Original Research Article

A study on influence of iron deficiency anemia over HbA1c levels

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Abstract

Background: Iron deficiency anemia is the commonest nutritional anemia worldwide. HbA1c, which is a valuable tool in monitoring glycemic control, has been recently recommended for diagnosing diabetes. HbA1c can be affected by other non-glycemic parameters like hemoglobin variants, anemia, uremia, pregnancy, and acute blood loss. Reports on the effects of iron deficiency anemia on HbA1c levels were inconsistent.
Aim of study: To study the levels of HbA1c in iron deficiency anemia patients and to study the changes in HbA1c levels after the correction of anemia.
Materials and methods: 120 patients confirmed to have iron deficiency were enrolled in this study. Complete blood count, anemia profile including serum ferritin and HbA1c levels were measured at baseline and after treatment of anemia. These values were compared with those in the control population.
Results: The mean HbA1c level in iron deficiency anemia patients (4.619 ± 0.308%) was significantly lower than the control group (5.446 ± 0.281%). A significant increase (5.816 ± 0.323%) was observed in the mean HbA1c of the anemia group after treatment.
Conclusion: Our study showed that HbA1c levels were affected by iron deficiency anemia. The HbA1c levels are lower in iron deficiency anemia patients and it increases after treatment with iron supplements. So iron deficiency anemia has to be kept in mind before using the HbA1c to diagnose diabetes.

Key words

Iron deficiency anemia, HbA1c, Ferritin.

Introduction

Iron deficiency anemia is the commonest form of nutritional anemia worldwide. WHO (World Health Organization) reported that globally there are 2.1 billion cases of iron deficiency anemia, which is approximately 30% of the world population [1]. Anemia is a late indicator of iron deficiency. It is estimated that iron deficiency is 2.5 times more common than anemia [2]. Anemia and iron deficiency leads to significant productivity losses in adults. Iron deficiency in pregnant women is associated with increased maternal mortality, preterm labor, low birth weight, and increased infant mortality. Iron deficiency in children leads to defective cognitive and motor development and increases susceptibility to infections [3]. Hemoglobin A1c (HbA1c) or glycated hemoglobin is the predominant fraction of hemoglobin A. It is used as the gold standard method for assessing glycemic control. It reflects the glycemic status of the individual over the past 3 months [4]. In addition to blood glucose level, HbA1c is affected by multiple factors like genetic, hematologic, and illness-related factors. Initial studies suggested a relationship between HbA1c levels and iron deficiency anemia [5]. They tried to explain that based on structural modifications and alterations in HbA1c levels in old and new red blood cells. Few studies reported no differences in the HbA1c levels of anemic patients compared to healthy controls [6]. Few studies stated that higher HbA1c levels were seen in iron deficiency anemia patients and it decreased significantly after treatment. The results of various studies on the relationship between HbA1c and iron deficiency anemia were conflicting. Only fewer studies have been conducted in the Indian population on this topic [7].

Materials and methods

This cross-sectional study included 240 patients. In the group – A was a control group of 120 patients and in the group – B, 120 patients diagnosed with iron deficiency anemia, who were attending the outpatient Department of Madha Medical College and Hospital, were included in the study in the year 2017-2018.

Inclusion criteria: All consented Iron deficiency anemia patients attending Medical OPD and medical wards in our hospital, aged between 18 to 60 years.

Exclusion criteria: Age <18 years or >60 years, patients with diabetes/IFG/IGT, patients with chronic renal failure/ liver disease, patients with hemolytic anemia, pregnancy, chronic alcoholism, known case of malignancy.

A detailed history was recorded along with a complete clinical examination. The provisional diagnosis was made and this was subsequently revised after completion of the investigations.

