Effect of Iron Deficiency Anemia on Glycosylated Hemoglobin Levels in Non Diabetic Indian Adults

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ABSTRACT
Glycosylated hemoglobin (HbA1c) is used as a gold standard for monitoring glycemic control and as a predictor of diabetic complications. Conditions that effect erythrocyte turnover affect HbA1c concentration. Although many forms of anemia are associated with lowering of HbA1c, iron deficiency anemia tends to increase HbA1c. However, reports on the effects of iron deficiency anemia on HbA1c are inconsistent. So we conducted a study to analyze the effects of iron deficiency anemia on HbA1c levels in non diabetics. 40 Non diabetic anemia patients and 40 age matched healthy subjects were enrolled in the study. HbA1c and absolute HbA1c levels were measured in patients and controls. The mean HbA1c and absolute HbA1c levels in anemic non diabetic patients were significantly lower than that in the control group. 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 / prediabetics.

KEYWORDS: Glycated hemoglobin, Hemoglobin A1c, Iron deficiency anemia.

INTRODUCTION
Glycosylated hemoglobin (HbA1c) is used as the gold standard for monitoring glycemic control and as a predictor of diabetic complications [1]. Throughout the circulatory life of the red blood cell glycohemoglobin is formed continuously by addition of glucose to the N–terminal of the hemoglobin beta–chain. This process is non-enzymatic and reflects the average exposure of hemoglobin to glucose over a period of nearly 2-3 months. Glycohaemoglobin has been defined as the fast fraction haemoglobin (HbA1a, A1c) which elute first during column chromatography with cation exchange resin. HbA1c levels are not affected by blood glucose levels alone. Any condition that shortens the life span of erythrocytes is likely to decrease HbA1c level. They are acute or chronic blood loss, sickle cell anemia, thalassaemias, hemolytic anemia, aplastic anemia, splenectomy, pregnancy, chronic kidney diseases, vitamin-B12 and folate deficiency anemia [2,3].

Falsely elevated HbA1c concentration can be encountered when there is increased circulating erythrocyte life span. They are alcoholism, hyperbilirubinemia and iron deficiency anemia [4,5]. According to WHO, iron deficiency is the commonest of all deficiency diseases worldwide [6]. Iron deficiency anemia is the most common in India [7]. Some studies show that HbA1c levels are increased in iron deficiency anemia and attempted to explain on the basis of both modifications to the structure of hemoglobin and levels of HbA1c in old and new red blood cells [8,9]. According to some studies, there were no differences between HbA1c levels of anemia patients and controls[10,11]. Study done by El –Agouza L et al showed that HbA1C level were higher in patients with iron deficiency anemia and decreased significantly upon treatment with iron [12]. Recently Sinha et al showed that HbA1c levels and absolute HbA1c levels increased with treatment of iron deficiency anemia [13]. The results of all these studies are conflicting; hence we were prompted to study the effects of iron deficiency anemia on HbA1c levels in Indian non-diabetic adults.

MATERIALS AND METHODS
Blood sample were obtained from 40 anemic patients (both men & women) aged between 20-50 yrs. Among them 36 were women & 4 men and 40 age & sex matched healthy subjects as control. The patients were from both Outpatient and Inpatient Departments of Navodaya Medical College and research centre, Raichur, Karnataka. The study was approved by Human ethical clearance committee. The anemia patients were selected based on their haemoglobin levels (Hb < 12gm/dl, MCV < 80 fl and MCH <26 pg/cell) and on their peripheral blood smears (mostly microcytic hypochromic). Patients with history of acute or chronic blood loss, hemolytic anemia, haemoglobinopathies, kidney diseases, diabetics, pregnancy, chronic alcohol ingestion and impaired glucose tolerance were excluded.
The levels of haemoglobin, mean corpuscular haemoglobin (MCH), hematocrit, mean corpuscular volume(MCV), mean corpuscular haemoglobin concentration (MCHC), platelet count, total leucocyte count (TLC) and differential leucocytes count(DLC) were measured by an automated counter. Peripheral blood smear examinations were performed to define the anemia type. On the basis of haemoglobin level patients were categorized as having mild, moderate or severe anemia. Mild anemia (male patients 12-12.9 gm/dl & female patients 11-11.9 gm/dl), Moderate anemia (male patients 9-11.9 gm/dl & female patients 8-10.9 gm/dl) and Severe anemia (male patients < 9gm/dl & female patients < 8 gm/dl). Those with predominantly microcytic indices (MCV <80 fl), hypochromic indices (MCH < 26 pg/cell) were considered to have iron deficiency anemia. HbA1C levels were measured by using the glycohaemoglobin ion exchange resin method kit (Erba diagnostics, Mannheim, Germany). Hemolysed preparation of the whole blood is mixed continuously for 5 minutes with weak bonding cation–exchange resin. During this HbA0 binds to the resin after the mixing period, a filter is used to separate the supernatant containing the glycohemoglobin from the resin. The glycohemoglobin percent is determined by measuring the absorbance at 450 nm of the glycohaemoglobin fraction and total haemoglobin fraction. The ratio of the two absorbances gives the percentage glycohemoglobin.

