Original Research Article

Effect of Iron deficiency anemia on glycated hemoglobin levels in non-diabetes patients: Prospective Case-Control study

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Abstract

Background: Glycated hemoglobin (HbA1c) has become the gold standard for the diagnosis and scale for measuring the glycemic control among the physicians. The level of HbA1c is influenced by several factors, including red blood cell turnover. There are various types of anemia that affect HbA1c readings, but iron deficiency anemia (IDA) is one of the most frequent. IDA has been shown to exhibit HbA1c readings that are greater than normal.**Aims and objectives:** To study the effect of IDA on HbA1c in subjects with normal fasting and postprandial blood glucose levels. **Materials and Methods:** Fifty subjects with IDA were studied attending OPDs in a tertiary care hospital, and findings were compared with a control arm having 50 subjects without anemia. Patients with IDA defined per WHO [Hb: <13.0 g/dl (adult males), <12 g/dl (non-pregnant women) and those with the microcytic, hypochromic picture in peripheral blood smear, serum ferritin <15 ng/ml suggestive of iron deficiency, subjects with normal fasting and postprandial plasma glucose level, normal blood urea and serum creatinine level were included. Patients who were being quantified underwent a comprehensive history, clinical examination, and pertinent biochemical examination, including HbA1c.**Results:** The study observed a statistically significant difference in Hba1c levels in non-diabetic patients withIDA compared with the normal population. The mean baseline serum ferritin level was significantly lower in patients with IDA than in controls (P<0.01). The mean HbA1C levels in patients with moderate anemia were 6.74. In severe anemia, the mean HbA1c was 7.07, significantly higher than the control group (P < 0.01). there was a significant negative correlation between the following parameters and Hba1C, which are Hb (r= -0.727), serum ferritin (r=-0.827) ,(r = -0.909), MCV levels (r = -0.839), between % saturation (r = -0.592). The study has shown a significant positive correlation between total iron-binding capacity and hbA1c levels (correlation co-efficient r = 0.743).**Conclusion:** HbA1c is not affected by blood sugar levels alone. There are various confounding factors when HbA1c is measured, especially iron deficiency, which is the commonest of the deficiency diseases worldwide. Hence, it is prudent to rule out IDA before making a therapeutic decision to treat diabetes mellitus based on the HbA1c levels.

Keywords: iron deficiency anemia, diabetes, serum ferritin, glycemic control, iron stores

Introduction

One of the most common kinds of malnutrition is iron deficiency. Iron deficiency is responsible for 50% of anemia worldwide.Ferritin is the iron storage form, and it properly reflects the iron state. According to a previous study, reduced iron stores have been linked to increased glycatedhemoglobin (HbA1c), resulting in falsely high HbA1c levels in non-diabetic people[1].The glycation of terminal valine at the β-chain of hemoglobin produces HbA1c, which is the most common component of HbA1. It shows the patient's glycemic status during the last three months. HbA1c is a commonly used diabetes mellitus screening test, and the American Diabetes Association recently recommended HbA1c >6.5 percent as a diagnostic criterion for diabetes mellitus[2].In a study conducted by Sinha et al., its modification in various situations such as hemolyticanemia, hemoglobinopathies, pregnancy, and vitamin B12 insufficiency was explained[3]. Even though iron insufficiency is the most frequent nutritional deficiency, data on its clinical impact on HbA1c levels have been mixed[4].Brooks et al. measured HbA1 values in 35 non-diabetic patients with iron deficiency anemia (IDA) before and after therapy with iron in research. HbA1c levels were shown to be considerably higher in IDA patients and to be significantly lower after treatment with iron.[5] Gram-Hansen et al.[6], and Coban et al[7]both reported similar results in their research.Increased HbA1c levels in iron deficiency anemia were found in diabetic chronic renal disease patients and diabetic pregnant women, which were reduced after iron therapy. When compared to iron-sufficient controls matched for fasting plasma glucose (FPG) levels, Tarim et al. found that iron shortage increased HbA1c levels in diabetes individuals[8].HbA1c is a commonly used glycemic control measurement, and it is critical to rule out any causes that could artificially raise its values. As a result, we conducted a study in iron-deficient persons with FPG levels below 126 mg/dl to see if anemia affects HbA1c levels. If so, if anemia should be considered before making any therapeutic decisions based purely on HbA1c values.

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Materials and Methods

Present prospective Case-Control study was performed on 50 subjects with iron deficiency anemia admitted in various medicine wards and attending Out-Patients Department in a tertiary care Hospital. A control arm was kept having 50 subjects without anemia.

