Effect of Iron deficiency on glycation ofhemoglobin in non diabetics

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Abstract

BACKGROUND

Hemoglobin A1C (HbA1c) has been adopted by physicians as a surrogated for monitoring glycemic control. The use of HbA1c for the diagnosis of diabetes is now widely advocated despite caveats to its use. HbA1c concentrations are influenced by several factors including red blood cells (RBC) turnover. There are many types of anemia that affect glycated hemoglobin (HbA1c) values, but iron deficiency anemia (IDA) one of the most common. Further research needs to focus on the mechanistic reasons why HbA1c is higher in IDA. The study attempts to discern clinical differences in HbA1c levels in patients with IDA, compared to patients without IDA & showing the direction of such differences.

METHODS

50 non diabetic anemic patients and 50 age matched healthy subjects were involved in this study. Hematological investigations were done and fasting & post prandial (PP) glucose and HbA1c level were measured in all the subjects.

RESULTS

The mean HbA1c [9.10±1.08%] in the patients with IDA was higher than that in the control non anemic group [5.21±0.45%] [P<0.001] There was no differences in the levels of fasting and PP glucose between the IDA & the control groups. [P>0.05]

CONCLUSIONS

HbA1c is likely to be affected by IDA with a spurious increase in HbA1c values, conversely non IDA may lead to confusion when diagnosing diabetes using HbA1c. This article clearly identifies especially the types of anemia likely to have significantly impact on the reliability HbA1c.

KEY WORDS: HbA1c, hemoglobin, IDA

Introduction

Chronic hyperglycemia is the hallmark of diabetes mellitus [DM] [1].HbA1c is used as gold standard for monitoring glycemic controls and as a predictor of diabetic complications [2]. HbA1c is a hemoglobin variant that is formed when glucose bind covalently to the β chain of HbA [3]. The number "1C" represents the orders of Hb detection on chromatography. The normal life span of the erythrocyte is 120 days [4]. The glycation of Hb occurs over the entire 120 days life span of erythrocyte.

HbA1c measures the binding of circulating glucose to Hb protein within RBC.Higher levels of glucose in the blood, contribute to more binding and consequently higher levels of glycated Hb. The major form of glycated Hb is HbA1c [5].

The international Expert committee and American Diabetes Association (ADA) has recommended using HbA1c to diagnosis DM [6].The traditional role of HbA1c is the most prominent biomarker for assessing and management of glycemic status of people with diabetes [7].Over the years many health care providers have come to view the HbA1c values as a "magic number" that comprises all the information required for blood glucose concentration to prevent complications in people with diabetes, the concept "the lower the better" was considered a tempting approach.HbA1c was considered as " an abnormal Hb in diabetes " by Samuel Rohbar.

Human Hb in its structural and functional features in the most extensively studied protein. The major component of its HbA_0 ($\alpha_2\beta_2$) comprises over 90% the total protein with the other two minor components being Hb genes. HbA1c is the most abundant minor components arises from spontaneous, non enzymatic, post translational modification reaction of HbA_0. HbA1c on the contrary, in unique set of conditions represents the formation of glycosylated form of human Hb [8]. Glycated Hb is produced by a ketoamine reaction between glucose and NH_2 terminal of both β chain of the hemoglobin molecule [9].HbA1c is majorly affected by the blood glucose level alone. However, certain studies have proven that, the HbA1c levels are altered by various other existing factors along with or without diabetes, especially that of IDA [10].Which is major public health problem in developing countries like India. According to World Health Organization (WHO) IDA is one of the most common types of anemia found worldwide [11].The WHO estimated that 2.1 billion people globally have IDA, which is approximately 30% of the world population at that time. The prevalence of IDA is higher in low & high income countries, Adolescents, children and women are the most susceptible. In these countries, diabetes is also a rapidly increasing issue [12].In India, it is estimated that 52% of non-pregnant women of reproductive age are anemic [13].

The RBC turnover can be decreases too and this will increases glycation rate of the Hb [14].The pathogenesis of anemia varies depending on the cause and influence on HbA1c is also variable. The value of HbA1c influenced by certain physiological and pathological condition depends on 3 main factors, the Hb present in reticulocytes when there are released from the bone marrow, the glycation rate is a function as the erythrocyte age (since Hb glycation rate of Hb is a function of glucose concentration to which Hb is exposed) and the mean age of erythrocytes in the circulation [15].

Anemia and its effect on HbA1c has not been studied extensively. Although IDA is a commonly encountered disease in clinical practice. In the current study we performed a cross sectional analysis to investigate the association between IDA and HbA1c levels in adults.The study attempted to discern clinical difference in HbA1c levels in patients with anemia compared to non anemic populations as well as to quantify and show the direction that asglucose is covalently bound to HbA1c glycosylated Hb, HbA1c levels in non diabetic iron deficiency anemic population is significantly higher than in non diabetic non anemic population.

