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Effect of Iron Deficiency Anemia on Glycation of Hemoglobin in Non-diabetics

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Abstract

Introduction: Glycosylated hemoglobin (HbA1c) is commonly used for monitoring glycemic control and as predictor of diabetes complications. Recently, HbA1c has also been recommended as a diagnostic test for diabetes mellitus by the American Diabetes Association.

Materials and Methods: About 63 non-diabetic, anemic patients, and 63 age-matched healthy subjects were enrolled in this study.

Results: The mean HbA1c (6.13% ± 0.6%) level in the patients with iron deficiency anemia (IDA) was higher than that in the control group (5.12% ± 0.5%) (P < 0.001).

Conclusion: It was found that IDA was common among the reproductive age group women. It was associated with shift in the HbA1c levels to higher side, primarily between 6.0% and 6.5%.

Key words: Diabetes mellitus, Glycosylated hemoglobin, Iron deficiency anemia

INTROCUCTION

Glycosylated hemoglobin (HbA1c) is on its way to celebrate the 50 years of existence and is being considered as one of the best achievements in the history of diabetes mellitus (DM).

HbA1c has been in use since 1980s as the “gold standard” for monitoring glycemic control and as a predictor of diabetic complications. Even though several conditions such as hemolytic anemia (lowers HbA1c) and aplastic anemia (raises HbA1c) tend to confound and interfere with HbA1c measurement, in most circumstances, HbA1c is a valid and reliable index of glycemic status. Recently, HbA1c has also been recommended as a diagnostic test for DM by the American Diabetes Association. HbA1c offers logistical advantages over the conventional oral glucose tolerance test as it requires a non-fasting random sample.

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Iron deficiency is one of the most prevalent forms of malnutrition. Globally, 50% of anemia is attributed to iron deficiency. Ferritin is the storage form of iron, and it reflects the iron status accurately.[1] An earlier study showed that reduced iron stores have a link with increased HbA1c, leading to false-high values of HbA1c in non-diabetic individuals.[2] HbA1c is the most predominant fraction of HbA1, and it is formed by the glycation of terminal valine at the β-chain of Hb.[3] It reflects the patient’s glycemic status over previous 3 months.

Its alteration in other conditions such as hemolytic anemia, hemoglobinopathies, pregnancy, and vitamin B12 deficiency has been explained in a study conducted by Sinha *et al*.[4] Although iron deficiency is the most common nutritional deficiency, reports of the clinical relevance of iron deficiency on HbA1c levels have been inconsistent.

HbA1c is widely used as an important marker of glycemic control, and it is of utter importance to exclude factors which could spuriously elevate its levels. Hence, we conducted a study in iron-deficient individuals with

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normal fasting blood sugar (FBS) and post lunch blood sugar (PLBS) levels to assess whether anemia has any effect on A1c levels, and anemia can be considered before making any therapeutic decisions based solely on HbA1c levels.[5]

MATERIALS AND METHODS

The present study was done over a period of about 1-year from July 2014 to August 2015 after approval by the Hospital Ethics Committee. Adult patients attending the outpatient clinics of Department of Medicine and patients admitted into medical wards of Kakatiya Medical College, MGM Hospital, Warangal, were screened for anemia as defined by WHO guidelines.

A total of 128 cases of anemia were detected of which 71 cases were diagnosed to have iron deficiency anemia. Among the 71 cases, the initial 63 consecutive patients satisfying the inclusion criteria were enrolled in the study.

Inclusion Criteria

- Presence of anemia as defined by WHO
  Hb: <13.0 g/dl (adult males)
  <12 g/dl (non-pregnant women)
- Microcytic, hypochromic picture in peripheral blood smear
- Serum ferritin:
  <9 ng/ml (in females)
  <15 ng/ml (in males) suggestive of iron deficiency
- Normal fasting and postprandial plasma glucose levels
- Normal liver function tests
- Normal blood urea, serum creatinine levels.

Exclusion Criteria

The following patients were excluded from this study, those with:

- Glucose tolerance abnormalities (impaired glucose tolerance or DM)
- Hemoglobinopathies
- Hemolytic anemia
- Chronic alcohol ingestion
- Chronic renal failure
- Pregnant females
- History of blood transfusion in the past 3 months.

A detailed history was taken and physical examination was done.

The blood specimens were drawn after an overnight fast. Venous blood samples (3 ml) were drawn; 0.5 ml was taken into an EDTA-treated tube. A RIPL - 5000 fully automated analyzer and cell counter was used for the whole blood counts (Hb, hematocrit, mean corpuscular volume [MCV], and mean corpuscular Hb [MCH]); the serum ferritin levels were measured by using a Diatek kit in automated analyzer, and the peripheral blood smears were examined in all the patients. The HbA1c levels were determined by turbidimetric immunoinhibition by using AIA 360 TOSOH AUTOMATED IMMUNOASSAY ANALYSER. Various biochemical tests were evaluated by standardized laboratory techniques.

