

# A Study of the Effect of Iron Deficiency Anemia on the Levels of Glycosylated Hemoglobin in Diabetics

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## ABSTRACT

**Introduction:** Assessing the effect of IDA on HbA1c in diabetic patients is of paramount significance from different perspectives. Misinterpretation of the concentration of HbA1c in diabetic patients could affect in monitoring the diabetic patients. Study aimed to demonstrate the effect of IDA on HbA1c in diabetic patients.

**Material and methods:** This hospital based study was conducted in the Department of Pathology AIMS, Bathinda over a period of one and half years on 50 cases & 50 controls.

**Results:** There was a significant difference between the values of hemoglobin between the cases and controls as the p value was 0.001 which is highly significant. The mean MCV in the cases was 70.44 fl while in the control group the mean MCV value was 86.41 fl which is statistically significant. Mean value of MCH among the cases was 21.50 pg and in the controls, the mean MCH was 28.53 pg which was statistically significant. The data shows that MCV, MCH and MCHC were lower in cases and RDW was raised in cases which was statistically significant. We found a negative correlation of HbA1c with MCV and MCHC.

**Conclusion:** HbA1c is shown to be a predictor of complications of diabetes as well as mortality and morbidity. Iron deficiency anemia is the most prevalent nutritional anemia in India. Our study showed that IDA spuriously elevates HbA1c levels in the diabetic patients independent of plasma glucose concentration. Hence the iron status must be considered during the interpretation/diagnosis of Diabetes Mellitus.

**Keywords:** Diabetes, HbA1C, Anemia

## INTRODUCTION

Diabetes mellitus (DM) is a complex metabolic disorder caused by variable interactions between hereditary and environmental factors. DM is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels.<sup>1</sup> Diabetes can be classified into the four general categories; Type 1 diabetes, type 2 diabetes, gestational diabetes mellitus (GDM) and the specific types of diabetes due to other causes like monogenic diabetes syndromes (such as neonatal diabetes and maturity-onset diabetes of the young [MODY]), diseases of the exocrine pancreas (such as cystic fibrosis), and drug or chemical-induced diabetes.<sup>2</sup> Prevalence of diabetes for all age groups worldwide was estimated to be 2.8% in 2000 and 4.4% in 2030. According

to latest World Health Organisation (WHO) report India has 31.7 million diabetic patients and the number is expected to increase to a staggering 79.4 million by 2030.<sup>3</sup>

It is associated with considerable mortality and morbidity from a variety of complications which tend to worsen with time. Its main features are abnormal insulin secretion, high level of blood glucose and a variety of complications such as retinopathy, nephropathy, neuropathy and arteriosclerosis<sup>3</sup>, so early diagnosis of DM is important for the prevention of complications.<sup>4</sup>

Blood and urine glucose and urine ketone testing provide useful information for day-to-day management of diabetes. Measurements of glycosylated proteins, primarily hemoglobin and serum proteins, have added a new dimension to the assessment of glycemia. With a single measurement, each of these tests can quantify average glycemia over weeks and months, thereby complementing day-to-day testing.<sup>5</sup>

Glycosylated hemoglobin is widely used as a gold standard for monitoring glycemic control over the previous 3 months. It also serves as the predictor of complications of diabetes.<sup>6</sup>

HbA1c level of  $\geq 6.5\%$  is sufficiently sensitive and specific to identify individuals who are at risk for developing retinopathy and who should be diagnosed as diabetic.<sup>7</sup>

HbA1c may be affected by a variety of genetic, physiological, hematological and illness-related factors. Falsely elevated HbA1c concentrations encountered when there is increased circulating erythrocyte life span (decreased red cell clearance) or impaired reticulocyte production. Alcoholism, iron deficiency, renal failure, and hyperbilirubinaemia and also in pre-menopausal women HbA1c level increase.<sup>8,9,10</sup> On the other hand, falsely decreased HbA1c level is seen in conditions with a reduced erythrocyte life span (increased hemoglobin turnover) or where a large number of reticulocytes are produced. These conditions include acute

