Iron Deficiency Anemia- Does it affect the Glycosylated Hemoglobin levels in Non- diabetic Patients?

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Abstract

Background: Glycosylated hemoglobin (HbA1c) is used in diabetic patients as an index of glycemic control. Reports on the effects of iron deficiency anemia (IDA) on HbA1c levels have shown inconsistent results. This study investigated the effects of IDA on HbA1c levels in non- diabetic patients.

Methods: Fifty non- diabetic patients diagnosed as having IDA, and an equal number of age- and sex- matched healthy controls, were included in this study. Hematological and biochemical parameters, including HbA1c levels were measured, and compared between cases and controls.

Results: Mean baseline HbA1c (%) level in anemic patients (6.1 ± 0.2) was significantly higher than in control group (4.1 ± 0.3) ($p < 0.0001$, 95% CI 1.89 to 2.1). A significant positive correlation of HbA1c was observed with total iron binding capacity (TIBC) ($r = 0.36$, $p = 0.009$, 95 % CI 0.09 to 0.58), but not with hemoglobin ($r = -0.015$, $p = 0.915$, 95% CI −0.29 to 0.26), MCV ($r = -0.082$, $p = 0.571$, 95 % CI −0.35 to 0.20), MCH ($r = -0.11$, $p = 0.453$, 95% CI −0.38 to 0.18), hematocrit ($r = -0.0032$, $p = 0.982$, 95% CI −0.28 to 0.28) and ferritin ($r = -0.052$ $p = 0.721$, 95% CI −0.33 to 0.23).

Conclusions: HbA1c levels were higher in patients with IDA than in controls and had a significant positive correlation with TIBC. This may have a practical application in diabetic individuals with IDA where HbA1c alone may give a false picture of poor glycemic control.

***Keywords*: Iron deficiency anemia, Glycosylated hemoglobin, HbA1c.**

Introduction

Hemoglobin A1c (HbA1c) is a glycated hemoglobin that can be used as an indicator of a patient's glycemic status over the previous 2 - 3 months. [1] Glycated hemoglobin does not include HbA1c alone. It includes other hemoglobins as well and together these constitute the HbA1 fraction of adult hemoglobin HbA. Among the various glycated hemoglobins, HbA1c is the predominant fraction. According to the American Diabetes Association Guidelines published in 2007, HbA1c levels should be maintained below 7% in all diabetic patients in order to prevent the development of diabetic microvascular complications. [2]

HbA1c levels are not affected by blood glucose levels alone. They are also altered in hemolytic anemias [3], hemoglobinopathies [4], acute and chronic blood loss [5], uremia [6] and patients on drugs like aspirin [7]. Vitamin B_{12} and iron deficiency anemia have also been shown to affect HbA1c levels. [8]

Iron deficiency anemia (IDA) is the most common form of anemia globally. [9] Initial studies revealed a relationship between iron deficiency anemia and HbA1c levels measuring high levels of HbA1c in IDA and attempted to explain it on the basis of both modifications in the structure of hemoglobin

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and levels of HbA1c in old and new red blood cells. [10-12] Later, other studies reported that there were no differences between the HbA1c levels of anemic patients and controls. [13, 14] A recent study showed that HbA1c levels were lower in patients with iron deficiency anemia and increased significantly upon treatment with iron. [15]

Therefore, in view of lack of clear cut evidence and paucity of such studies on the Indian population, we investigated the effect of iron deficiency anemia on HbA1c levels in non-diabetic patients having iron deficiency anemia.

Methods

This prospective cross- sectional study was conducted in the Department of Medicine, Himalayan Institute of Medical Sciences (HIMS), Dehradun, India. Fifty non- diabetic patients of more than 18 years of age, with iron deficiency anemia were selected out of 134 patients initially screened for inclusion in the study. An equal number of age- and sex- matched healthy individuals were included in the study. Subjects were recruited from patients admitted in the medical wards and those attending the Medicine OPD of Himalayan Institute Hospital, Dehradun, India with a primary diagnosis of iron deficiency anemia after obtaining written informed consent. Ethical clearance was obtained from the institutional ethics committee.

Iron deficiency was defined as per WHO guidelines with hemoglobin (Hb) < 13g/dl in males and <12g/dl in females, RBC morphology showing microcytic hypochromic picture on a peripheral blood smear, serum TIBC (Total Iron Binding Capacity) ≥ 400 µg/dl, and serum ferritin ≤ 15 ug/L.