This study was approved by the Ethics Committee of Kakatiya Medical College, Mahatma Gandhi Memorial Hospital, Warangal, Telangana State, India. An informed consent was obtained from all the subjects.

RESULTS

Estimation of complete hemogram including MCV, MCH, packed cell volume along with peripheral smear, serum ferritin levels, FBS, PLBS, HbA1c levels was done in all the 126 patients (63 cases and 63 controls) included in the study, during the study period.

All the patients enrolled in the study had normal FBS and PLBS levels, which confirmed their non-diabetic status.

- The Hb, serum ferritin levels (index of iron deficiency status) were low among the cases and the peripheral blood smear showed hypochromic microcytic picture in them.
- The Hb, serum ferritin and the HbA1c levels were normal in the control group ($P > 0.05$).
- The liver function tests, blood urea and serum creatinine levels were normal in all the patients.
- The serum HbA1c levels were significantly increased among the IDA patients as compared to those in the controls.

The mean HbA1c level in the patients with IDA (6.13% ± 0.57%) was higher than that in the control group (5.12% ± 0.30%) ($P < 0.001$).

Of the total of 126 patients enrolled in the study, case group consisted of 63 patients and the control group consisted of 63 patients. The youngest patient was 15 years and the oldest patient was 82 years old. The mean age of the study population was 38.41 ± 17.6 years and the mean of the control group was 39.17 ± 17.9 years. Those who were more than 40 years of age constituted 33% and the rest 67% were less than or up to 40 years of age. 21 patients, i.e., 33% with IDA were in the age group of 31-40 years and constituted the largest group (Table 1).

Out of 63 cases, 47 were females constituting about 75% of the study group and 16 were males constituting about

25%. Sex distribution was similar in the control group. Thus, iron deficiency anemia was more common among the females than the males (Table 2).

Out of 47 females with iron deficiency anemia, 13 females (28%) were in the age group of 31-40 years constituting the highest number, and out of 16 males with iron deficiency anemia, 8 males (50%) were in the age group of 31-40 years. In the study, iron deficiency anemia was more common in the age group of 31-40 years (Table 3).

Almost all patients were symptomatic with easy fatigability and tiredness. Easy fatigability was the most common symptom, present in all the 63 cases (100%). Dyspnea was the presenting complaint in 34 patients (54%). along with fatigability. Giddiness was presenting complaint in 11 patients constituting about 17%. Gastrointestinal blood loss was present in 10 cases (15%). Palpitations were present in 5 patients (8%) along with easy fatigability. Fever was the predominant complaint in 4 patients (6%) along with fatigability. Menorrhagia was present in 12 females (25% of females) (Table 4).

The most common clinical sign in the study group was pallor of tongue and conjunctiva which was present in all the 63 cases (100%) followed by ejection systolic murmur in the pulmonary area which was present in 32 cases (51%). Nail changes (koilonychia/platonychia) were present in 30 cases (48%) along with pallor. Koilonychia were present in 18 cases and platynychia in 12 cases. Along with pallor, bald tongue was present in 20 cases (32%), venous hum in 11 cases (17%), and pedal edema in 10 cases (16%) (Table 5).

The range of Hb was from 4.1 to 10.5 g/dl. The average 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 group was 6.84 ± 1.63 g/dl. The mean Hb in the control group was 12.87 ± 1.3 g/dl. These data provided evidence that Hb was indeed lower in anemic patients than in healthy controls, and the observed difference was statistically significant ($P < 0.001$) (Table 6).

In the study group, among 63 cases, 50 cases (79%) had severe anemia and 13 cases (21%) had moderate anemia. There were no mild anemia cases in the group. Out of 50 cases with severe anemia, 40 cases were females and 10 cases were males. Out of 13 cases with moderate anemia, 7 cases were females and 6 cases were males (Table 7).

Table 1: Age distribution

Age (in years)	Number of cases	Number of controls	% of total
≤20	12	12	19
21-30	9	9	14
31-40	21	21	33
41-50	8	8	13
51-60	4	4	6
>60	9	9	14
Total	63	63	100
Mean±SD	38.41±17.63	39.17±17.93	

SD: Standard deviation

Table 2: Sex distribution

Sex	Number of cases	Number of controls	% of patients
Male	16	16	25
Female	47	47	75
Total	63	63	100

Table 3: Age and sex-wise distribution

Age (in years)	Number of patients		% of patients	
	Male	Female	Male	Female
≤20	2	10	13	21
21-30	1	8	6	17
31-40	8	13	50	28
41-50	2	6	13	13
51-60	1	3	6	6
>60	2	7	13	15
Total	16	47	100	100