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or chronic blood loss, sickle cell anemia, thalassaemias, glucose-6-phosphate dehydrogenase (G6PDH) deficiency, hemolytic anemia, aplastic anemia, splenectomy and paroxysmal nocturnal hemoglobinuria (PNH).<sup>9,11</sup>

Anemia is defined as a decrease in the concentration of circulating red blood cell or in the hemoglobin concentration and a concomitant impaired capacity to transport oxygen.<sup>12</sup> Anemia may be classified according to their physiology or their morphology. The morphological classification is based on red blood cell indices, while the physiological classification is determined based on symptoms and bone marrow response.<sup>13</sup> In blood cell morphology, iron-deficiency anemia (IDA) will manifest as microcytic, hypochromic (small, pale) RBC.

It is the most common nutritional disorder worldwide and accounts for half of the cases of anemia. IDA can result from inadequate iron intake, decreased iron absorption, increased iron demand and increased iron loss.<sup>14</sup> Patients with diabetes may be more vulnerable to the effects of anemia. The definitive test for diagnosis of IDA is bone marrow aspiration however the procedure is invasive, difficult and expensive. Thus, serum ferritin is found to be the best test for distinguishing those with IDA from those who are not iron deficient.

According to WHO criteria, IDA was defined as Hgb < 120 g/l for non-pregnant women (above 15 years of age) and < 130 g/l for men (above 15 years of age), mean cell volume (MCV) < 80 fl, mean cell hemoglobin (MCH) < 27 pg, mean corpuscular hemoglobin concentration (MCHC) < 32% and serum ferritin level < 15 ng/ml was defined as IDA.<sup>15</sup>

One of the well-studied pathological ill-effects of IDA in the biological system is the glycation of proteins. The nonenzymatic glycation of proteins has pronounced effects on the structure and the function of proteins. The pathological consequences of these alterations depend on the nature of the proteins which are involved, as well as on their functions and concentrations in specific tissue localizations.<sup>16</sup>

Even though, HbA1c is the most accurate, precise diagnostic tool for diabetic patients there are different factors like IDA which can give spuriously result of HbA1c.

Assessing the effect of IDA on HbA1c in diabetic patients has a relevant significance from different perspectives. Misinterpretation of the concentration of HbA1c in diabetic patients could affect in monitoring the diabetic patients. Therefore, this result will help the patient and also clinician or other health care provider to consider IDA before making any diagnostic or therapeutic decision. This study is aimed to demonstrate the effect of IDA on HbA1c in diabetic patients. Current research aimed to study the effect of iron deficiency anemia on glycosylated hemoglobin in diabetics and to measure the levels of HbA1c in diabetic patients with iron deficiency anemia and their comparison with controls i.e., diabetic patients without iron deficiency anemia and to correlate the values of HbA1c with the RBC indices

## MATERIAL AND METHODS

This hospital based study was conducted in the Department

of Pathology AIMS, Bathinda. The samples were run through the Biorads haemoglobin testing system for HbA1c by HPLC method over a period of one and half years.

### Sample size calculation:

The following formula was used to calculate the sample size for matched case control study:

$$N1 = (Za + Zb)^2 p q (r + 1)$$

$$r = \frac{p1 - p2}{p2} \text{ and } N2 = rN1$$

Where:

N1 = Number of cases

N2 = Number of controls

Za = Standard normal deviate for two-tailed test based on alpha level (relates to the confidence interval level) (here 95%)

Zb = Standard normal deviate for one-tailed test based on beta level (relates to the power level) (here 80%)

r = ratio of controls to cases (here, 1)

p1 = proportion of cases with exposure and q1 = 1 - p1

p2 = proportion of controls with exposure and q2 = 1 - p2

$$p = \frac{p1 + r p2}{r + 1} \text{ and } q = 1 - p$$

$$r + 1$$

The minimum sample size to be taken came out to be 33.

For the study to be more significant, 50 cases & 50 controls were taken which were well above the estimated sample size.

