

Study of Glycated hemoglobin level in Non-Diabetic Iron Deficiency Anemia

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ABSTRACT

Background: The traditional role of HbA1c analysis has been for assessing glycaemic control in patients with diabetes. The results of seminal studies demonstrated that early, intensive glycaemic control could significantly reduce the risk of a range of diabetes-related complications, and permitted the establishment of precise HbA1c target values for treatment goals.

Methods: Study involves total 140 participants and among them 70 are non-diabetic, anaemic patients and 70 age-matched healthy subjects. Haematologic investigations were done and the fasting and postprandial glucose and HbA1c levels were measured in all the subjects.

Result: The mean HbA1c ($9.1 \pm 1.7\%$) level in the patients with IDA was higher than that in the control group ($5.5\% \pm 0.8$) ($p < 0.05$). There were no differences in the levels of fasting and postprandial glucose between the IDA and the control groups ($p > 0.05$).

Conclusion: HbA1c is not affected by the blood sugar levels alone, and there are various confounding factors when HbA1c is measured, especially that of iron deficiency, which is the commonest of the deficiency diseases worldwide. It is hence important to rule out IDA before making a therapeutic decision, based on the HbA1c levels.

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Introduction

Diabetes mellitus is a metabolic disease which is caused by absolute or relative insulin deficiency. About 10% of the Indian population suffers from this disease. Various factors play a role in the aetiopathogenesis and in the glycaemic control among the type 2 diabetic patients.^[1] The term HbA1c refers to glycated haemoglobin. It develops when haemoglobin, a protein within red blood cells that carries oxygen throughout your body, joins with glucose in the blood, becoming 'glycated'. By measuring glycated haemoglobin (HbA1c), clinicians are able to get an overall picture of what our average blood sugar levels have been over a period of weeks/months.

HbA1c is likely to be affected by iron deficiency and iron deficiency anemia with a spurious increase in HbA1c values, the researchers wrote. This may lead to confusion when diagnosing diabetes using HbA1c. This review clearly identifies the need for more evidence, especially in identifying the types and degrees of anemia likely to have significant impact on the reliability of HbA1c.^[2] According to the American Diabetes Association (ADA) guidelines, the value of HbA1c should be kept below 7% in all the diabetics.^[3] The values which are greater than 7% indicate an increased chance of progression to the diabetic complications, especially the microvascular ones. When plasma glucose is consistently elevated, the nonenzymatic glycation of haemoglobin increases; this alteration reflects the glycaemic history over the previous 2–3 months, since erythrocytes have an average lifespan of 120 days.^[4]

Evidence has accumulated, which supports the hypothesis that the glycation reaction, apart from the traditional chronic hyperglycaemia, can be modulated by the iron status of the patient. If the degree of glycation of other proteins in anaemic patients was similar to that of the glycated haemoglobin, it would have important clinical implications. Thus, the main aim of the present study was to determine whether the HbA1c levels were increased among the anaemic patients without diabetes. If so, the iron deficiency has to be corrected before any diagnostic or therapeutic decision was made based on the HbA1c level.^[5]

Materials and Methods

This study was conducted at B.J. Medical College, Ahmedabad, Gujarat. from 2012-2013 in department of pathology.

Blood samples (3ml) were obtained from 70 anaemic patients of the mean age, 45±5 years, among which 35 were males and 35 were females and 70 age-matched healthy subjects. The anaemic patients were recruited from the Medicine Outpatients Department of our institute.

All collected sample were analysed for complete blood count (CBC), Diabetic profile (FBS, PP2BS) and HbA1c. CBC was done in 3 part Nihon kohden Celtac Alpha hematology analyser.

The anaemic patients were selected, based on their haemoglobin levels (Hb < 11 g/dl), and on their peripheral blood smears (mostly microcytic hypochromic), which suggested iron deficiency anaemia and on their haematologic investigations (Like MCV, MCH, MCHC) and serum fasting and postprandial glucose levels.

The HbA1c levels were determined by turbidimetric immunoinhibition. The peripheral blood smears were examined in all the patients. Estimation of blood sugar was done by GOD-POD method in semi automated biochemistry analyser along with Biorad Quality control sera.

