

Effect of Iron Deficiency Anemia on HbA1c in Diabetics and Non Diabetics

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Abstract

Background: Iron deficiency anemia (IDA) is one of the most common anemias. HbA1c has been cited as one of the good laboratory parameters to diagnose and monitor type II diabetes mellitus (DM). The publications of the recent past have observed that the value of HbA1c is influenced by a state of anemia. Anemia with various underlying causes influences HbA1c value including anemias in pregnancy. These states of anemia may result in spuriously high values of HbA1c. This may lead to the wrong stratification of the patients as pre-diabetics or overt diabetics.

Method: 150 patients were taken and divided into three groups. Group A was taken as a control group, group B as patients with IDA but no DM type II and group C had patients who were suffering from both IDA and DM type II. All underwent OGTT to know and define type II DM for its presence. Intergroup comparison of HbA1c

was done with the results of OGTT, total serum iron levels, serum ferritin and total iron-binding capacity.

Result: There were twelve cases (24%) that were spuriously labelled to be prediabetic (2 cases) and diabetic (10 cases) in group B subjects of the study who had IDA but normal OGTT results. Group C patients who were diabetic with IDA showed higher HbA1c values when compared with controls.

Conclusion: The definition based on HbA1c values of the American Diabetes Association in the diagnosis of pre-diabetes or DM type II may be interpreted in the light of the state of IDA. It’s found that IDA influences Hb1c levels.

Keywords: Iron Deficiency Anemia, Diabetes Mellitus, and HbA1c.

Introduction

The metabolic disease of diabetes mellitus type II and anemia of nutritional deficiencies are the common

clinical problem prevailing in the Indian population. The glycemic control in type II diabetes mellitus has primarily been monitored by estimating HbA1c levels [1]. However, recent studies have shown that multiple factors influence HbA1c levels. One such common factor that influences the level of HbA1c is the state of anemia and hemoglobinopathies [2].

HbA1c being glycated hemoglobin formed by glycation of -NH₂ terminal of the beta chain of globin is linked to lifespan of red blood cells and pathologies of it [3]. Iron deficiency anemia (IDA), is one of the most common anemia in India [4-7]. Diabetes and anemia are coexisting public health problems all over the world [8-12]. Recent literature has quoted that anemia influences HbA1c [13-17]. There are reports that IDA interferes with the value of HbA1c in non-diabetic anaemic patients and diabetic anemic patients. Iron deficiency anemia increases glycosylation of proteins, including haemoglobin glycation (HbA1c) [13-17].

Occasional reports observed that iron supplementation can lower the value of HbA1c. A few reports observe the effects of iron supplementation on levels of HbA1c pre and post iron supplementation in diabetic patients [5, 18]. The effect of iron deficiency on hemoglobin glycation in non-diabetics has also been studied [19]. It has been concluded that HbA1c is not affected by blood sugar levels alone but has confounding factors, especially of iron deficiency states, which may alter therapeutic decisions upon HbA1c levels [20].

Therefore, the definition of type II diabetes mellitus as suggested by the American Diabetes Association may result in erroneous stratification of patients to DM, especially in the states of iron deficiency anemia.

Therefore, the study has the objectives of:

1. The relationship between HbA1c levels in iron deficiency states with results of oral glucose tolerance test in knowing the erroneous diagnosis of pre-diabetic and diabetic states in patients who had IDA but no type II DM on OGTT.
2. To know the relationship between HbA1c levels and serum iron status in patients of IDA but no type II DM on OGTT.
3. Intergroup comparison of HbA1c in group A (control group), group B (IDA + NDM) and group C (IDA +DM).

Material and Methods

Patients recruited in the study were recorded for preliminary medical data. The study design was prospective, observational and analytical. A total of 150 subjects were divided into three groups:

Group A: (Control group): The 50 healthy subjects were taken. The patients had no iron deficiency anemia and no type II diabetes mellitus on OGTT. These patients underwent HbA1c and iron studies. Results of these groups were taken for standard referencing and comparison of the other two groups of study.

Group B: (IDA + NDM): Fifty patients were included who had iron deficiency anemia but were non-diabetic on OGTT.

Group C: (IDA + DM): The 50 patients included in this group had iron deficiency anemia and diabetes mellitus type II by OGTT. This group did not include the cases that had complications of DM types II.

Anemia was defined when haemoglobin was below 13 g/dl for adult males [22, 8] and below 12 g/dl for adult females [21,22, 8]. The patients were defined as iron deficiency anemia when total iron-binding capacity was greater than 450 mcg/dl, total serum iron less than 50 mcg/dl in females [13, 23, 24] less than 65 mcg/dl in

males [25, 13, 22] and total serum ferritin was less than 20 ng/mL in male [25, 26] and 10 ng/mL in female [25, 27].