Table 4: Presenting symptoms

Symptoms	Number of patients	% of total
Easy fatiguability	63	100
Dyspnoea on exertion	34	54
Fever	4	6
Giddiness	11	17
GI blood loss	10	15
Palpitations	5	8

GI: Gastrointestinal

Table 5: Clinical signs

Clinical signs	Cases (%)
Pallor	63 (100)
Bald tongue	20 (32)
Platynychia/koilonychia	30 (48)
Pedal oedema	10 (16)
Ejection systolic murmur	32 (51)
Venous hum	11 (17)

Table 6: Hb

Hb (g/dl)	Cases	Controls
Male	7.59	14.03
Female	6.54	12.47
Total	6.81	12.87
Mean±SD	6.84±1.63	12.87±1.3

Hb: Hemoglobin, SD: Standard deviation

Table 7: Degree of anemia

Severity of anemia	Cases (%)		
	Male	Female	Total
Severe (<8)	10 (62.5)	40 (85.1)	50 (79)
Moderate (8-10.9)	6 (37.5)	7 (14.9)	13 (21)
Mild (11-11.9)	0 (0.0)	0 (0.0)	0 (0)
Total	16 (100)	47 (100)	63 (100)

DISCUSSION

Our results suggested that IDA was associated with higher concentrations of HbA1c. The earliest study to investigate the effects of iron deficiency anemia on HbA1c levels was conducted by Brooks *et al.*[6] who assessed HbA1c levels in 35 non diabetic patients having iron deficiency anemia both before and after treatment with iron. They observed that HbA1c levels were significantly higher in iron deficiency anemia patients and decreased after treatment with iron. The mechanisms leading to increased HbA1c levels were not clear. It was proposed that, in iron deficiency, the quaternary structure of the Hb molecule was altered, and that glycation of the globin chain occurred more readily in the relative absence of iron.[6]

- Sluiter *et al.*[7] tried to provide an explanation for the above findings. They proposed that the formation of HbA1c is an irreversible process and hence, the concentration of HbA1c in erythrocyte will increase linearly with the cell's age. For example, they found that in patients with normal blood glucose levels, but with very young red cells, as would be found after treatment of iron deficiency anemia, HbA1c concentration was reduced. However, if iron deficiency has persisted for a long time, the red cell production rate would fall, leading not only to anemia but also to a higher-than-normal average age of circulating erythrocytes and, therefore, increased HbA1c levels.[7]
- Further studies by El-Agouza *et al.*[8] and Cogan *et al.*[9] showed that HbA1c levels were higher in patients with iron deficiency anemia and decreased significantly upon treatment with iron. They argued that elevated HbA1c levels in iron deficiency anemia could be explained by the assumption that if serum glucose remains constant, a decrease in the Hb concentration might lead to an increase in the glycated fraction.[8,9] As evident from the above studies, the exact mechanism through which iron deficiency anemia affects HbA1c levels still remains unclear. The explanations provided in the studies quoted above are merely speculation. Due to the variation in results obtained from these multiple studies, we were prompted to conduct our own study to investigate the effects of iron deficiency anemia on HbA1c levels.
- Gram-Hansen *et al.*[10] showed normal HbA1c concentrations in iron deficiency, which dropped to subnormal levels after iron supplementation.
- Rai and Pattabiraman[11] investigated the different methods and no difference was detected among the colorimetric methods, ion-exchange chromatography, and affinity chromatography. The commonly performed immune turbidometric method was performed to determine the HbA1c levels in this study.
- Coban *et al.*[9] found that among non-diabetic adults with iron-deficiency anemia, the A1c was 7.4% ± 0.3% before treatment and 6.2% ± 0.6% after treatment. Similar results were also found in studies carried out by Gram-Hansen *et al.*[10] and Coban *et al.*[9] Investigations performed on diabetic chronic kidney disease patients, and diabetic pregnant women showed increased A1c levels in iron deficiency anemia, which was reduced following iron therapy.
- Likewise, Tarim *et al.*[12] found that HbA1c in iron-deficient patients decreased from 7.6% ± 2.6% to 6.2% ± 1.4% after iron therapy ($P < 0.05$), despite similar glucose levels. We did not find such large shifts in HbA1c associated with iron deficiency, either because of the population-based nature of the sample or differences in HbA1c assays. In addition, we did not examine pregnant patients, and the previous studies of no pregnant patients may have included some adults with undiagnosed diabetes, as suggested by the HbA1c levels.
- Kim *et al.*[13] investigated the influence of iron deficiency on HbA1c distribution among adults who were not known to have diabetes, over 7 years of the National Health and Nutrition Examination Survey. Of the 6666 female participants, 13.7% had iron deficiency and 30% of these had IDA. A much lower proportion of males (1.6%) had iron deficiency and 33% of these had IDA. When HbA1c values in women were adjusted for age and ethnicity, the difference between iron deficient and non-iron deficient became significant (5.33% vs. 5.27% [35 vs. 34 mmol/mol], $P = 0.002$). The authors found that iron deficiency in women of reproductive age was associated with a shift in HbA1c from<5.5% to 5.5-6.0% (<37 to 37-42 mmol/mol), although no association was found at higher levels, possibly owing to the lower number of participants in those groups. After adjusting for age and ethnicity, the authors concluded that HbA1c was higher in iron-deficient individuals and was likely to result in an upward shift of HbA1c distributions.