Laboratory investigations: Samples were collected from all the participants to estimate complete blood count, blood urea, serum creatinine, serum electrolytes, blood sugar-FBS/PPBS/GTT, urine R/E, HbA1c level, anemia profile including serum ferritin, vitamin B12, and folic acid levels, based on standard tests available in our hospital. Also, ECG, chest x-ray, and ultrasonogram abdomen were done in necessary cases. The final data was entered onto the Microsoft excel sheet 2007 version. Study protocol: Patients with iron deficiency anemia based on WHO criteria cut off point and age, sex-matched control patients were assigned for study. History, clinical assessment, and investigations including serum ferritin, HbA1c were done.

Statistical analysis

The clinical parameters were compared and analyzed using the Pearson chi-square method. The diagnostic accuracy of all the parameters was then compared and interpreted concerning clinical data. In the present study, the statistical methods for quantitative data, descriptive statistics were presented by N, Mean, Standard Deviation, and Range. For qualitative data, frequency count, N, and percentage were put in a tabular manner. To analyze the data, appropriate statistical tests were applied. The significance of

the difference between means in the two groups was calculated using the student t-test and the significance of the difference in proportions using a chi-square test. 2 x 2 tables were constructed for each variable and the chi-square value for the degree of freedom calculated.

Results

Mean age (±SD) in the control group was 32.1 (8.34) and in the study group was 34.1 (8.398). P-value was 0.064 which was not significant i.e. the age distribution among the control and study group were equal. About 67% of study subjects were in the age group of 21-40 years while 28% were aged 41-60 years. Minimum age was 19 years, maximum age was 49 years. The majority of the study subjects were females (68.3%) while the remaining 31.7% were males. It confirmed the fact that iron deficiency anemia was more common in females. P-value was 0.146 which was not significant i.e. sex distribution among the control group and study group was equal (**Table – 1**).

Table – 1: Age distribution.

Age (years)	Control group N (%)	Study group N (%)
≤20	12(10)	5(4.2)
21-30	51(42.5)	40(33.3)
31-40	28(23.3)	41(34.2)
>40	29(24.2)	34(28.3)
Total	120(100)	120(100)
Mean	32.06	34.067
SD	8.34	8.398
P value	0.064 Not Significant	

Table – 2: FBS distribution.

FBS mg/dl	CONTROL GROUP N (%)	STUDY GROUP N (%)
≤80	11(9.2)	10(8.3)
81-90	66(55)	68(56.7)
91-110	43(35.8)	42(35)
N	120	120
Mean	88.508	88.525
SD	5.312	5.231
P value	0.98 not significant	

Mean (±SD) in the control group was 88.508 (5.312) and in the study group was 88.525 (5.231). P-value was 0.98 which is not significant i.e. FBS distribution among the control and study groups were the same (**Table – 2**).

Table – 3: PPBS distribution.

PPBS mg/dl	CONTROL GROUP N (%)	STUDY GROUP N (%)
≤110	48(40)	57(47.5)
111-120	29(24.1)	21(17.5)
121-130	32(26.7)	32(26.7)
131-140	11(9.2)	10(8.3)
N	120	120
Mean	114.65	112.352
SD	12.522	13.725
P value	0.185 Not significant	

Table – 4: Distribution of hemoglobin between control and study group pre-correction.

HB (g/dl)	CONTROL GROUP	STUDY GROUP PRE
≤13	03	120
>13	97	0
N	120	120
Mean	13.408	6.778
SD	0.354	1.085
P value	<0.001 Significant	

Table – 5: Distribution of hemoglobin in study group pre and post correction.

HB (g/dl)	STUDY GROUP PRE	STUDY GROUP POST
≤13	120	82
>13	0	38
N	120	120
Mean	6.778	12.659
SD	1.085	0.446
P value	<0.001 Significant	

Mean (±SD) in the control group was 114.65 (12.522) and in the study group was 112.352 (13.725). P-value was 0.185 which was not significant i.e. PPBS distribution among the control and study groups were the same (**Table – 3**).

Table – 6: Distribution of MCV.