Patients with hemoglobinopathies and hemolytic anemia, chronic renal failure, chronic liver disease, malignancies, bone marrow disorders, connective tissue disorders, chronic alcohol ingestion and any history of long term therapy with steroids were excluded. Those with established diabetes, impaired fasting glucose, or impaired glucose tolerance (as per ADA diagnostic guidelines) were excluded.

A detailed history was taken and physical examination was done. The complete hemogram, peripheral blood smears, stool for occult blood, serum ferritin, TIBC, serum creatinine, blood urea nitrogen (BUN), and fasting and postprandial blood glucose levels were measured. The glycosylated hemoglobin was measured using Bio Rad D-10 Hemoglobin A1c Program utilizing principles of ion- exchange high-performance liquid chromatography (HPLC).

Statistical Analysis

The data are presented as mean ± SD for continuous variables. Student's Unpaired 't'-Test was used for comparison of continuous data. Fisher's exact test was used for testing the significance of differences in proportions. Pearson's Product Moment Correlation Coefficient was used for establishing the correlation between glycosylated hemoglobin and other parameters including hemoglobin, MCV, MCH, hematocrit, serum ferritin, and TIBC. A 'p' value of <0.05 was taken as statistically significant. All reported 'p' values are two- sided.

Results

The baseline characteristics of patients with IDA and controls are shown in table 1. There was no significant difference between the baseline characteristics of cases and controls. Age range of the patients with IDA was 20 to 75 years.

	No. of Patients with IDA (%) (n=50)	**No. of Controls (%) (n=50)**	**p value**
Males	15 (30)	15 (30)	NS
Females	35 (70)	35 (70)	NS
Age groups			
0- 34 years	13 (26)	13 (26)	NS
35- 49 years	13 (26)	13 (26)	NS
50- 64 years	17 (34)	17 (34)	NS
≥65 years	7 (14)	7 (14)	NS
Socio- economic status [a]			
High	5 (10)	7 (14)	NS
Middle	17 (34)	14 (28)	NS
Low	28 (56)	29 (58)	NS

Dietary habits			
Vegetarian	38 (76)	34 (68)	NS
Mixed	12 (24)	16 (32)	NS
Smokers [b]	4 (8)	6 (12)	NS
Smokeless tobacco users [c]	7 (14)	8 (16)	NS

NS- Not significant ($p > 0.05$);
[a] High: annual income >Rs. 850 000; Middle: annual income Rs. 50000 to Rs. 850000; Low: annual income < Rs. 50000 (1 Indian Rupee = 20.4 Korean Won approximately);
[b] Defined as a person who had ever smoked cigarettes/ beedis for at least one year;
[c] Defined as a person who was currently using a smokeless tobacco product for more than one year at least once daily.

Table 1.Baseline Characteristics of the Patients with IDA and Controls

Clinical features of patients with IDA are shown in table 2. Easy fatiguability and malaise were the commonest symptoms while pallor and bald tongue were the commonest signs.

Clinical Features	No. of Patients (%)
Symptoms	
Easy fatigability	49 (98%)
Malaise	44 (88%)
Dyspnea on exertion	17 (34%)
GI blood loss	16 (32%)
Palpitation	12 (24%)
Menorrhagia	3 (6%)
Signs	
Pallor	50 (100%)
Bald tongue	21 (42%)
Platynychia / Koilonychia	17 (34%)
Pedal edema	15 (30%)
Cheilosis	10 (20%)
Ejection systolic murmur	11 (22%)

Table 2.Clinical features of the Patients with IDA (n=50)

Various hematological and biochemical parameters in various age groups of patients with IDA have been shown in table 3**.**

Parameters	Age group (years)				
	20- 34	35- 49	50- 64	≥65	All patients
Hb (g/dl)	7.4 ± 2.3	7.6 ± 1.4	8.4 ± 2.1	7.2 ±2.0	7.8 ±2.0
MCV (fl)	72.6 ± 6.8	66.0 ± 8.8	69.8 ± 8.3	62.8 ± 5.4	68.6 ± 8.2
MCH (pg)	22.1 ± 2.7	21.1 ± 4.6	20.9 ± 3.2	18.5 ± 2.3	20.9 ± 3.5
Hct (%)	24.1 ± 7.6	25.3 ± 5.14	28.3 ± 7.6	27.4 ± 8.1	26.3 ± 7.1
TIBC (µg/dl)	465.9 ± 90.9	470.6 ± 63.1	469.2 ± 117.7	498 .7± 80.2	472.8 ± 91.8
Ferritin (ng/ml)	7.9 ± 3.9	6.7 ± 4.6	10.1 ± 3.8	7.9 ± 5.1	8.3 ± 4.3
HbA1c (%)	6.1 ± 0.1	6.0 ± 0.1	6.0 ± 0.2	6.2 ± 0.3	6.1 ± 0.2