CONCLUSION

Our results showed that iron deficiency anemia was associated with higher proportions of HbA1c, which could cause problems in the diagnosis of uncontrolled DM in iron-deficient patients. This may have a practical application in diabetic individuals with IDA where HbA1c alone may give a false picture of poor glycemic control. The iron status must be considered during the interpretation of the HbA1c concentrations in DM. The iron replacement therapy is thus especially important in diabetic patients with iron deficiency, as it would also increase the reliability of the HbA1c determinations.

What does this mean on a wider scale?

Recent data has shown a surge in the incidence and prevalence of young onset obesity, many of whom are premenopausal females, at increased risk of iron deficiency. There has been a concomitant increase in HbA1c values in these participants considered at high risk of diabetes, owing to factors such as obesity. Data from England shows that the prevalence of pre-diabetes rose from 11.6% in 2003 to 35.3% in 2011 and in view of the rising prevalence of obesity; we would anticipate identifying a larger proportion of patients, with higher HbA1c values. If the estimated upward shift in HbA1cvalues seen with iron deficiency is combined with the apparent increased prevalence of pre-diabetes, it may result in a significant number of patients where the combination of the two will be sufficient to shift HbA1c values to move from a diagnosis of pre-diabetes to diabetes.

REFERENCES

1. John A. Iron deficiency and other hypoproliferative anemias. In: Longo D, Fauci A, Kasper D, Hauser S, Jameson J, Loscalzo J, editors. Principles of Internal Medicine by Harrisons. 17th ed. United States of America: McGraw-Hill; 2008. p. 628-35.
2. Brooks AP, Metcalfe J, Day JL, Edwards MS. Iron deficiency and glycosylated haemoglobin A. Lancet 1980;2:141.
3. Kim C, Bullard KM, Herman WH, Beckles GL. Association between iron deficiency and A1c levels among adults without diabetes in the National Health and Nutrition Examination Survey, 1999-2006. Diabetes Care 2010;33:780-5.
4. Sinha N, Mishra TK, Singh T, Gupta N. Effect of iron deficiency anemia on hemoglobin A1c levels. Ann Lab Med 2012;32:17-22.
5. Goode KM, John J, Rigby AS, Kilpatrick ES, Atkin SL, Bragadeesh T, Clark AL. Elevated glycated haemoglobin is a strong predictor of mortality in patients with left ventricular systolic dysfunction who are not receiving treatment for diabetes mellitus. Heart 2009;95(11):917-23.
6. Huisman TH, Dozy AM. Studies on the heterogeneity of hemoglobin. V. Binding of hemoglobin with oxidized glutathione. J Lab Clin Med 1962;60:302-19.
7. Sluiter WJ, van Essen LH, Reitsma WD, Doorenbos H. Glycosylated haemoglobin and iron deficiency. Lancet 1980;2:531-2.
8. El-Agouza I, Abu Shahla A, Sirdah M. The effect of iron deficiency anaemia on the levels of haemoglobin subtypes: Possible consequences for clinical diagnosis. Clin Lab Haematol 2002;24:285-9.
9. Coban E, Ozdogan M, Timuragaoglu A. Effect of iron deficiency anemia on the levels of hemoglobin A1c in nondiabetic patients. Acta Haematol 2004;112:126-8.
10. Gram-Hansen P, Eriksen J, Mourits-Andersen T, Olesen L. Glycosylated haemoglobin (HbA1c) in iron- and Vitamin B12 deficiency. J Intern Med 1990;227:133-6.
11. Rai KB, Pattabiraman TN. Glycosylated haemoglobin levels in iron deficiency anaemia. Indian J Med Res 1986;83:234-6.
12. Tarim O, Küçükerdogan A, Günay U, Eralp O, Ercan I. Effects of iron deficiency anemia on hemoglobin A1c in type 1 diabetes mellitus. Pediatr Int 1999;41:357-62.
13. McGill JB, Bell DS. Anemia and the role of erythropoietin in diabetes. J Diabetes Complications 2006;20(4):262-72.

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