Is HbA1c a reliable diagnostic marker for diabetes mellitus in patients with iron deficiency anaemia?: A cross sectional study

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ABSTRACT

Purpose of the study: The high prevalence of diabetes in the rural population of India required an early and accurate diagnostic tool. There were several techniques standardized and recommended by the American Diabetes Association. Some of the techniques were plasma glucose, oral glucose tolerance test, and HbA1c. The efficacy of HbA1c is questionable in individuals having a low haemoglobin content. This situation is further aggravated due to high anaemic individuals in rural population. This study was therefore designed for analyzing the efficacy of HbA1c in diagnosing diabetes in anaemic individuals.

Brief description of the experiment: The experiment involves the enrolment of individuals who are diabetic. Further, these individuals were divided into anaemic and non-anaemic individuals. Here, anaemic individuals were termed as cases and non-anaemic individuals were termed as controls. Samples were collected from both the cases and controls and analyzed for fasting plasma glucose, serum iron and direct TIBC, HbA1c levels, and oral glucose tolerance test.

Results: The results obtained in this study shows that the HbA1c levels were lower in the anaemic individuals as compared to the non-anaemic diabetic individuals. There was a positive correlation between Hb level and HbA1c level in an individual, thus indicating that an increase in the Hb level will cause an increase in the HbA1c level in diabetic individuals.

Conclusion: The study provides a baseline that HbA1c can’t be considered as a reliable tool for diagnosing diabetes in anaemic individuals. Further extensive studies are required to strengthen these findings with respect to a larger population.

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1. Introduction

Diabetes is a chronic disease characterized by an increased glucose concentration in the blood. This disease has a long history with its symptoms mentioned in the Ebers papyrus and ayurvedic texts.¹ The characterization and description of this disease has been credited to Jospeh von Mering and Oskar Minkowski in the year 1889.² There are several reasons behind the onset of this dreaded disease, but the most common among them is the reduced production of insulin or the inability of the body to effectively use the produced insulin. There are different types of diabetes based on the causative agent viz. type 1, type 2, and gestational diabetes. The type 1 diabetes is an inborn error due to defective pancreatic β cells. The gestational diabetes is the form of diabetes characterized by increased glucose levels in the blood due to hormonal variation during pregnancy. The most common form of diabetes is the type 2 diabetes.

Type 2 diabetes is caused primarily due to the lethargic lifestyle of an individual. Lack of physical activity, or over consumption of carbohydrates are some of the reasons for the occurrence of this dreaded disease.³ Technological advancement has reduced the physical activity and indirectly aggravated this disease to such level that now it has become a global burden. The World Health Organization has started various initiatives for countering the spread of this disease. The developing countries such as India are more prone for this disease due to lack of
education, poor health infrastructure, etc. As per the World Health Organization, around 69.2 million people in India had diabetes in the year 2015 and this population is projected to increase to 98 million by the year 2030.

The seriousness and near epidemic status of this disease has motivated the health care professionals to provide different diagnostic techniques for an early and accurate diagnosis. Till date, the most common techniques used for diagnosing diabetes are plasma glucose, oral glucose tolerance test, and HbA1c levels. The diabetes diagnostic criterion as mentioned by the American Diabetes Association is: HbA1c level more than 6.5%; oral glucose tolerance test (OGTT) value of more than 120 mg/dL in fasting sample and more than 140 mg/dL after 2 hours. HbA1c level is a preferred technique among these owing to the ability to provide the glucose concentration of up to 3 months. In 2009, an expert cabinet gave their recommendation for using HbA1c as a diagnostic marker for diabetes mellitus over conventional diagnostic tests, such as fasting plasma glucose and 2-hour plasma glucose, owing to more subtle results with less day-to-day discrepancies with a forewarning that any conditions that could affect the red blood cell turnover could lead to inaccurate assessment of HbA1c levels. This test is based on the property of a Hb component bound to glucose molecule. A minor haemoglobin component of human red blood cell hemolysate, HbA1c, is formed as a result of a non-enzymatic ketoamine reaction between glucose and alpha-amino groups of valine residues at the N-terminal of beta-chains. Initially, the bond formed between haemoglobin molecule and glucose is a weak bond, but with time this bond rearranges itself and forms a more stable complex. As the blood glucose rises, the unstable form rises far more than the stable form which rises linearly, this modification provides us with a time-average integrated glycemic history of plasma glucose over the period of previous 60-90 days. Since erythrocytes have an average life span of 120 days under normal physiological and biochemical conditions, thus HbA1c levels provide a definite measure of average glycemic control over time.