Absolute HbA1C levels were calculated from the measured HbA1C levels by using the formula

\[
\text{Absolute HbA1c (gm/dl)} = \frac{\text{HbA1C (\%)} \times \text{Hb (gm/dl)}}{100}
\]

**Statistical Analysis:** The data are presented as mean ± SD for continuous variables. A student t-test was applied for comparison of group means. Pearson’s coefficient of correlation was calculated to determine the correlation between two variables. P value < 0.05 was considered statistically significant.

**RESULTS**

Of 40 patients, 36(90%) were females and 4(10%) were males. The mean age of the patients was 35.6 ±10.97 (20-50 yrs). Severe anemia was seen in 16(40%) patients, Moderate anemia in 22(55%) and Mild anemia in 2(5%) patients. The mean Hb, HCT, MCV and MCH levels in patients and control were 8.74±1.98, 26.99±5.42, 66.40±10.66, 21.66±4.46 and 12.90±0.54, 36.57±1.92, 85.20±6.76, 30.01±2.40 respectively. These data provided evidence that Hb, HCT, MCV and MCH was lower in anemic patients than in healthy controls and the observed difference was statistically significant [p<0.01] as shown in Table No 1. The mean HbA1C levels in anemia patients were 5.91±0.47, while that in control was 6.54±0.39. The HbA1c levels were significantly lower in patients than controls .The mean absolute HbA1c levels in patients and control was 0.52±0.11 and 0.84±0.14. We observed a significant correlation only between hemoglobin and HbA1c in patients and controls [coefficient of correlation=0.859; p<0.01].

<table>
<thead>
<tr>
<th>parameters</th>
<th>IDA (n=40)</th>
<th>Controls(n=40)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>35.6±10.97</td>
<td>34.85±11.57</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>FBS(mg/dl)</td>
<td>86±9.42</td>
<td>84±10.6</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>PPBS(mg/dl)</td>
<td>102±6.4</td>
<td>98±6.82</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Hb(gm/dl)</td>
<td>8.74±1.98</td>
<td>12.90±0.54</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>HCT (%)</td>
<td>26.99±5.42</td>
<td>36.57±1.92</td>
<td>&lt; 0.01</td>
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<tr>
<td>MCV (fl)</td>
<td>66.40±10.66</td>
<td>85.20±6.76</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>MCH (pg/cell)</td>
<td>21.66±4.46</td>
<td>30.01±2.40</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>HbA1C (%)</td>
<td>5.91±0.47</td>
<td>6.54±0.39</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Absolute HbA1C (gm/dl)</td>
<td>0.52±0.11</td>
<td>0.84±0.14</td>
<td>&lt; 0.01</td>
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Data are presented as mean ± SD values
DISCUSSION

The result of the present study show that Hb concentrations are positively corrected with HbA1c concentration and that HbA1c concentration tended to be lower in the presence of iron deficiency anemia. In an early study from the US, the mean HbA1c concentration for 4 patients with IDA was 4.9% compared with a mean HbA1c concentration of 5.3% among 14 healthy adults[3]. The work done by brooks et al, revealed that the mean total HbA1c concentration was 9.9% among 35 non-diabetic patients and women with IDA levels higher than the normal mean of 7.9%[8].It was proposed that in the iron deficiency the quaternary structure of the hemoglobin molecule was altered and the glycation of the globin chain occurred more readily.

A subsequent report of 14 non-diabetics with IDA noted a mean concentration of HbA1c of 6.9% compared to a normal mean of 7.0%[10].Another study from India, that included 15 non-diabetic patients with IDA and 12 controls also failed to find a difference in mean concentration of HbA1c[14]. In a report from Denmark, there were no differences found in HbA1c concentration between 10 non–diabetic patients with IDA and to healthy controls [11]. They were of the opinion that in iron deficiency anemia the erythrocyte survival rate is normal.

Hence they thought that normal levels of glycated Hb are expected. In another study by E S Ford et al, they did not detect a significant difference in mean HbA1c concentration according to IDA status [15]. A previous analysis of NHANES data reported small differences in HbA1C concentration depending on iron deficiency status [16]. The exact mechanism of iron deficiency anemia affecting HbA1c level still remains unclear and the explanations provided in the studies are just assumptions. Due to large variations in the study results, we were prompted to conduct our own study to investigate the effects of iron deficiency anemia on HbA1c levels.

Among the 40 patients, 36 were females suggesting that iron deficiency anemia is more common in women. In our study the HbA1c levels were found to be significantly lower in patients with iron deficiency anemia than controls. The hemoglobin and HbA1c levels were positively correlated in anemia patients. Our observation of decreased HbA1c level in IDA patients were in accordance with Horton BF and Huisman TH [3], van Heyningen et al [10] . Hansen et al [11] . Rai et al [14] and Sinha et al [13]. Saudek et al considered measurements of HbA1C to be invalid in the presence of anemia [17]. The International Expert Committee warned to be aware of any condition that would increase or decrease the life span of RBCs [18].

Our analysis suggest that iron deficiency anemia is unlikely to be a major concern in diagnosing diabetes using concentration of HbA1c according to the American Diabetes Association (ADA) guideline. Our study population mainly belongs to low socioeconomic group. The cause of iron deficiency is mainly nutritional. Any other unknown variables like racial, geographical factors may have a bearing on our results. Further studies in large number are needed to confirm our findings.

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