Subjects with the presence of anemia as defined by World Health Organization [Hb: <13.0 g/dl (adult males), <12 g/dl (non-pregnant women), microcytic, hypochromic picture in peripheral blood smear, serum ferritin <15 ng/ml suggestive of iron deficiency, subjects with normal fasting and postprandial plasma glucose level, normal blood urea and serum creatinine level were included.Subjects with glucose tolerance abnormalities (impaired glucose tolerance or diabetes mellitus), hemoglobinopathies, hemolytica nemia, chronic alcohol ingestion, chronic renal failure, pregnant females, and history of blood transfusion in the past 3 months were excluded. Patients who were being quantified underwent a comprehensive history, clinical examination, and pertinent biochemical examination, including HbA1c.IBM SPSS ver. 20 software was used for all types of data analysis. All continuous variables have been expressed as mean ± SD (normally distributed). All categorical variables have been described as frequency and percentage. Categorical variables were analyzed by Chi-square test. Continuous variables were analyzed by an independent t-test (normally distributed). The linear relationship between discrete variables has been measured using Pearson Correlation. A p-value of <0.05 was considered significant.

Results

The mean age of the cases and control subjects was 28.34±4.1 years, ranging from 18 to 50. Peak age noted for iron deficiency anemia was between 18 to 30 years (70%), while a minimum number of patients are 45-55 years (10%). Of the 100 people in our study (50 patients and 50 controls), 39 (78%) were females, and 11 (22%) were males in each group. Female preponderance in reproductive age was noted.

Table 1: Comparison of lab parameters between cases and controls

Parameters	Case	Control	t-test	P-value
MCV	60.22± 8.676	83.22±6.735	14.807	<0.001
Serum Iron	31.56±8.676	81.56±6.125	17.855	<0.001
TIBC	456.60±14.609	293.88±91.330	-11.995	<0.001
% Saturation	8.06±2.456	18.08±29.333	17.551	<0.001
Vitamin B 12	499.52±203.203	499.52±210.397	.000	1.000
Serum Creatinine	.6028±.6028	.6028±.13843	.000	1.000

Data are expressed as mean± standard deviation, MCV; mean corpuscular volume, TIBC; total iron-binding capacity, %; percentage. P value of <0.05 is considered as significant.

Among cases, mean Hb ranged from 4.1 to 9.9 g/dl. The mean Hb level among males with IDA was 7.59 g/dl and among females with IDA was 6.54 g/dl. The average Hb level among males in the control group was 14.03 g/dl and among females in the control group was 12.47 g/dl. The mean Hb in the case and control groups was 6.84 ± 1.63 g/dl and 12.87 ± 1.3 g/dl, respectively.

Table 2: Pearson Correlation between Hba1c and lab parameters

Parameters	Correlation coefficient (r)	P value
Serum Iron	-0.905	<0.001
MCV	-0.839	<0.001
Hemoglobin	-0.937	<0.001
% Saturation	-0.865	<0.001
TIBC	0.743	<0.001

MCV; mean corpuscular volume, TIBC; total iron-binding capacity, %; percentage. P value of <0.05 is considered as significant.

In the present study, 38(76%) patients had severe anemia, while moderate anemia was seen in 12 (24%) patients. We found no patient with mild anemia. We found that the more severe the anemia higher is the value of HbA1c. The mean HbA1c levels in patients with moderate anemiawere 6.74.In severe anemia, the mean HbA1c was 7.07. The mean HbA1c level in moderate and severe anemia was significantly higher compared to the control group (P < 0.01).