MCV (fl)	CONTROL GROUP N(%)	STUDY GROUP PRE N(%)	STUDY GROUP POST N(%)
<80	0(0)	120(100)	0(0)
80-90	45(37.5)	0(0)	44(36.7)
91-100	75(62.5)	0(0)	76(63.3)
N	120	120	120
Mean	91.316	64.46	91.349
SD	2.851	6.674	2.81
P value	<0.001 Significant		<0.001 Significant

Table – 7: Distribution of MCH.

MCH (pg/cell)	CONTROL GROUP N(%)	STUDY GROUP PRE N (%)	STUDY GROUP POST N(%)
<26	0(0)	119(99.2)	0(0)
26-28	50(41.7)	0(0)	49(40.8)
>28	70(58.3)	1(0.8)	71(59.2)
N	120	120	120
Mean	28.277	19.615	28.283
SD	0.836	3.018	0.833
P value	<0.001 Significant		<0.001 Significant

Table – 8: Distribution of serum iron.

SERUM IRON (mcg/dl)	CONTROL GROUP N(%)	STUDY GROUP PRE N(%)	STUDY GROUP POST N(%)
<30	0(0)	120(100)	0(0)
31-60	0(0)	0(0)	0(0)
61-90	3(2.5)	0(0)	4(3.3)
91-120	53(44.2)	0(0)	54(45)
>120	64(53.3)	0(0)	62(51.7)
N	120	120	120
Mean	117.167	21.257	117.147
SD	13.091	4.688	12.661
P value	<0.001 Significant		<0.001 Significant

P value was less than 0.001 which was highly significant i.e. mean hemoglobin level in the study group was significantly lower than the control group as expected (**Table – 4**).

P value was less than 0.001 which was highly significant i.e. mean hemoglobin level had increased significantly in study subjects after iron treatment as expected (**Table – 5**).

P-value between the control group and study group before anemia correction was less than 0.001 which was highly significant. It conveys that MCV was significantly lower in the anemia group. P-value between study group pre and post-correction was less than 0.001 which was highly significant. It indicated that MCV improved after iron treatment as expected (**Table – 6**).

Table – 9: Distribution of serum ferritin.

SERUM FERRITIN (mg/dl)	CONTROL GROUP N(%)	STUDY GROUP PRE N(%)	STUDY GROUP POST N(%)
<15	0(0)	120(100)	0(0)
16-50	0(0)	0(0)	0(0)
51-150	0(0)	0(0)	0(0)
151-300	119(99.2)	0(0)	120(100)
>300	1(0.8)	0(0)	0(0)
N	120	120	120
Mean	232.264	6.871	237.239
SD	28.394	1.5	25.267
P value	<0.001 Significant	<0.001 Significant	

Table – 10: Distribution of HbA1c between control and study group pre-correction.

HBA1C (%)	CONTROL GROUP N(%)	STUDY GROUP PRE N(%)
≤5	10(8.3)	104(86.7)
5.1-5.5	66(55)	16(13.3)
5.6-6.0	42(35)	0(0)
6.1-6.5	2(1.7)	0(0)
>6.5	0(0)	0(0)
Mean	5.446	4.619
SD	0.281	0.308
P value	<0.001 Significant	

Table – 11: Distribution of HbA1c in study group pre and post correction.

HBA1C (%)	STUDY GROUP PRE N(%)	STUDY GROUP POST N(%)
≤5	104(86.7)	0(0)
5.1-5.5	16(13.3)	32(26.7)
5.6-6.0	0(0)	55(45.8)
6.1-6.5	0(0)	33(27.5)
>6.5	0(0)	0(0)
Mean	4.619	5.816
SD	0.308	0.323
P value	<0.001 Significant	

Table – 12: Mean difference result in pre and post correction for Hb & HbA1c values.