The requirement of haemoglobin for the measurement of diabetes makes it prone to inaccuracy in anaemic patients. Anaemia is a serious condition characterized by a reduced number of red blood cells in an individual. In India, anemia has been known to affect about half the women of reproductive age between 15 to 49 years. Iron deficiency has been attributed to be the most common cause of anaemia and is estimated to affect approximately 42% of all the cases of anaemia in children under 5 years of age and about 50% of all cases among pregnant and non-pregnant women, and worldwide. The symptoms of this disease primarily arise due to deranged oxygen carrying capacity of red blood cells. Iron deficiency anaemia is a prevalent form of malnutrition especially in developing countries such as India. India has a huge burden of anaemia with the highest prevalence in the world. Despite observing a decrease of 3.5% in all the cases of Iron deficiency anaemia across India from 1998 to 2016, there was an increase in the cases between the states of Delhi-National Capital Territory, Himachal Pradesh, Kerala and Punjab. Such deficiency is reported probably due to malnutrition and consumption of non-nutritious diet.

There are conflicting studies on the role of anaemia on HbA1c levels. Some studies have completely ruled out any effect of iron deficiency anaemia on HbA1c levels. On the contrary, some studies have indicated a negative impact of iron deficiency anaemia on HbA1c levels, and some positive thus contradicting the efficiency of this diagnostic tool. This study has therefore been designed for evaluating the efficacy of HbA1c in detecting diabetes in anaemic patients.

2. Material and Methods
2.1. Sample population
The present observational study was conducted in the Department of Biochemistry in collaboration with the Department of Medicine, Sri Guru Ram Das Institute of Medical Sciences and Research, Sri Amritsar. After taking clearance from the institutional ethical committee and informed consent from the patients, 200 diabetic patients in the age group of 30-65 were enrolled in the present study. The participants were selected by randomization technique and divided into two groups.

Group I: Newly diagnosed diabetic patients with anaemia

Group II: Newly diagnosed diabetic patients without anaemia

2.2. Sample collection
The blood of the selected patients was collected in three different vials viz. 2 ml blood in grey top, 4 ml in red top and 2 ml in lavender top. The grey top vacutainer contain sodium fluoride and oxalate for collecting plasma, red top was a simple vacutainer for collecting serum and the lavender top was an EDTA vacutainer for collecting whole blood. The samples were immediately processed for the following parameters: HbA1c test was run on Biorad D10 HPLC system using the dedicated reagent supplied by Biorad using HPLC method. The serum iron and direct TIBC was analyzed on Orthoclinical Diagnostics vitros 5600. Iron was analyzed using ferrozine no deproteinization method, and direct TIBC was studied using chromazurol B-vitros. Glucose was analyzed using dedicated reagent on Siemens Dimension RxL Max by hexokinase method. Oral glucose tolerance test was conducted as per the protocol given by Lopez-Lopez et al.
2.3. Inclusion criteria

1. Patients with newly diagnosed diabetes having fasting plasma glucose more than 120 mg/ dL and plasma glucose level after two hours (as per OGTT protocol) being more than 140 mg/ dL

2. Patient with Hb levels below 13 g/ dL in males, and 12 g/ dL in females.

3. Patients having iron concentration less than 37 μg/ dL

4. Patients having direct TIBC concentration more than 462 μg/ dL

2.4. Exclusion criteria

1. Patients diagnosed with β-thalassemia minor or β-thalassemia major. Other patients were diagnosed for β-thalassemia using mathematical formula on red cell parameters from an automatic counter.

2. Patients who are pregnant

3. Patients having recent blood transfusion

4. Patients having malignancies

5. Patients taking iron supplements

2.5. Statistical tests

The cases and controls enrolled in the study were selected using randomization technique in SPSS v17.0. The obtained data was processed for mean and standard deviation. The cases and controls were compared using independent sample t test. The sex of the participants was compared with each other using chi square analysis. The correlation between HbA1c and direct TIBC, and iron concentration, and the correlation between oral glucose tolerance test and direct TIBC, and iron concentration was analyzed by Pearson’s correlation using SPSS v17.0.

3. Results

The study was conducted for highlighting the efficacy of HbA1c in diagnosing diabetes. There are several advantages of this test and it has been recommended by the American Diabetes Association. As this test is based on the levels of Hb component therefore its accuracy in anaemic population can be relooked. The study was conducted on 200 patients that were included in the study after selecting through randomization and taking informed consent. Among the patients, 100 patients who were diabetic and anaemic were marked as cases, while 100 patients who were diabetic and without anaemia were marked as controls. The case group included 56 males and 44 females, while the control group included 60 males and 40 females. The groups were compared with each other using chi square (Chi square = 1.667). The difference among the group was found to be non-significant with $p \leq 0.197$ (Table 1).