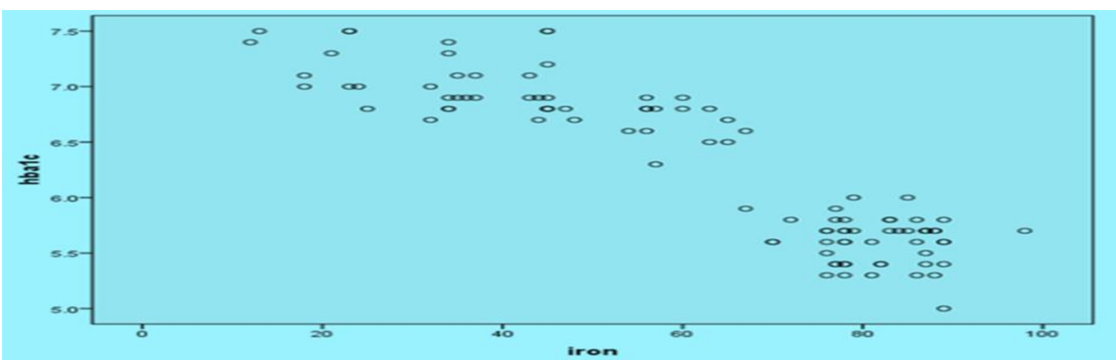

Fig 1: Scattered diagram showing the correlation between HbA1C and iron

Our study showed the mean serum ferritin level of anemic patients to be 7 ng /ml. The mean baseline serum ferritin level was significantly lower in cases (P<0.01). In our study, the iron level in the case group was 31.56±8.676 and 81.56±6.125 among the control group. We observed a significant negative correlation between Hband HbA1c (r = -0.723). Ferritin showed a notable negative correlation with hbA1c (r =- 0.827). In our study S. Iron level and HbA1c showed a correlation co-efficient = -0.909, MCV levels and hbA1c show a correlation co-efficient = -0.839 and % saturation and HbA1c show a correlation coefficient r = -0.592. There is a significant positive correlation between total iron-binding capacity and hbA1c levels (r = 0 .743).