The other criteria to categorise the patients as IDA when mean corpuscles volume (MCV) was less than 80 fl, mean cell haemoglobin (MCH) less than 27 pg/cell, and peripheral smear showing predominantly microcytic hypochromic red blood cell morphology. The patients were defined as diabetic if fasting blood sugar was greater than 126 mg/dl [8] and postprandial blood glucose (2 hours after glucose intake) was greater than 200 mg/dl [8]. HbA1c values were taken according to American Diabetes Association criteria as normal was <5.7%, pre-diabetics from 5.7 to 6.4 % and diabetics > 6.5 % [20].

The blood sugar was performed by the colourimetric method by GOD PAP underlying principle. HbA1c was determined by latex agglutination inhibition assay. The iron studies were performed by standard method meant for serum iron, serum ferritin, and total iron-binding capacity. The red blood cell studies were done by an

automated cell coulter. The peripheral smear examination was performed on Leishman's stained blood smear.

The study was a comparative study carried out within three groups. Group A (control group), group B (IDA + NDM) and group C (IDA + DM). Comparative statistics were used. The statistical tests performed onset of results were of Pearson coefficient (p-value) and mean + SD.

Results

The distribution in 150 cases of groups A, B, and C included 68 females (45.3%) and 82 males (54.7%). There were 61 cases (40.7%) in the age range of 25-44 years and 39 cases (26.0%) in the age range of 45 – 59 years. The mean age of group A was 41.56 ± 16.69 years, group B was 41.54 ± 16.69 years And group C was 41.78 ± 15.79 years.

The result of the comparison of HbA1c with blood sugar levels by OGTT in three groups of the study is shown in table 1 (Fasting Blood Sugar) and table 2 (Postprandial Blood Sugar):

Table 1: HbA1c levels comparison with Fasting blood sugar among the groups:

FBS	HbA1C	GROUP			Total	p-value
		Control (Group A)	IDA + NDM (Group B)	IDA + DM (Group C)		
Less than 126	Less than 5.7	50(100%)	38(76%)	0(0%)	88(58.67%)	0.0011,S
	5.7 to 6.4	0(0%)	2(4%)	0(0%)	2(1.33%)	
	6.5 and above	0(0%)	10(20%)	0(0%)	10(6.67%)	
126 to 160	6.5 and above	0(0%)	0(0%)	8(16%)	8(5.33%)	-
	Total	0(0%)	0(0%)	8(16%)	8(5.33%)	
More than 160	5.7 to 6.4	0(0%)	0(0%)	1(2%)	1(0.67%)	-
	6.5 and above	0(0%)	0(0%)	41(66%)	41(22%)	
	Total	50(100%)	50(100%)	50(100%)	150(100%)	

Table 2: Post meal blood sugar wise comparison of HbA1C level among the groups

PMBS		GROUP			Total	p-value
		CONTROL (Group A)	IDA + NDM (Group B)	IDA + DM (Group C)		
Less than 200 mg/dl	Less than 5.7	50 (100%)	38 (76%)	0 (0%)	88 (58.6%)	< 0.001 S
	5.7 to 6.4	0 (0%)	2 (4%)	0 (0%)	2(1.33%)	
	6.5 and above	0 (0%)	10 (20%)	0 (0%)	10 (6.67%)	
	Total	50 (100%)	50 (100%)	0 (0%)	100(66.67%)	
200 TO 300 mg/dl	5.7 to 6.4	0 (0%)	0 (0%)	1 (2%)	1(0.67%)	-
	6.5 and above	0 (0%)	0 (0%)	26 (52%)	26(17.33%)	
	Total	0 (0%)	0 (0%)	27 (54%)	27 (18%)	
More than 300 mg/dl	6.5 and above	0 (0%)	0 (0%)	23 (46%)	23 (15.33%)	-
	Total	50 (100%)	50 (100%)	50 (100%)	150	

The 12 patients (24%) were having the abnormally increased value of HbA1c in the patients who had FBS <126 mg/dl in group B. Two patients were observed to have HbA1c in pre-diabetic range (5.7 to 6.4%) and 10 patients had HbA1c in diabetic range (> 6.5%).