Exclusion Criteria: Patients having Diabetes mellitus and any type of hemoglobinopathies were excluded from our study.

Result

All the parameters which were tested in both the groups have been reported in [Table 1]. The fasting and the postprandial blood glucose levels confirmed the non-diabetic status. The peripheral blood smears showed a hypochromic microcytic picture. The HbA1c levels were significantly increased among the IDA patients as compared to those in the controls. [Table 2] The mean HbA1c ($9.1 \pm 1.7\%$) level in the patients with IDA was higher than that in the control group ($5.5 \pm 0.8\%$) ($p < 0.05$) [Table 3]. There were no differences in the levels of fasting and postprandial glucose between the IDA and the control groups ($p > 0.05$).

Table 1: Age wise distribution of participant.

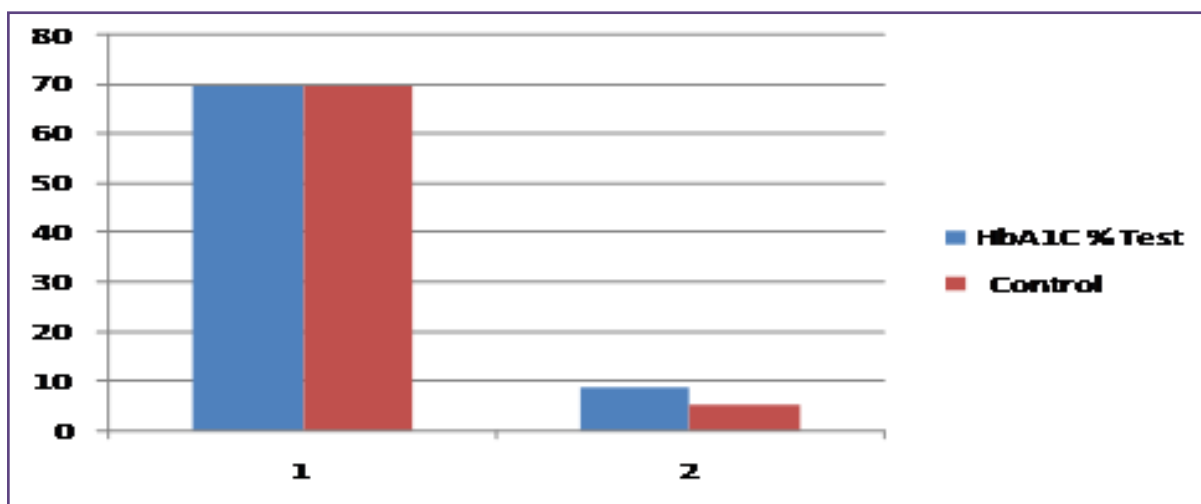
Group	Number(n)	Age(year) Mean \pm SD
Test (IDA)	70	45 \pm 5
Control	70	43 \pm 4

Table 2: Laboratory data of study group.

Parameter	Test Group (IDA) (N=70)	Control Group (N=70)
Hemoglobin gm/dl	9.9 \pm 1.5	12.1 \pm 1.3
PCV %	30.1 \pm 4.3	41.4 \pm 2.7
MCV fl	79.1 \pm 4.9	83.2 \pm 4.6
MCH pg	24.5 \pm 2.2	32.9 \pm 1.7
Fasting blood Glucose mg/dl	96.5 \pm 9.4	93.5 \pm 9.8
Postprandial blood sugar Mg/dl	114.8 \pm 6.1	116.5 \pm 5.8
HbA1C %	9.1 \pm 1.7	5.5 \pm 0.8

Table 3: Comparisons of various parameter between Test(n=70) and control(n=70) Group.

Parameter	Group	Number(n)	Result(Mean)	P-value
Hb (gm/dl)	Test	70	9.9	<0.05
	Control	70	12.1	
Fasting blood sugar (mg/dl)	Test	70	96.5	>0.05
	Control	70	93.5	
Postprandial blood sugar (mg/dl)	Test	70	114.8	>0.05
	Control	70	116.5	
HbA1C %	Test	70	9.1	<0.05
	Control	70	5.5	

**Graph 1: Showing comparison of HbA1c (%) concentration between test and control group.**

Discussion

The results of the present study show that Hb concentrations are positively correlated with HbA1c concentrations, that HbA1c concentrations tended to be higher in the presence of iron deficiency, and that participants with IDA had similar HbA1c concentrations as participants with normal concentrations of Hb and a normal iron status. The positive association between HbA1c concentrations and Hb may have offset the small inverse association between HbA1c concentrations and iron status. We observed erythrocyte size and Hb to have a negative correlation with HbA1c, compatible with the hypothesis that iron deficiency increases Hb glycation, perhaps related to an association between insulin resistance and higher glucose levels.

