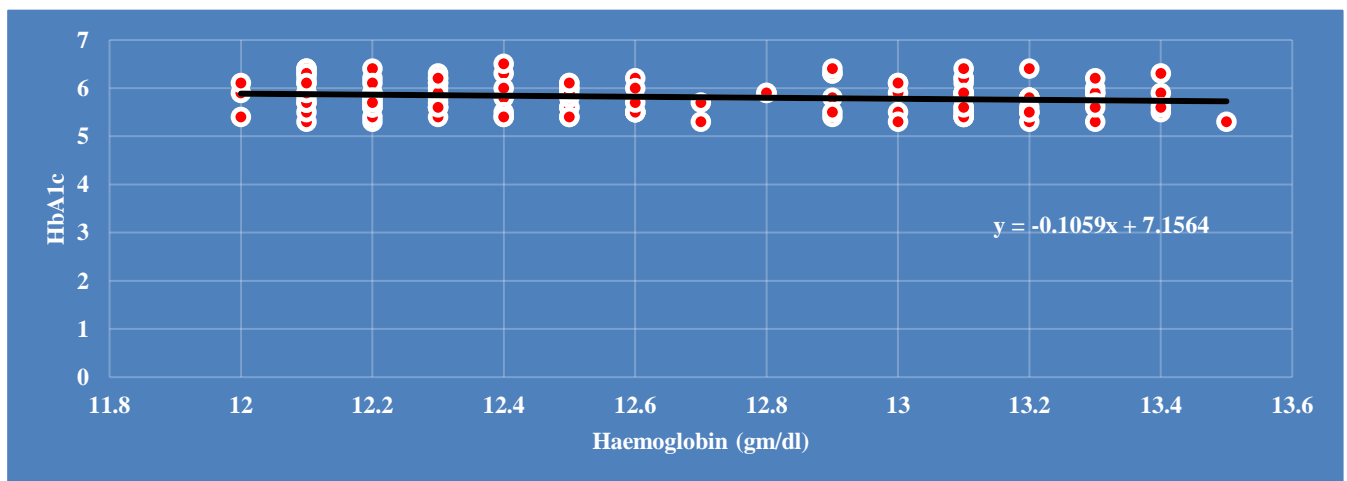
## DISCUSSION

Iron deficiency anaemia is the most common nutritional anaemia. It contributes to more than half of the global anaemia burden. HbA1c was used not only to assess the glycaemic control but also for diagnosis and screening for diabetes. HbA1c can be affected by multiple non-glycaemic parameters. Recently, researchers have become interested in studying HbA1c levels in more

commonly encountered anaemias like iron deficiency anaemia.<sup>15</sup>



**Figure 2: Correlation between haemoglobin and HbA1c in study group before correction.**



**Figure 3: Correlation between haemoglobin and HbA1c in study group after correction.**

The earliest study to find the effects of iron deficiency anaemia on HbA1c levels was done in 1965 by Horton and Husiman, showed that the decreased mean concentration of HbA1c in iron deficiency patients were due to reduced lifespan of red blood cell.<sup>16</sup> In 1980, Brooks et al, discovered that HbA1c levels were slightly increased in iron deficiency patients which decreases on treatment with iron and they postulated “in iron deficiency state quaternary structure of haemoglobin molecule get altered and readily undergo glycation due to low iron levels”.<sup>10</sup> Sluiter et al, proposed that glycated haemoglobin is an irreversible process and hence if the cell’s age increases, the HbA1c concentration also increases i.e. in chronic iron deficiency anaemia, the red cell production will decrease with a longer lifespan which would lead to an increased HbA1c.<sup>11</sup> Mitchell et al, calculated the absolute amount of HbA1c and found that there was no significant difference in HbA1c levels

before and after iron therapy. Also suggested the changes in HbA1c levels in iron deficiency with red cell age were unlikely.<sup>17</sup> Van heyningen et al, proved no differences in HbA1c concentrations in patients with iron deficiency anaemia before and after iron supplementation to healthy controls. They speculated the use of different assay methods for estimating HbA1c be the reason for differences in HbA1c concentrations.<sup>13</sup> But Rai et al used colorimetric assays, ion exchange chromatography and affinity chromatography for measuring HbA1c levels and found no significant differences.<sup>18</sup> Further studies also demonstrated a baseline increase in HbA1c levels in patients with iron deficiency and decreased with iron treatment.<sup>19,20</sup> The explanation given them was if serum glucose is accepted to remain constant, a decrease in the haemoglobin concentration might lead to an increase in the glycated fraction. Nitin Sinha et al showed different results by saying that values of HbA1c decreasing with

fall in haemoglobin values and increases with iron treatment. The reason given was the study population belongs to low socioeconomic status and cause of iron deficiency was nutritional deficiency rather than malabsorption and bleeding.<sup>15</sup> A similar observation was seen in Bhardwaj K et al.<sup>21</sup> From the above studies, the exact mechanism remains unclear. The theories projected by them were also just conjecture. Hence we were prompted to conduct a study on influence of iron deficiency anaemia over HbA1c levels.

Among the 120 patients studied, 82 were female, suggesting that the prevalence of iron deficiency anaemia is more common in women. The mean haemoglobin, mean serum iron and mean serum ferritin levels increased in anaemia patients after iron treatment. However, the HbA1c levels were found to be significantly lower in patients with iron deficiency anaemia than in controls. After treatment, the HbA1c level increased significantly in patients with iron deficiency anaemia was in concordance with previous studies.<sup>15,21</sup> The haemoglobin and HbA1c levels were positively correlated in anaemia patients before treatment, but no positive correlation was observed after treatment.

Authors observation of decreased HbA1c levels in iron deficiency anaemia patients and subsequent rise in HbA1c with iron treatment was different from other studies which were all done in western countries. Only few studies were conducted in our country. Nutritional deficiency as a cause of iron deficiency anaemia in a low social economic status and racial-ethnic variations which affect HbA1c value (or other unknown variables) may be responsible for this confounding results.<sup>15,22</sup>

## CONCLUSION

People with anaemia who are close to the diagnostic threshold may need to be retested or to be diagnosed with an alternative method.<sup>23</sup> Further studies with a large sample size at the community level with other markers of glycaemic control like glycated albumin and fructosamine in iron deficiency anaemia patients to be done to assess their usefulness.

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*Conflict of interest: None declared*

*Ethical approval: The study was approved by the Institutional Ethics Committee*

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