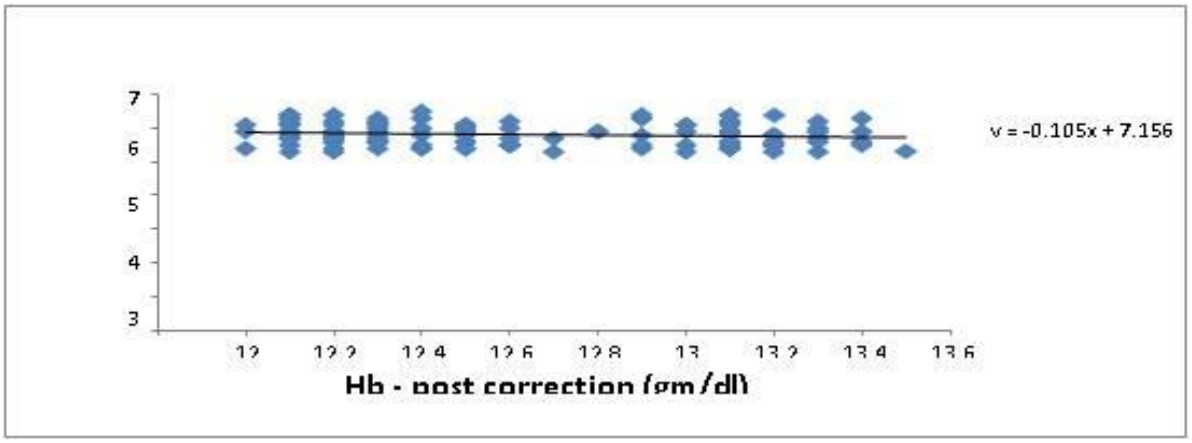
Parameters	Mean Difference
Hb (gm%)	5.88***
HbA1c (%)	1.20***

*** Highly Significant (P=0.0001)

P value between the control group and the study group before anemia correction was less than 0.001 which was highly significant. It conveys that MCH was significantly lower in the anemia group. P-value between study group pre and post-correction was less than 0.001 which was highly significant. It indicated that MCH improved after iron treatment as expected (**Table – 7**).

P value between the control group and the study group before anemia correction was less than 0.001 which was highly significant. It conveys that serum iron was significantly lower in the anemia group. P-value between study group pre and post-correction was less than 0.001 which was highly significant. It indicated that serum iron improved after iron treatment as expected (**Table – 8**).

Graph – 1: Correlation between Hb and HbA1c in study group after anemia correction.



P-value between the control group and study group before anemia correction was less than 0.001 which was highly significant. It conveyed that serum ferritin was significantly lower in the anemia group. P-value between study group pre and post-correction was less than 0.001 which was highly significant. It indicated that serum ferritin improved after iron treatment as expected (**Table – 9**).

Mean HbA1c of iron deficiency anemia patients (4.619 ± 0.308) was significantly lower than the control population (5.446 ± 0.281). About 55% of control subjects had an HbA1c level between 5.1-5.5% while 35% had an HbA1c level between 5.6-6.0%. About 86.7% of study subjects had HbA1c levels between ≤5% while 13.3% had HbA1c levels between the 5.1-5.5%.P value of HbA1c distribution between the control group and study group pre-correction was less than 0.001 which is highly significant. It reveals that HbA1c was lower in the anemia group (**Table – 10**).

The mean HbA1c level in the study group increased from 4.619 (±0.308)% to 5.816 (±0.323)% after the correction of anemia. After the correction of anemia, about 45.8% of study subjects had an HbA1c level between 5.6-6.0% while 27.5% had an HbA1c level between 6.1-6.5%. The P-value of HbA1c in the study group pre and post-correction was less than 0.001 which was highly significant. It indicates that HbA1c increased after anemia correction (**Table – 11**).