## STATISTICAL ANALYSIS

T test for calculation of p value and Pearson coefficient test to find the correlation was applied. P value < 0.05 was taken significant.

## RESULTS

The present study was conducted in the Department of Pathology at Adesh Institute of Medical Sciences and Research, Bathinda for a period of one and a half year. Our case control study included 50 cases and 50 controls. The controls were age and sex matched.

In our study, the mean age of the cases and controls was 52.70 ± 7.96 and 52.74 ± 7.93 years respectively. There was no significant difference between the ages of cases and control groups as the p value was 0.980 (> 0.05). Out of the 50 diabetic patients with iron deficiency anemia, i.e., the cases, this study included 27 (54%) males and 23 (46%) females. Sex matched controls, i.e., diabetics without iron deficiency anemia were taken. There was no significant difference between the two groups as the p value was 0.6714.

The mean hemoglobin value in cases was found to be 10.54 g/dl with the standard deviation of 0.91 while in controls the mean hemoglobin was 13.70 g/dl and standard deviation was 0.84. There was a significant difference between the values of hemoglobin between the cases and controls as the p value was 0.001 which is highly significant.

The mean MCV in the cases was 70.44 fl and standard deviation was 2.96 while in the control group the mean MCV value was 86.41 fl and standard deviation was 1.92. This difference was statistically significant as the p value was < 0.05.

The mean value of MCH among the cases was 21.50 pg with

a standard deviation of 1.59 and in the controls, the mean MCH was 28.53 pg with a standard deviation of 1.02 which was statistically significant.

The mean MCHC in cases was 24.38 g/dl with a standard deviation of 1.21 and the mean MCHC in controls was 32.20 g/dl with a standard deviation of 0.94 which was statistically significant.

The data shows that MCV, MCH and MCHC were lower in cases and RDW was raised in cases. The difference observed was statistically significant. The correlation of HbA1C with the red cell indices and Hb are being shown in Table 1 and Figure 1

The mean HbA1c in cases was  $7.91 \pm 1.20$  and in controls was  $7.11 \pm 0.89$ , which was significant statistically as the p value was 0.0003 ( $>0.05$ ).

In our study, we found a negative correlation of HbA1c with MCV and MCHC which was not significant statistically and a positive correlation of HbA1c with MCH and Hb which was also not significant statistically.

## DISCUSSION

Diabetes is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction and failure of different organs, especially the eyes, kidneys, nerves, heart and blood vessels. Long-term complications of diabetes include retinopathy with potential loss of vision; nephropathy leading to renal failure; peripheral neuropathy with risk of foot ulcers, amputations and Charcot joints; and autonomic neuropathy causing gastrointestinal, genitourinary, and cardiovascular symptoms and sexual dysfunction. Patients with diabetes have an increased incidence of atherosclerotic, cardiovascular, peripheral arterial, and cerebrovascular disease.<sup>17</sup>

Hemoglobin A1c (HbA1c) is a glycosylated hemoglobin that can be used as an indicator of a patient's glycemic status over the previous 3 months. According to the American Diabetes Association (ADA) Guidelines 2007, the value of HbA1c should be kept below 7% in all diabetics. Values greater than 7% indicate an increased chance of progression to diabetic complications especially microvascular ones. Glycosylated haemoglobin does not include HbA1c alone. It includes other haemoglobins as well and together, these constitute the HbA1 fraction of adult haemoglobin HbA. Among the various glycosylated haemoglobins, HbA1c is the predominant fraction. It appears that HbA1c is affected by blood glucose levels alone.<sup>18</sup>

However, certain studies have proven that HbA1c levels are altered in haemolytic anaemias, haemoglobinopathies and nutritional anaemias such as iron deficiency anaemia.