Methods

Blood sample were obtained from 50 anemic patients of the mean age of patients (37.44±5.11years) among which 12 were males and 38 were females and 50 age matched healthy subjects. The anemic patients were recruited from the medicine Outpatients Department of our institute, medical college & Hospital Warudi. District Jalana, Maharashtra, India from September 2015 to February 2016, constituted material for this study. The anemic patients their peripheral blood smears (mostly microcytic hypochromic) which suggested IDA, their hematological investigation like PCV, MCV, MCH & MCHC.The blood samples were drawn after on overnight fast. A sysmax automated hematology analyzer was used for the whole blood count Hb, haematocrit (Hct), mean carpuscular volume (MCV) and mean carpuscular hemoglobin (MCH) and fasting & PP glucose levels by GOD-POD method.The HbA1c levels were determined by SDA1c Care System based on the Immunoassay and reflectometry technology and the peripheral blood smear were examined in all the patients.The anemic

patients were selected, based on their Hb levels (Hb<11.0 g/dl) and the peripheral blood smears (mostly microcytic hypochromic) which suggested IDA, their hematological investigation like PCV, MCV, MCH & MCHC. serum F & PP glucose levels. The study assumed a consistent HbA1c assay method at the study center over the study period.Patients with history of acute chronic blood loss, hemolytic anemia, haemoglobinopathies, kidney diseases, diabetes, pregnancy, chronic alcohol ingestion and impair glucose tolerance, gestational diabetes, history of endocrinopathy with affect for glycemic control. Hb concentration <6.0 g/dl or >11g/dl.

Results

All the results are presented as mean ± SD. The statistical significance of the difference between the groups was evaluated by the students 't' test. The correlation was assessed by the partial correlation analysis. A P value of 0.05 was considered statistically significant. All the parameters which were tested in both the groups have been reported in (Table No.1). Of 50 patients 38 (76%) were females & 12(24%) were males. The mean age of the patient (37.44±5.11years) The F and PP blood glucose level confirmed the **Table No. 1: Comparision of various parameters between patients and control**

Parameters	IDA (n=50) mean±SD	Controls (n=50) mean±SD
Age	37.44±5.11	37.54±4.77
FBSL(mg/dl)	83.38±6.57	81.80±5.64
PPBSL(mg/dl)	114.88±8.33	116.67±8.57
Hb (gm/dl)	8.07±1.07	12.27±1.00
HCT (%)	30.40±3.36	38.53±5.05
MCV fl	71.23±5.02	82.43±2.37
MCH (pg/cell)	21.66±2.54	31.28±1.41
HbA1c (%)	9.10±1.08	5.21±0.45

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. The mean HbA1c levels in anemic patients were significantly increased among the IDA patients as compared to those in the controls. The mean HbA1c (9.10±1.08%) level in patients with IDA was higher than in the control group. (5.21±0.45%) (P<0.001) There were no difference in the levels of fasting and PP glucose between the IDA and the control groups (P>0.05).

Discussion

We observed a significant increase in the HbA1c concentration in the IDA patients, when compared to that of healthy controls. We found a significant reduction in the activity of Hb among the IDA patients. (Table no. 1 P< 0.001)According to some investigators, the increase in the glycated Hb in non-diabetic anemic patients has been mainly attributed to the decrease in the Hb levels in these patients. El-Agouza et al 2002 [16].This study has got a significant relevance because IDA is very highly prevalent in a tropical country like India [17].The current study was also supported by Brooks et al [18].IDA was associated with higher concentration of HbA1c.Higher HbA1c concentration in iron deficiency non diabetic adults which decreased to normal after iron replacement Hansen et al [19].The mechanism that lead to increase glycosylated HbA1c level in IDA was not clear, the explanation provided in the studies are just assumptions. There is a dearth of literature on HbA1c levels in the anemic population. There are a few documented studies on this matter the finding of which are at best, in consistent.But it was postulated that the quaternary structure of the Hb molecule was altered in IDA. This allowed the β globin chain to be more readily glycosylated in the face of low Hb or iron levels.Sluiter et al postulated that formation of glycosylated Hb is irreversible with Hb and the HbA1c in a RBC increase with the cell age [20]. IDAis chronic disorder, RBC production will decrease leading to anemia and a longer life span of red blood cells in the circulation. This would lead to an increase HbA1c [21].Dr. Chittaranjan S. Yajnik of king Edward Memorial Hospital Research Center in Pune, India wrote that," A1c concentration depends not only on prevailing glycemia but also the life span of

erythrocyte [22].Iron deficiency increases erythrocyte survival and therefore disproportionate elevated HbA1c concentrations at a given glycemic level.

Among the 10 genetic loci associated with HbA1c level in Caucasians, three are in loci associated with fasting glucose level, where as seven are in genes related to erythrocyte physiology. A decision to use HbA1c is must also consider the probability that the patient has Hb variants, that may interfere with the analysis.IDA has been known to cause a rise in HbA1c of up to 2% and this has been shown to be reversed with iron supplementation. Some studies show that, HbA1c levels are increase in IDA and attempted to explain on the basis of both modification to the structure of Hb and levels of HbA1c in old and new red blood cells [23].Hence extending target HbA1c values to certain anemia may be erroneous due to potential differences in glycation rates and some physiological challenges. (markedly decreased red cell survival)

Conclusion

From this study it can be concluded that, glycation of Hb is definitely affected in anemia particularly IDA, even in non diabetic patients. It is found that HbA1c level in IDA increased almost in linear proportion and increase in HbA1c level is statistically significant.

It is also seen that, as soon as IDA is treated, HbA1c levels tend to reach to normal levels, changes in abnormal levels of HbA1c to normal levels individually suggested that anemia has been treated sufficiently.

The present case lies at the intersection of clinical chemistry, medicine and hematology. This study has contributed to a better understanding of its appropriate use and interpretation.

It so the IDA has to be corrected before any diagnostic or therapeutic decision was made based on the HbA1c level. Also the clinician should or must considered the overall patient profile and a number of local variations and disorders as anemia, so HbA1c cannot be used as sole and independent test to diagnosis diabetes mellitus. But the clinician must consider the overall profile of the patient before taking into consideration the abnormal HbA1c value**.**

Conflicts of interest

None declared.

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