Mean difference result in pre and post correction for Hb & HbA1c values was as per **Table – 12**. In this study, HbA1c significantly increased after correction of anemia. In this study group, post correction Hb and HbA1C showed negative, poor correlation (r = -0.15) which was statistically not significant (p= 0.111). In this scatter diagram, the trend line shows negative, poor, and statistically not significant correlation. For this line, Regression equation obtained, Y (HbA1C) = -0.105 (X = Hb) + 7.156. That is, If we put the value of Hb (X) = 10gm %, the predicted HbA1C would be 6.11%. And for Hb = 12 gm%, the predicted HbA1C would be 5.9%. i.e. there was a decrease of 0.21% HbA1C level for each 2gm of Hb level (**Graph – 1**).

Discussion

In India, about 50% of anemia is attributed to iron deficiency. Children and women are the

most vulnerable population. The factors contributing to iron deficiency anemia varies in different population. Physiologically, HbA1c undergoes glycosylation in a slow and non-enzymatic manner [8]. The degree of glycosylation depends on the concentration of glucose. HbA1c is the predominant form of glycated hemoglobin. Glucose gets attached to the NH2 group in the terminal valine of the β-globin chains irreversibly. The glycosylation process occurs throughout the life span (120 days) of red cells. Hence the measured glycohemoglobin levels reflect the glycemic status of the preceding 3 months [9]. HbA1c levels can be affected by multiple factors other than the plasma glucose level. Several conditions can result in falsely lower or higher values. Hemolytic anemia, hemoglobinopathies, uremia, and chronic blood loss influence the HbA1c assays. So far HbA1c has been used as a valuable tool in monitoring glycemic control in diabetics [10]. Recently American Diabetic Association and International Expert Committee recommended HbA1c for diagnosing diabetes. HbA1c level of 6.5% has been proposed as a diagnostic cut-off point. Multiple studies were investigating the relationship between iron deficiency anemia and HbA1c [11]. But the results were inconsistent. In this study, about 120 patients were allotted to the study group. The same number of age and sex-matched controls were taken. The mean age group of the study population was 34.1±8.4 years. The minimum age was 19 years and the maximum was 49 years. About 67% of the study subjects were in the age group of 21-40 years while 28% were aged 41-60 years. Thus in our study, the prevalence of iron deficiency anemia is more common in the 2nd to 4th decade of life. The mean hemoglobin of the study population was 6.8(±1.1) gm/dl. About 85.8 % of the study population had severe anemia i.e. less than 8 gm/dl. The minimum hemoglobin observed in the study population was 2.9 gm/dl and the maximum was 8.3 gm/dl. The p-value of the unpaired t-test between the study group hemoglobin and control group was less than 0.001 which is highly significant. It indicates that the mean hemoglobin level in the study group was significantly lower than the control group as expected. The mean hemoglobin level in the study group increased from 6.8(±1.1) gm/dl to 12.7(±0.4) gm/dl after the correction of anemia with iron. The minimum hemoglobin observed in the study population after iron treatment was 12gm/dl and the maximum was 13.5gm/dl. The p-value of the paired t-test in the study group hemoglobin before and after iron treatment was less than 0.001 which is highly significant. It indicates that the mean hemoglobin level had increased significantly in study subjects after iron treatment as expected. The mean MCV and MCH of the control and study group were 91.316 (±2.851), 28.277 (±0.836) and 64.46 (±6.674), 19.615 (±3.018) respectively. This shows that MCV, MCH were lower in the study group compared to the control group. The observed difference was statistically significant ($p<0.001$).The mean MCV and MCH of the study group after iron treatment were 91.349 (±2.81) and 28.283 (±0.833) respectively. That was a statistically significant improvement. The mean serum iron and ferritin levels of the control and study group were 117.167 (±13.091), 232.264 (±28.394) and 21.257 (±4.688), 6.871 (±1.5) respectively. This shows that serum iron and ferritin levels were lower in the study group compared to the control group. The observed difference was statistically significant ($p<0.001$).The mean serum iron and ferritin levels of the study group after iron treatment were 117.147 (±12.661) and 237.239 (±25.267) respectively. That was a statistically significant improvement. The mean HbA1c of the study population was 4.619 (±0.308) %. About 86.7% of study subjects had an HbA1c level of ≤5% while 13.3% had an HbA1c level between 5.1-5.5%. The mean HbA1c of the control group was 5.446 (±0.281) %.The p-value of the unpaired t-test between the study group HbA1c and the control group was less than 0.001 which is highly significant. It indicates that the mean HbA1c level in the study group was significantly lower than the control group. The mean HbA1c level in the study group increased from 4.619(±0.308) % to 5.816(±0.323)% after correction of anemia with iron. After the correction of anemia, about