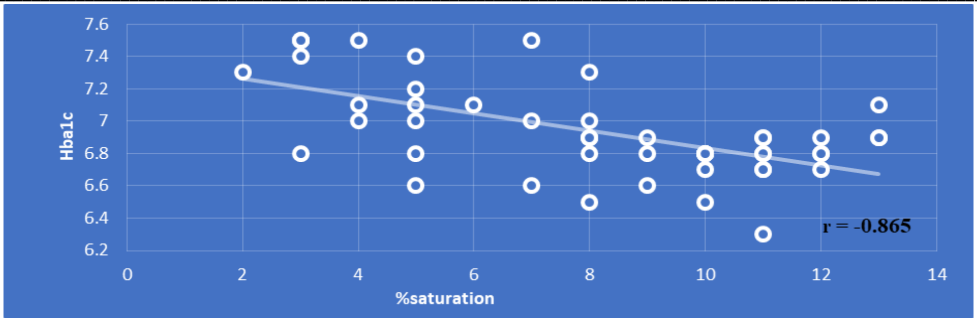

Fig 2: Scattered diagram showing the correlation between Hba1c and % saturation

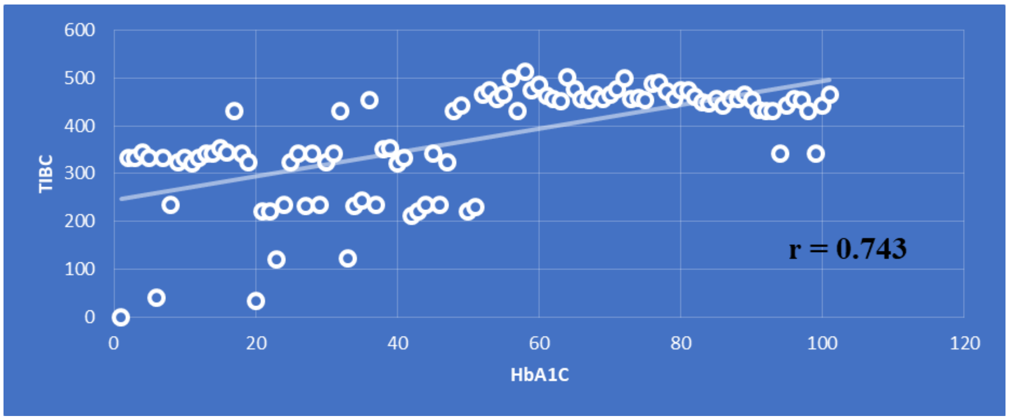

Fig 3: Scattered diagram showing a correlation between TIBC and HbA1c

Discussion

According to the researchers, iron insufficiency and IDA are likely to cause a false increase in HbA1c readings. This may confuse when using HbA1c to diagnose diabetes. Evidence has developed to support the concept that the patient's iron status can influence the glycation reaction in addition to classic chronic hyperglycemia. It would have significant clinical implications if the degree of glycation of other proteins in anemic patients were equivalent to glycated haemoglobin[9].As a result, we conducted a study in iron-deficient persons with FPG levels below 126 mg/dl to see if anemia affects A1C levels. If so, if anemia should be considered before making any therapeutic decisions based purely on HbA1c values.The present study demonstrated a mean age of 28.34±4.1 years in IDA patients, ranging from 18- 50 years. In line with this, Faldu et al. noted IDA in 45 years[9].Though iron deficiency anemia has been observed in all age groups of cases, we saw a peak between 18 years to 30 years. At the same time, only 10% population were between the age of 45 to 55 years. A study done by Barraganetal.demonstrated the peak age range to be 18- 45 years.[10] The mean age of the study population reported by Parlapally et al. was 38.41 ± 17.6 years, and the mean of the control group was 39.17 ± 17.9 years which is in line with the present study[11].Female preponderance in reproductive age was noted in the present study. The anemia in the female group can be attributed to poor nutritional status and female reproductive physiology. In agreement with this, Parlapally et al. studied 63 non-diabetic, anemic patients and reported female's preponderance constituting about 75% of the study group. The Hb level ranged from 4.1 to 9.9 g/dl in the present study. The mean Hb level among males with IDA was slightly higher (7.59 g/dl) than the female's IDA (6.54 g/dl). The mean Hb level among males in the control group was 14.03 g/dl and of females in the control group was 12.47 g/dl. Total 76% of patients were with severe anemia and 24% with moderate anemia. A study done between 1999-2006 under the United States National Health and nutrition examination survey (US NHANES) showed 60% with severe anemia and 25% with moderate anemia, and 15%with mild anemia[12].The fact that IDA was linked to increased HbA1c levels was a notable finding. Brooks et al. conducted one of the first studies to examine the effects of iron deficiency anemia on HbA1c levels, assessing HbA1c values in 35 non-diabetic patients with iron deficiency anemia before and after therapy with iron. HbA1c values were considerably higher in iron deficiency anemia patients and decreased after treatment with iron, according to the researchers. The processes causing elevated glycated HbA1c levels remained unknown.The quaternary structure of the hemoglobin molecule was thought to be altered in iron deficit, and that glycation of the globin chain proceeded more readily in the absence of iron.Sluiter et al.attempted to explain the findings described above. They suggested that because the development of HbA1c is an irreversible process, the concentration of HbA1c in erythrocytes will rise in lockstep with the cell's age.[13]HbA1c values were more significant in individuals with IDA and dropped significantly after therapy with iron, according to studies by El-Agouza et al. [14]and Cogan et al[7]. They proposed that the hypothesis might explain high HbA1c values in IDA that if serum glucose remains constant, a drop in hemoglobin concentration will increase the glycated fraction. As the research above shows, the specific mechanism by which IDA impacts HbA1c levels is yet unknown.

HbA1c concentrations were normal in iron deficiency but declined to subnormal levels after iron supplementation; according to Gram-Hansen et al[6]Rai and Pattabiraman looked at the numerous methods for estimating HbA1C and found no differences between colorimetric techniques, ion-exchange chromatography, and affinity chromatography[15].In this investigation, the HbA1c levels were determined using the widely used immunological turbidometric method.

Another study by Coban et al. reported that the HbA1c was 7.4 percent 0.3 percent before treatment and 6.2 percent 0.6 percent after therapy in non-diabetic persons with iron-deficiency anemia. [7]Gram-Hansen et al. observed similar results in their research as well. [6] HbA1c levels in IDA were higher in diabetic patients with chronic renal disease and diabetic pregnant women, but this was reduced with iron therapy. The research mentioned above explanations is purely hypothetical. We were compelled to perform our study to evaluate the effects of IDA on HbA1c because of the disparity in results acquired from these several research.HbA1c was determined using a High-Performance Liquid Chromatography technique in our study. Hb and HbA1c have a substantial negative connection, according to our findings. Falduetal.found that the lower the Hb, the higher the HbA1c (Hb-5.11.5, HbA1c- 9.11.7).Ferritin was also discovered to have a negative relationship with HbA1c. Bindel found a negative association as well[16].The study's findings were found to be similar to those of the majority of research mentioned in the literature.

Conclusion

Iron deficiency is the most prevalent nutritional deficiency and the most common cause of anemia in India. Previous studies have shown the effect of iron therapy on HbA1c levels and found a characteristic reduction in HbA1c levels in non-diabetic iron-deficient anemic patients. Similar to the previous studies, we too have shown that the IDA is associated with higher levels of HbA1C in non-diabetics, and with severe anemia, higher is the HbA1c level. Therefore,problems could occur during the diagnosis of diabetes mellitus in patients suffering from IDA. The patient's iron status must be considered during the interpretation or diagnosis of diabetes mellitus based on the concentration of HbA1c. The iron replacement therapy is paramount in diabetic patients with iron deficiency, as it would also increase the reliability of the Hba1c determinations. It should be stressed that though this study was performed in the non-diabetic group of patients and the impact of anemia on HbA1c levels was assessed, it may be of supreme clinical importance in patients with diabetes mellitus and co-existing IDA, particularly in the presence of poor metabolic control.

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