The observation in group B for comparison between postprandial blood sugar and HbA1c were similar to that

Table 3 describes the observation of TIBC and its relationship with HbA1c:

Table 3 - Serum TIBC level wise distribution of HbA1C level among the groups

SERUM TIBC (mcg/dl)	HbA1C	Group			Total	p-value
		CONTROL (Group A)	IDA + NDM (Group B)	IDA + DM (Group C)		
240 to 450	Less than 5.7	50 (100%)	0(0%)	0(0%)	50	-
	Total	50(100%)	0(0%)	0(0%)	50	
More than 450	Less than 5.7	0(0%)	38(76%)	0(0%)	38	< 0.001 S
	5.7 to 6.4	0(0%)	2(4%)	1(2%)	3	
	6.5 and above	0(0%)	10(20%)	49(98%)	59	
	Total	0(0%)	50(100%)	50(100%)	150	

Pearson correlation r = 0.413, p-value< 0.001 S

There were 12 patients who had HbA1c levels of more than 5.6%. The distribution was as follows: two patients had HbA1c in the range of 5.7 to 6.4 (pre-diabetic) and 10 patients had HbA1c >6.5 (diabetic) when TIBC was >

of observation of fasting blood sugar. There were 12 patients who had increased HbA1c values when compared with the postprandial blood sugar (<200mg/dl). The distribution of HbA1c levels were as follows: 2 cases in the pre-diabetic range and 10 cases in the diabetic range.

450 mcg/dl. The p-value for these observations was significant.

These observations confirmed that the state of iron deficiency anaemia influences HbA1c levels which may

be erroneously interpreted as a pre-diabetic state or overt diabetic state while its OGTT results are in the non-diabetic range.

It was further observed that the group C (IDA +DM) patients (table 1 and 2) had HbA1c values over 6.5 % when their fasting and postprandial blood sugar suggesting the influence of iron deficiency anaemia in increasing the glycated haemoglobin percentage.

P-values for these observations of IDA increasing HbA1c levels who had a normal OGTT result has been found to be significant so also for the observation of TIBC ($p < 0.001$, S).

It was observed that HbA1c has been influenced for its increased percentage by MCV and MCH (in the range of IDA). So also the total serum iron levels and serum ferritin level (in range of IDA) in group B and group C patients with significant p-value ($p < 0.001$, S).

Discussion

Twelve cases (24%) were wrongly classified as diabetic and prediabetic by ADA definition based on HbA1c when their OGTT-FBS was less than 126 mg/dl in group B (IDA + NDM). These 12 patients (24%) with FBS less than 126 mg/dl had a concomitant pathology of iron deficiency anemia. Thus, the state of iron deficiency anemia appeared to affect the value of HbA1c. This observation of the present study is in agreement with the observation of Son et al. (8) that in the diagnosis of diabetes mellitus in the state of iron deficiency anemia, HbA1c cut off needs to be increased as compared to the normal ones given by ADA to eliminate the population whose HbA1c influenced by the state of iron deficiency anemia. The present study observed a significant p-value of 0.0001, S for the correlation between iron deficiency anemia and HbA1c in patients with normal fasting blood sugar carried out by OGTT.

The study of Silva et al. [19] has observed the negative correlation between mean corpuscular volume (MCV) and HbA1c ($r = -0.448$, $p < 0.01$, S) levels in anemic patients. The study of Madhu et al. [26] also observed the negative correlation between MCV and HbA1c ($r = -0.156$). The study of Hardikar et al. observed higher HbA1c with lower MCV value ($r = -0.22$). The present study made the concomitant observation to the study of Silva et al., Madhu et al. [26], Hardikar et al. [20] that with the reduction of MCV, HbA1c values were raised for group B population (IDA+NDM) with significant p-value ($r = -0.43$, $p < 0.001$, S). However, the study of Solomon et al. [17] has observed no correlation between MCV and HbA1c.

The study of Madhu et al. [26] observed the negative correlation between the value of (mean corpuscular hemoglobin) MCH and HbA1c ($r = -0.236$). The study of Hardikar et al. [20] has observed the higher level associated with lower MCH ($r = -0.37$). The observation of the present study is in agreement with the observation of Madhu et al. [26] and Hardikar et al. [20]. The present study observed a significant p-value < 0.001 , S with Pearson correlation ($r = -0.355$) when attempting the relationship between MCH with HbA1c.

Study group B (IDA + NDM) had 21 females in it, of which five females (23.18%) had HbA1c levels more than 6.5% with their serum iron level less than 50 mcg/dl. There were 18 females (100%) in group C who demonstrated an HbA1c value of more than 6.5 % when their serum iron level was less than 50 mcg/dl. In males with the cut off of 65 mcg/dl, the observation made in group B (IDA + NDM) showed 2 (6.9%) and 5 (17.24%) patients had HbA1c in prediabetic and diabetic levels, respectively.