The mean age of diabetic patients with anaemia was 45.72, while the non diabetic controls had a mean age of 50.76. The difference was found to be statistically significant with $p$ value 0.001. The $t$ value among the two groups was 3.302 (Table 2). The selection of controls and cases was done on the basis of their glucose levels, since diabetes was the most important criteria. The plasma glucose levels of the controls and cases were measured from their fasting sample (12 h fasting). The cases in the study had mean fasting plasma glucose level 157.54 mg/ dL and the mean fasting plasma glucose levels in the controls was found to be 158.36 mg/ dL. The difference between the two groups was non-significant ($p = 0.922$), indicating that both the groups have almost diabetic status (Table 3).

The diabetic patients were then further selected on the basis of their level of haemoglobin. The anaemic patients (Hb less than 13 g/ dL in males and less than 12 g/ dL in females) were selected as cases. The non anaemic patient group were selected as controls. The mean Hb level was found to be 8.97 g/ dL in cases, while the level was 13.15 g/ dL in controls. The difference among the cases and controls was significant ($p \leq 0.001$) and the $t$ value among the groups was 12.485 (Table 4).

The confirmation of anaemia among the cases and controls was done by testing the concentration of iron and direct total iron binding capacity (dTIBC). The iron concentration more than 37 μg/ dL and the dTIBC concentration less than 462 μg/ dL was considered as normal. The iron concentration in the controls was observed to be 67.26 μg/ dL, while the cases had mean iron concentration 32.22 μg/dL. The difference among the cases and controls was significant ($p \leq 0.001$) and the $t$ value was 8.670 (Table 5). The dTIBC concentration of the cases was 475.15 μg/ dL and the dTIBC concentration in the controls was 297.02 μg/ dL. The difference among the cases and controls was significant ($p \leq 0.001$) and the $t$ value was 18.313 (Table 6).

The whole blood sample was collected from the cases and controls and was used for analyzing the HbA1c concentration. It was observed that the diabetic patients without anaemia (controls) had mean HbA1c level 7.55%, while the mean HbA1c level of diabetic patients with anaemia (cases) was 6.26%. The HbA1c level in cases was significantly lower than the controls as confirmed through $t$ test with $t$ value 5.143 and $p$ value 0.001 (Table 7). The results obtained through HbA1c were confirmed using the gold standard diagnostic tool for diagnosing diabetes i.e. oral glucose tolerance test (OGTT). There are several methods of conducting this diagnosis, but for the ease of the participants 2 sample OGTT was performed. The fasting plasma glucose was considered as the baseline for the tool. A fresh blood sample was collected after 2 hours of giving oral glucose. The plasma glucose levels among the cases and controls were compared in the reading obtained after 2 hours. The mean plasma glucose level after 2 hours in the cases was 198.08 mg/ dL, while the mean plasma glucose
Table 1: Sex distribution in controls and cases

<table>
<thead>
<tr>
<th>Group</th>
<th>Sex</th>
<th>Number</th>
<th>Chi square value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetes with anaemia</td>
<td>Male</td>
<td>28</td>
<td>0.164</td>
<td>0.685</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>22</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes with anaemia</td>
<td>Male</td>
<td>30</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>20</td>
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Table 2: Mean age group of controls and cases

<table>
<thead>
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<th>Group</th>
<th>Mean Age</th>
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<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetes with anaemia</td>
<td>45.72 ± 7.91</td>
<td>3.302</td>
<td>0.001</td>
</tr>
<tr>
<td>Diabetes without anaemia</td>
<td>50.76 ± 7.34</td>
<td>0.098</td>
<td>0.922</td>
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</tbody>
</table>

Table 3: Mean fasting plasma glucose levels of controls and cases

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean Fasting Plasma Glucose (mg/dL)</th>
<th>t value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetes with anaemia</td>
<td>157.69 ± 35.63</td>
<td>0.098</td>
<td>0.922</td>
</tr>
<tr>
<td>Diabetes without anaemia</td>
<td>158.36 ± 47.31</td>
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<td></td>
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</table>

Table 4: Mean Hb level of cases and controls

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean Hb (g/dL)</th>
<th>t value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetes with anaemia</td>
<td>8.97 ± 1.51</td>
<td>12.485</td>
<td>0.001</td>
</tr>
<tr>
<td>Diabetes without anaemia</td>
<td>13.15 ± 1.82</td>
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<td></td>
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</table>