It is the most common form of anemia observed in Indian settings and India being the diabetes capital of the world, it was imperative to find out the relationship that exists between iron deficiency anemia and HbA1c which is the most common investigation carried out routinely in diabetic patients. So it becomes prudent to show the effect of iron

deficiency anaemia on HbA1c levels before any decision or guidelines are made based on HbA1c levels.<sup>17</sup>

The earliest study to investigate the effects of iron deficiency anemia on HbA1c levels was conducted by Brooks et al. who assessed HbA1 levels in 35 non-diabetic patients having iron deficiency anemia both before and after treatment with iron and observed that HbA1c levels were significantly higher in iron deficiency anemia patients and decreased after treatment with iron. The mechanisms leading to increased glycosylated HbA1 levels were not clear. It was proposed that, in iron deficiency, the quaternary structure of the hemoglobin molecule was altered, and that glycosylation of the globin chain occurred more readily in the relative absence of iron.<sup>19</sup>

Sluiter et al.<sup>20</sup> proposed that the formation of glycosylated hemoglobin is an irreversible process and hence, the concentration of HbA1 in 1 erythrocyte will increase linearly with the cell's age. 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 HbA1 levels.

In Turkey, in 1999 Tarim et al revealed that iron deficiency anemia is associated with higher concentrations of HbA1c among type 1 DM patients and after iron treatment the concentration decreased.<sup>21</sup>

Coban et al. in 2004 conducted a study on 50 patients with iron deficiency anemia and 50 controls and a reduction in the levels of HbA1c was observed after iron treatment in iron deficient patients and correction of iron deficiency anemia was suggested before making a diagnostic or therapeutic decision based on HbA1c.<sup>22</sup>

In Mexico, a study conducted by Francisco *et al* to analyze the advantage and disadvantage of using HbA1c as a diagnostic method for diabetes, in 2010 concluded that it is insufficient to recommend it as the method of choice for diagnosis and the major arguments was lack of a universal threshold for the diagnosis of diabetes, the cost of the test, abnormal hemoglobin, anemia and iron deficiency and renal failure.<sup>23</sup>

Study conducted by Ford et al., in 2011 showed that Hb concentration appears to be positively correlated with HbA1c value. HbA1c appears higher in the patients with iron deficiency and similar to values in patients with normal iron and normal Hb.<sup>24</sup>

Hardikar et al., in 2012 investigated the diagnostic performance of HbA1c against a standard OGTT, and looked at the haematological, nutritional and other factors influencing HbA1c concentration and concluded that there was higher prevalence of iron deficiency in the participants classified by HbA1c as prediabetic or diabetic. The authors suggested that diagnosing diabetes and prediabetes in iron-deficient populations may lead to spuriously high HbA1c concentrations and potential for increased mis-diagnosis of diabetes.<sup>25</sup>

Christy et al, in 2013 showed a positive correlation between iron deficiency anemia and increased HbA1c levels, especially in the controlled diabetic women and individuals

having FPG between 100-126 mg/dl.<sup>26</sup>

Shanthi et al, in 2013, concluded that HbA1c is not affected by blood sugar levels alone, and there are various confounding factors when HbA1c is measured, especially that of iron deficiency.<sup>17</sup>

In 2015, Silva et al carried out a control-case study in Brazil to investigate the effect of iron deficiency anemia on HbA1c levels in non-diabetic individuals. HbA1c results were higher in patients with moderate and severe anemia unlike mild anemia and thus concluded that IDA affects HbA1c results and the effect is dependent on anemia degree.<sup>27</sup>

Shekhar et al conducted a study to determine whether the HbA1c levels were increased among the anemic patients without diabetes. The finding suggested that the mean HbA1c in IDA was higher  $9.5 \pm 1.8\%$  compared to the control subjects  $5.5\% \pm 0.8$ . Thus, IDA could cause problems in the diagnosis of uncontrolled diabetes mellitus, so iron status must be considered during the interpretation of the HbA1c concentrations in DM.<sup>28</sup>