In males, in group C (IDA + DM) of 32, all had serum iron levels less than 65 mcg/dl with HbA1c level in diabetics (more than 6.5%) and one case in prediabetic range (5.7% to 6.4%). Thus, it is inferred from the present observation that total serum iron level when in males and females below cut off of less than 65 mcg/dl and 50 mcg/dl, respectively, affects HbA1c for its elevation. The observation similar to the present study was done by Bharadwaj et al. [22] and Rajagopal et al. [23] However, Madhu et al. [26] observed a negative but weak correlation ($r=-0.173$) between serum iron and HbA1c, which is dissimilar to the observation of the present study.

The present study adopted total iron-binding capacity (TIBC) as one of the criteria to classify patients for iron deficiency anemia. TIBC more than 450 mcg/dl was considered one of the indicators for criteria of iron deficiency anemia. Accordingly, the present study observed 12 such cases in group B (24%), where the value of TIBC was less than 450 mcg/dl with an increase in HbA1c prediabetics (2 cases) and diabetics (10 cases) with the significant p-value. However, not many studies are available in the literature that correlates serum TIBC levels with HbA1c. The study by Madhu et al. [26] established a negative but weak correlation between the TIBC and HbA1c level ($r = -0.035$).

The present study divided the population into all three groups to define iron-deficiency anemia based on gender cut off values and serum ferritin levels. The serum ferritin value less than 10 ng/ml in females and less than 20 ng/ml is considered characteristic of iron deficiency anemia in males. The present study observed the relationship between the serum ferritin level in the female and increased value of HbA1c in group B (IDA + NDM) in 5 cases (23.81%) out of 21 females in that

group with the significance of Pearson correlation ($r=-0.491$; $p < 0.001$, S). Similarly in group B (IDA + NDM) of 29 subjects, 2 subjects (6.90 %) showed HbA1c in prediabetic level as 5.7% to 6.4% and 5 cases (17.4%) were in diabetic level (more than 6.5%). In group C of 32 patients of the current study, 31 patients showed a further elevated level of HbA1c, with 98.66% patients showing HbA1c more than 6.5% when serum ferritin level was less than 20 ng/ml ($p = 0.12$, NS). This observation of the present study concordance with the observation made by Christy et al. [13] ($r= 0.05$, $p=0.295$), Madhu et al. [26] ($r=- 0.441$), Intra et al. [27] ($p < 0.0001$), who observed that reduction in levels of ferritin is associated with elevated values of HbA1c leading to misdiagnosis of diabetes mellitus in these patients of iron deficiency anemia. However, the study of Ford et al. [11] found no correlation between reduced levels of serum ferritin with an elevated level of HbA1c in iron deficiency anemia patients.

Conclusion

It is concluded that the state of iron deficiency anemia influences HbA1c levels. The HbA1c levels may be increased to the pre-diabetic or diabetic range in a state of IDA when OGTT results in the non-diabetic range. It is further concluded that IDA with its laboratory diagnosis parameters such as total serum iron levels, serum ferritin, and TIBC correlates for the influence on HbA1c.

Therefore, it is suggested that the definition of type II diabetes mellitus based on HbA1c may wrongly categorise patients to pre-diabetic and diabetic states if concomitantly suffering from iron deficiency anaemia.

What is already known on this topic:

- There are few studies that investigated the effect of anemia on HbA1c levels.

- These studies investigated the value change of HbA1c in a state of anemia affecting the treatment protocol and its follow up.

What this study adds:

- The present study adds to the knowledge for variations of HbA1c in the group of patients with iron deficiency anemia without diabetes mellitus type II and iron deficiency anemia with diabetes mellitus type II when compared with the control group.
- The valuation of the American Diabetes Association definition is based on HbA1c in patients with iron deficiency anemia without diabetes resulting in prediabetic and diabetic diagnosis compared to WHO Oral Glucose Tolerance Test criteria of diabetes mellitus type II.
- The study observed that a few patients may be categorised as prediabetic and diabetic if HbA1c criteria of ADA is utilised in the group of patients with IDA without abnormal OGTT.

Limitations:

- The study is carried out in a single centre.
- The population selected for the study is adults and cannot be applied its results in pediatric populations.
- The study is also limited by the type of diabetes mellitus. The study included the subjects with type II diabetes mellitus, and we cannot apply its results to type I diabetes mellitus.
- A comparative data of HbA1c levels for other types of anemia besides iron deficiency anemia is another limiting factor for the present study.
- The study also limited that the cases recruited were of type II diabetes mellitus without complications. Thus, findings of HbA1c concerning anemia in type

II cannot be applied in the opposite population with complications.

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