Table 5: Mean serum iron concentration in the cases and controls

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean Iron (μg/dL)</th>
<th>t value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetes with anaemia</td>
<td>32.22 ± 10.78</td>
<td>8.670</td>
<td>0.001</td>
</tr>
<tr>
<td>Diabetes without anaemia</td>
<td>67.26 ± 26.46</td>
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<td></td>
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</table>

Table 6: Mean serum dTIBC concentration in the cases and controls

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean dTIBC (μg/dL)</th>
<th>t value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetes with anaemia</td>
<td>475.15 ± 22.01</td>
<td>-18.313</td>
<td>0.001</td>
</tr>
<tr>
<td>Diabetes without anaemia</td>
<td>297.02 ± 67.32</td>
<td></td>
<td></td>
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</table>

level after 2 hours in the controls was 186.64 mg/dL. The difference between the two groups was non-significant with p ≤ 0.269 (Table 8). This value indicates that the mean plasma glucose levels after 2 hour is presumably similar among the cases and controls.

The HbA1c level in the cases and controls was correlated with different parameters viz. age, fasting plasma glucose, glucose reading after 2 hours, Hb, iron, and dTIBC concentration (Table 9). The HbA1c level was observed to have a positive correlation with age in both cases and controls. This suggests that the HbA1c levels increase with an increase in age. The HbA1c levels showed a significantly positive correlation with fasting plasma glucose and glucose reading after 2 hours. Interestingly, r values are much higher in the controls in comparison to the cases. This pattern clearly indicates that HbA1c is much reliable in controls in comparison to cases. The Hb levels showed a positive correlation with HbA1c in cases, while a negative correlation was observed in controls. The iron and dTIBC concentration showed a negative correlation with HbA1c. This indicates that the concentration of iron and dTIBC would decrease with an increase in HbA1c levels.

4. Discussion

Diabetes is a serious pathological condition characterized by an increased concentration of plasma glucose both in fasting as well as random sample. There are several reasons for the diabetes that includes genetic history, inborn error, medication use, or lifestyle. The disease has become a global health burden, affecting a large
population of India as well.

The widespread nature and the associated symptoms of the disease requires an early diagnosis. There are different diagnostic tools that have been referred by American Diabetes Association for this purpose. Some of the tools are fasting plasma glucose, random plasma glucose, oral glucose tolerance test, and HbA1c. Among these tools, HbA1c has been widely explored due to its ability to interpret glucose levels of up to 3 months. This diagnostic tool relies on the component of haemoglobin for precise diagnosis. The requirement of haemoglobin for diagnosis makes it a vulnerable diagnostic tool in population having low haemoglobin level. Such individuals are referred to as anaemic individuals. Though there are rather large number of reasons that may affect the haemoglobin levels in an individual, the most common reason in a developing country is iron associated anaemia.

The current study is therefore focused on evaluating the efficacy of HbA1c in diagnosing diabetes in anaemic individuals.

The mean age of individuals enrolled in the study is around 50. The individuals in this age group are more prone to diabetes due to reduction in enzyme production and reduced physical activity. The mean fasting plasma glucose concentration in both controls and cases was higher than the normal upper limit of 120 mg/dL. The difference between the two groups was observed to be non-significant, thus indicating that both the groups were similar and no difference exists in their glucose levels. These results provided an insight that the cases and controls included in the study could provide a better highlight to the actual role of HbA1c in diagnosis of diabetes.

The mean Hb level was 8.97 g/ dL in cases thus indicating that the cases were anaemic, while the controls were non-anaemic with mean Hb level 13.15 g/ dL. Study conducted by Pasricha et al. highlighted the fact that the majority of anaemic cases were observed in developing countries, especially in preschool children and women. There are several side effects of iron deficiency anaemia such as reduced cognitive, physical, and psychomotor development. A retrospective observational study conducted by Alvarez-Uria et al. in rural India showed that anaemia is quite common in underdeveloped regions of India. There are several reasons associated with low Hb level in the Indian population. Some of these are lower life expectancy, lack of education and living standards, inequality in growth and development, and poor dietary quality.

Low iron intake in the diet is the major reason behind the low Hb content in the population. The result of the current study highlights low mean iron concentration (32.22 μg/dL) in controls with respect to the cases (67.26 μg/ dL). This disparity in the iron concentration is due to the fact that a majority of patients enrolled in the study were from rural background. Earlier research studies have pointed towards the role of iron deficiency in causing anaemia in rural population of India. This disparity is owed to the poor food quality and lower living standards. Lack of economic and social development is also responsible for aggravating such symptoms. Iron concentration in an individual is directly responsible for altering the direct total iron binding capacity (dTIBC) of an individual. There are plethora of studies that have shown high dTIBC content in individuals with low serum iron concentration. The current study has shown similar results and the patients with anaemia had high dTIBC content (475.15 μg/ dL) as compared to the controls (297.02 μg/ dL).