Our results shows that higher concentration of HbA1c is found in iron deficiency patients as compared to control group. Similarly, Brooks et al., showed higher HbA1c concentrations in iron-deficient nondiabetic adults, which decreased to normal after iron replacement.^[6] Hansen et al. showed normal HbA1c concentrations in

iron deficiency, which dropped to subnormal levels after iron supplementation.^[7]

In contrast with several previous studies, our results suggest that IDA has little population effect on concentrations of HbA1c or on diabetes prevalence. In an early study from the US, the mean HbA1c concentration for four patients with IDA was 4.9% compared with a mean HbA1c concentration of 5.3% among 14 healthy adults.^[8] In a subsequent study from the UK, the mean total HbA1c concentration was 9.9% among 35 non-diabetic men and women with IDA, levels higher than the normal mean of 7.9%.^[9] A case report from Australia noted that IDA was associated with a rise in the concentration of HbA1c.^[10] A subsequent report of 14 non-diabetic patients with IDA from the UK noted a mean concentration of HbA1c of 6.9% compared with a normal mean of 7.0%.^[11] The authors attributed the differences in findings with previous studies to differences in methodology for measuring HbA1c. Another study from India that included 15 non-diabetic patients with IDA and 12 controls also failed to find a difference in mean concentrations of HbA1c.^[12]

The exact reason for that is not found till date but it was suggested that in iron deficiency, the quaternary structure of the hemoglobin molecule was altered, and that glycation of the globin chain occurred more readily in the relative absence of iron. Sluiter et al. tried to provide an explanation for the above findings. They proposed that the formation of glycated hemoglobin is an irreversible process and hence, the concentration of HbA1c in 1 erythrocyte will increase linearly with the cell's age.^[13]

Several limitations should be noted. First, the results from the present study may not be fully generalizable to individuals with severe degrees of IDA or non-IDA. Second, although the sample size in the present study was large, the number of participants with IDA was nevertheless limited. Thus, additional studies with larger numbers of participants with IDA would be helpful in examining the impact of IDA on measurements of HbA1c.

Saudek et al considered measurements of HbA1c to be invalid in the presence of anemia.^[14] Although these warnings are sound, our analyses suggest that anemia is unlikely to be a major concern in diagnosing diabetes using concentrations of HbA1c in the US. The difference in concentrations of HbA1c between extremes of concentrations of Hb is in the order of an absolute 0.2%. This would suggest that primarily people with anemia who are close to the diagnostic threshold may require retesting or the use of another diagnostic method. Therefore, consideration should be given to performing glucose testing in patients who have low Hb concentrations and an HbA1c concentration just below the diagnostic threshold for diabetes and prediabetes or who have high Hb concentrations and an HbA1c concentration just above the diagnostic threshold for diabetes and prediabetes.^[14]

According to some investigators, the increase in the glycated haemoglobin levels in non-diabetic anaemic patients has been mainly attributed to the decrease in the haemoglobin levels in these patients But studies which have investigated the glycation levels of other proteins have not been carried out.

This study has got a significant relevance because iron deficiency anemia is very highly prevalent in a tropical country like India. IDA, being a common variable, influences the HbA1c levels when they are estimated by the most commonly employed methods like immunoturbidometry and so, the IDA must be corrected before making any diagnostic or therapeutic decision based on the HbA1c levels. HbA1c is commonly used to

assess the long-term blood glucose control in the patients with diabetes mellitus, because the HbA1c value has been shown to predict the risk for the development of many of the chronic complications in diabetes.

Conclusion

From our study we would like to conclude that iron deficiency was associated with higher proportions of HbA1c, which could cause problems in the diagnosis of uncontrolled diabetes mellitus in iron-deficient patients.

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Competing Interests

None Declared

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