Study conducted by Bhardwaj et al concluded that Iron deficiency anaemia has a straight forward correlation with HbA1c levels and the relationship is inverse between them. This signifies that with increasing severity of iron deficiency in anaemic subjects, HbA1c levels increase correspondingly. Moreover, with correction of iron deficiency in the anaemic subjects, the HbA1c levels decline to near normal values. Other than blood glucose, many other factors effect calculated HbA1c value which should be kept in mind before doing a therapeutic treatment modification. Iron deficiency anaemia being extremely common in Indian settings should always be ruled out when high HbA1c levels are detected and should be corrected on priority to achieve true levels of HbA1c.<sup>29</sup>

Rajagopal et al, concluded that IDA spuriously elevates HbA1c levels independent of blood glucose concentration in controlled-diabetics. HbA1c increases significantly as severity of anemia worsens.<sup>6</sup>

In our study there was a significant difference between the values of hemoglobin between the cases and controls as the p value was 0.001 which was highly significant. The data showed that MCV, MCH and MCHC were lower in cases and RDW was raised in cases. The difference observed was statistically significant.

Among the hematological parameters (Hb, MCV, MCH), our study showed statistically significant mean difference between cases and the control group.

Association between red cell indices and HbA1c were determined in the diabetic patients with IDA group and the result was not statistically significant. Similarly an Iranian study in 2014<sup>26</sup> showed no significant correlation between MCV and HbA1c but a borderline significant association was found between MCH and HbA1c in IDA diabetic patient (P=0.05).

Although association of elevated HbA1c with severity of iron deficiency anemia remains unexplained, its borderline association with red cell indices proves the role of erythrocyte morphology and lifespan in elevating HbA1c.

The mean HbA1c in cases was  $7.91 \pm 1.20$  and in controls

was  $7.11 \pm 0.89$ , which was significant statistically as the p value was 0.0003 ( $>0.05$ ). Our study was in concordance with the studies mentioned above.

According to Sinha *et al*, the reason for lower HbA1c was due to the severity of anemia in the study participants. This microcytic hypochromic cell have a short life span compared to the normal cell in the circulation so, glycosylated hemoglobin will decrease in iron deficient patients.<sup>30</sup>

Correspondingly, Hardikar et al<sup>25</sup> study reported that there is significant association between MCV, MCH, MCHC and HbA1c in IDA pre diabetic and diabetic patients.

The present study reported that there was no significant correlation between Hb and HbA1c in IDA group as  $r=0.17$  & p value was 0.214. Likewise, the other studies revealed there is no significant correlation between these parameters. On the contrary, Sinha et al study<sup>30</sup> observed significant correlation of Hgb and HbA1c in IDA patients this finding contradicts with the present study.

Even though, some of the studies contradict with our finding, our study depicts diabetic patients with IDA have significantly higher HbA1c compared to non-IDA diabetic patients. Therefore monitoring these patients using HbA1c could be misleading, hence physician and health care providers should take this into account before making any therapeutic decision. Though in our study, a correlation with severity of anemia and HbA1c levels was not taken into account, there is scope for further studies on this aspect.

## CONCLUSION

Insulin is the key hormone in substrate homeostasis and insulin deficiency results in wide variety of metabolic defects affecting carbohydrate, lipid and protein metabolism. Insulin deficiency and hyperglycemia affects the tissues as well, resulting in complications of diabetes. The HbA1c test has been used in diabetics, as HbA1c is formed over a period of two to three months and reflects the glycaemic status of a patient over the past two to three months. HbA1c is shown to be a predictor of complications of diabetes as well as mortality and morbidity.

Iron deficiency anemia is the most prevalent nutritional anemia in India. Our study showed that IDA spuriously elevates HbA1c levels in the diabetic patients independent of plasma glucose concentration. Therefore, problems could occur during the diagnosis and management of Diabetes Mellitus in the patients suffering from iron deficiency anemia. Hence the iron status must be considered during the interpretation/diagnosis of Diabetes Mellitus based on the concentration of HbA1c.

Hence, it is very important to exclude iron deficiency anemia and to correct it before making any diagnostic or therapeutic decision in a patient of Diabetes Mellitus.

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