There are several methods for diagnosing diabetes such as plasma glucose concentration and HbA1c levels. These diagnostic assays have been recommended by the American Diabetes Association (ADA) along with their specific cut-offs. Interestingly, ADA has not given any specific guidelines for anaemic individuals. Since HbA1c is directly

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### Table 7: Mean HbA1c concentration in the cases and controls

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean HbA1c (%)</th>
<th>t value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetes with anaemia</td>
<td>6.26 ± 1.25</td>
<td>5.143</td>
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<tr>
<td>Diabetes without anaemia</td>
<td>7.55 ± 1.27</td>
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### Table 8: Mean plasma glucose concentration reading after 2 hours in cases and controls

<table>
<thead>
<tr>
<th>Group</th>
<th>Reading After 2 Hours (mg/dL)</th>
<th>t value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetes with anaemia</td>
<td>198.08 ± 57.63</td>
<td>-1.113</td>
<td>0.269</td>
</tr>
<tr>
<td>Diabetes without anaemia</td>
<td>186.64 ± 44.33</td>
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### Table 9: Correlation between HbA1c and other parameters in cases and controls

<table>
<thead>
<tr>
<th>Correlation</th>
<th>r value</th>
<th>FPG</th>
<th>Glucose 2 hr</th>
<th>Hb</th>
<th>Iron</th>
<th>dTIBC</th>
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<tr>
<td></td>
<td>0.468</td>
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<td>0.666</td>
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<td>Controls</td>
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<td></td>
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<td></td>
<td>0.391</td>
<td>0.815</td>
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<td>0.001</td>
<td>0.850</td>
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</tr>
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</table>
related to the Hb content of an individual, therefore the anaemic individuals would have slightly lower HbA1c content as compared to the normal population. Several studies have researched on this topic and have concluded that HbA1c should not be used as a diagnostic tool in the anaemic individuals. 29–31 The current study has revealed that the selected population were confirmed diabetic as observed through high plasma glucose concentration after 2 hours in the oral glucose tolerance test. The controls had the plasma glucose concentration 198.08 mg/ dL, while the cases had the plasma glucose concentration 186.64 mg/ dL. The difference among the controls and cases was non-significant, thus suggesting that both the groups did not have significant difference among each other. The HbA1c level was observed to be much lower in the cases (6.26%) as compared to the controls (7.55%). These results corroborate with the earlier research.

The HbA1c levels were correlated with the different parameters in both cases and controls. The HbA1c level was positively correlated with age. Studies have suggested that with an increase in the age of an individual, the level of enzymes reduces drastically. This negatively impacts the different biochemical parameters in an individual. The low amount of insulin hormone may be responsible for the incidence of diabetes. This in turn results in an increased level of HbA1c. 32 The concentration of fasting plasma concentration and the reading obtained after 2 hours showed a positive correlation with the HbA1c levels of both controls and cases. These findings have been backed up by the previous studies that have highlighted an increase in HbA1c levels with an increase in the plasma glucose levels. 33–35 Similarly, HbA1c showed a positive correlation with Hb levels in the cases and controls. These findings suggest that the patients having low Hb will have lower HbA1c levels. Many research articles have confirmed such findings. 36,37 The iron concentration and direct total iron binding capacity shows a negative correlation with the HbA1c levels in both cases and controls. This suggests that a decrease in the iron and dTIBC levels will result in a decrease in the HbA1c levels in the individual.

5. Conclusion
The study focused on the efficacy of HbA1c as a diagnostic tool in anaemic population. Different research articles have focused on this topic, but no research was available in the population included in this study. The presence of a large number of anaemic individuals in this area encouraged this study. It was observed that the anaemic diabetic individuals had almost same glucose levels as the non-anaemic diabetic population. But the HbA1c levels varied in both the cases and controls. Such findings suggest that HbA1c can’t be considered a reliable marker for diagnosing diabetes in anaemic individuals. There were certain limitations in this study which would be addressed in the future studies. The research was done on a very limited number of individuals in a limited time duration. The other causative agents of diabetes were excluded from this study and will be included in the future studies. This study can act as a pave way for future research on standardizing the best method for an early diagnosis of diabetes in all the individuals in a population, irrespective of their haemoglobin concentration.

6. Source of funding
None.

6.1. Conflict of interest
None.

References


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