* Data are mean ± SD

Table 3.Mean values of various Hematological and Biochemical parameters in patients with IDA*

The range of hemoglobin was from 2.12 g/dl to 10.8 g/dl. The mean ± SD hemoglobin for males was 8.0 ± 1.6 g/dl, whereas for females it was 7.75 ± 2.1 g/dl, with a statistically insignificant difference ($p = 0.684$, 95% CI −0.97 to 1.87). The range of TIBC in patients with IDA was 400 to 768 µg/dl, ferritin 1.5to 15 ng/ml, and HbA1c 5.7 to 6.9 %. The mean ± SD TIBC, ferritin and HbA1c for males were 459.9 ± 88.1 µg/dl, 9.98 ± 4.3 ng/ml and 6.12 ± 0.23 % respectively. The

mean ± SD TIBC, ferritin, and HbA1c for females were 478.4 ± 94 µg/dl, 7.6 ± 4.2 ng/ml and 6.11 ± 0.20 % respectively. There was no significant difference in the values of HbA1c between males and females (p = 0.952). Stool test for occult blood was positive in 18 (36%) patients, of which 16 (32%) complained of malena while 2 (4%) patients had not noticed it.

In the control group, the mean ± SD hemoglobin for males was 14.3 ± 0.6 g/dl and for females 13.4 ± 0.5 g/dl. The mean ± SD TIBC, ferritin and HbA1c for females were 292.4 ± 35.0 µg/dl, 199.3 ± 39.3 ng/ml, and 4.16 ± 0.3 % respectively. The mean ± SD TIBC, ferritin and HbA1c for males were 291.1 ± 34.5 µg/dl, 200.9 ± 39.2 ng/ml, and 4.19 ± 0.4% respectively.

As compared to the control group, the values for each hematological parameter including hemoglobin, MCV, MCH, and Hct were significantly lower in the patients with IDA (p <0.0001 each). Biochemical parameters showed that serum ferritin was significantly lower, while TIBC and HbA1c were significantly higher in patients with IDA than in controls (p < 0.0001 each) (table 4).

Parameters	Cases (n=50)	Controls (n=50)	P value	95% C I
Hb (g/dl)	7.8 ±2.0	13.7 ± 0.6	<0.0001	−6.48 to −5.31
MCV (fl)	68.6 ± 8.2	87.5 ± 7.6	<0.0001	−22.03 to −15.76
MCH (pg)	20.9 ± 3.5	29.6 ± 1.1	<0.0001	−9.73 to −7.67
Hct (%)	26.3 ± 7.1	42.1 ± 2.5	<0.0001	−17.91 to −13.68
Ferritin (ng/ml)	8.3 ± 4.3	187.4 ± 40.6	<0.0001	−190.5 to −167.6
TIBC (µg/dl)	472.8 ± 91.8	288.6 ± 37	<0.0001	156.4 to 211.9
HbA1c (%)	6.1 ± 0.2	4.1 ± 0.3	<0.0001	1.89 to 2.1

* Data are mean ± SD

Table 4.Comparison of values of various hematological and biochemical parameters between patients with IDA and controls*

No significant correlation was seen between glycosylated hemoglobin and various parameters like hemoglobin, MCV, MCH, hematocrit and ferritin (p > 0.05 each). A significant positive correlation of HbA1c was seen only with TIBC (p = 0.009) (table 5).

Predictor variable	Dependent Variable (HbA1c)		
	Correlation coefficient 'r'	p Value	95% CI
Hemoglobin	− 0.015	0.915	−0.29 to 0.26
MCV	− 0.082	0.571	−0.35 to 0.20
MCH	− 0.11	0.453	−0.38 to 0.18
Hematocrit	− 0.0032	0.982	−0.28 to 0.28
Ferritin	− 0.052	0.721	− 0.33 to 0.23
TIBC	0.36	0.009	0.09 to 0.58

Table 5.Correlation of hematological and biochemical parameters with Glycosylated hemoglobin (HbA1c) of IDA cases

Discussion

In our study, iron deficiency anemia was more common among females (70%) compared to males (30%). Similar results were observed in other studies. [15-17]

In our study, IDA was most prevalent in individuals having lower socio- economic status, as has also been observed in surveys conducted by WHO, stating that nutritional deficiency and worm infestation were most commonly encountered in these populations. [18]

The values of various hematological parameters were lower in IDA patients than in controls. As for the values of biochemical parameters, ferritin values were low and TIBC was high. Coban et al. also had significantly lower values of hemoglobin,

hematocrit, MCV, MCH, and ferritin, in IDA patients than in control group ($p < 0.001$). [16]

In our study, HbA1c in IDA cases was significantly higher than in control group (6.1% vs. 4.1%) ($p < 0.0001$). Similar results were seen by Brooks et al. (9.9% vs. 7.9%) [10], Coban et al. (7.4% vs. 5.9 %) [16] and Tarim et al. (10.6% vs. 7.7 %) [19], ($p < 0.001$ each), and it was suggested that IDA should be corrected before interpreting HbA1c results.