45.8% of study subjects had an HbA1c level between 5.6-6.0% while 27.5% had an HbA1c level between 6.1-6.5% [12]. The p-value of paired t-test in the study group HbA1c before and after anemia correction was less than 0.001 which is highly significant. It indicates that the mean HbA1c level had increased significantly in study subjects after anemia correction. In this study group, pre-correction Hb and HbA1C showed positive, poor correlation (r = 0.26) which was statistically very significant (p= 0.005). That is when the hemoglobin decreases the HbA1c will also decrease and vice versa. And post-correction Hb and HbA1C showed negative, poor correlation (r = -0.15) which was statistically not significant (p= 0.111) [13]. That is when the hemoglobin increases the HbA1c will decrease, and vice versa, which is not significant statistically. Similar to this study, in 2014 a study was conducted by Sinha N, et.al on the effect of iron deficiency anemia on glycosylated hemoglobin levels in non-diabetic Indian adults. They postulated that Hb concentrations are positively connected with HbA1c concentration and that HbA1c concentration tended to be lower in the presence of iron deficiency anemia. But they concluded that iron deficiency anemia is unlikely to be a major concern in diagnosing diabetes using a concentration of HbA1c according to the American Diabetes Association (ADA) guideline [14]. In contrast to our study, a study was done by Kenneth Kaushansky et.al concluded that iron deficiency anemia elevates HbA1c levels in diabetic individuals with controlled plasma glucose levels. They postulated that iron-deficiency anemia has a positive correlation with increased HbA1c levels [15]. Longo DL, et al. conducted a study to evaluate the relationship between HbA1c and anemia in hypothyroid patients. They concluded that Non-diabetic hypothyroid individuals with anemia show elevated HbA1C levels in the prediabetes range. Hence care should be exercised while using HbA1C as a diagnostic tool for diabetes in such patients [16]. A study done by Tarim O, et al. found out that there was no significant influence of iron deficiency anemia over HbA1c concentrations. They suggested that differences observed in previous studies could be due to the various laboratory methods used in estimating the HbA1c. Hansen et al also observed similar results. Contradicting the conclusion of Van Heyningen, et al., Rai, et al. conducted a study using various assay methods to estimate HbA1c and found no significant alterations in HbA1c levels measured by those methods [17, 18]. In our study mean HbA1c of iron deficiency anemia patients (4.619 ± 0.308) was significantly lower than the control population (5.446 ± 0.281) and it increased (5.816 ± 0.323%) significantly after iron treatment [19, 20].

Conclusion

The prevalence of iron deficiency anemia is more common in females during the second to fourth decades of life.HbA1c was lower in patients with iron deficiency anemia compared to a healthy control group. After the correction of anemia, the HbA1c level increased significantly in iron deficiency anemia patients. Hemoglobin and HbA1c showed a statistically significant positive correlation in patients with iron deficiency anemia. After the correction of anemia Hemoglobin and HbA1c showed a statistically insignificant negative correlation. A longer period of study and a larger sample size may be required to show a statistically significant positive correlation. Iron deficiency anemia has to be kept in mind before using the HbA1c to diagnose diabetes.

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