We observed no correlation of HbA1c with hemoglobin, MCV, MCH, hematocrit and ferritin. However, a significant positive correlation of HbA1c was seen only with TIBC ($p = 0.009$). Thus, in spite of higher HbA1c values observed in non- diabetic patients with IDA, it is not possible to predict the actual rise in HbA1c levels for given hematological parameters.

Ford et al. found significant positive correlation of HbA1c with hemoglobin, hematocrit, ferritin, and negative correlation with transferrin saturation, MCV, and MCH. They suggested exercising caution while diagnosing diabetes and prediabetes among people with high or low Hb when the HbA1c level was near 6.5% or 5.7%, respectively. However, the trend for HbA1c to increase with iron deficiency does not appear to require screening for iron deficiency in ascertaining the reliability of HbA1c in the diagnosis of diabetes and prediabetes in a given individual. [20] Kim et al. observed that iron deficiency was associated with shifts in HbA1c distribution from < 5.5% to > 5.5%. However, whether iron deficiency is associated with shifts at higher HbA1c levels, is still not clear. [17]

Various mechanisms have been proposed for explaining the effects of IDA on the value of HbA1c. Brooks et al. showed higher HbA1c concentrations in non- diabetic anemic individuals and proposed that the quaternary structure of the hemoglobin molecule is altered, and that glycation of the β globulin chain occurs more readily in the relative absence of iron. [10] Hansen et al. have shown that normal glycosylated hemoglobin concentrations in iron deficiency decrease to subnormal levels after iron supplementation and it was proposed that it happened probably due to increase in bone marrow erythropoiesis on treatment leading to production of new immature erythrocytes. [8] Sluiter et al. explained it on the basis that formation of HbA1c is an irreversible process and hence the concentration of HbA1 in one erythrocyte will increase linearly with the cell's age. In patients with normal blood glucose values but with red cells younger than usual, as after treatment of IDA, HbA1 concentration falls. However, in persisting deficiency, the red cell production rate falls leading to not only anemia but also a higher than normal average age of circulating erythrocytes and therefore, of increased HbA1. [11] El-Agouza et al. explained it in terms of a balanced relationship between HbA1c and plasma glucose levels. So a decrease in hemoglobin concentration will lead to an increase in glycated fraction. [12] Tarim et al. speculated the cause for it to be emergence of young erythrocytes in the circulation after iron therapy that could have led to dilution and lowering the concentration of previously formed HbA1c and suggested that iron status of the patient must be considered during the interpretation of HbA1c concentrations. [19] Mitchell et al. disagreed with cell age concept to be a significant factor in explaining the change in HbA1 during the treatment of iron deficiency anemia. [13] In fact, no changes in HbA1c concentration were reported before and after therapy and the differences were said to be in the laboratory methods used for measuring HbA1c. [14] However, Rai et al. investigated different methods of estimating HbA1c, and no difference was detected among the colorimetric, ion- exchange chromatography, and affinity chromatography. [21] On the contrary, Sinha et al. showed that HbA1c concentration and absolute HbA1c levels were low in anemic population, suggesting nutritional deficiency to be responsible. [15]

So far, none of the proposed reasons for varying values of HbA1c in iron deficiency anemia has been verified and the exact cause remains elusive. Furthermore, to what extent iron deficiency anemia can affect the values of HbA1c is still not known.

Conclusion

Glycosylated hemoglobin (HbA1c) values were significantly higher in non- diabetic IDA patients than in controls implying that iron deficiency anemia leads to a rise in the glycosylated hemoglobin values. However, HbA1c had a significant correlation only with TIBC and not with other hematological and biochemical parameters. This may have a practical application in diabetic individuals with iron deficiency anemia where glycosylated hemoglobin alone may give a false picture of poor glycemic control. Therefore, iron status must be considered and iron deficiency must be corrected before making diagnostic or therapeutic decision based on glycosylated hemoglobin values in diabetes mellitus.

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