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Original article:

Study of iron and vitamin b12 deficiency anaemia at glycosylated hemoglobin level: a case control study

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ABSTRACT:

Introduction: Diabetes Mellitus (DM) refers to a group of common metabolic disorder. It is a chronic illness, complex requiring continuous medical care with multifactorial risk reduction strategies beyond glycemic control. HbA_{1c} which is primarily used as a test of glycemic control & as a diagnostic test. One condition that effect erythrocyte turnover is anemia. Vitamin B12 deficiency and iron inhibit erythropoiesis and increase the mean survival duration of erythrocyte, leading to increase HbA_{1c}.

Methods: The study was conducted in tertiary care hospital, JLN Medical college Ajmer. The study involved 180 patients out of which 60 were of Iron deficiency anemia and remaining 60 had Vitamin B 12 deficiency anemia. Permission was taken from the ethical committee before starting the study. A well informed written consent was taken prior to the study and the results of the same were compared with 60 non anemic, non diabetic controls using ANOVA and T test analysis.

Results: Out of 60 participants in each group, 36.67% were male and 63.335% were female in iron deficiency group, 53.33% were male and 46.67% were female in vitamin B12 deficiency group, 60% were male and 40% were female in control group. In Iron deficiency anemia 20 out of 60 participants (33.33%) had Hba_{1c} in the range of impaired glucose homeostasis(5.6 -6.5)[2] and in Vitamin B12 deficiency anemia 24 out of 60 participants (40%) had Hba_{1c} in the range of impaired glucose homeostasis. Interestingly 4 patients from iron deficiency anemia had Hba_{1c} in diabetic range (more then 6.5). Our study reported significant negative trend when Hemoglobin was compared with HbA_{1c} in patients with iron deficiency anemia, that is, patients with lower Hemoglobin had higher HbA_{1c}.

Conclusion: Anemia and Diabetes are a common problem in Indian scenario and use of HbA_{1c} to diagnose prediabetes and diabetes in iron-deficient populations may lead to a spuriously exaggerated prevalence of prediabetes.

INTRODUCTION

Diabetes Mellitus is group of common metabolic disorders that share the phenotype of hyperglycemia with an increasing incidence worldwide[1]. Diabetes Mellitus is a leading cause of morbidity and mortality. The prevalence of Diabetes Mellitus is rising rapidly in India presumably because of increase obesity, decrease activity levels and industrialization. HbA1c which was earlier primary used to test glycemic control has now been added as a diagnostic tool, therefore it is more important today than earlier[2]. HbA1c levels are affected by not only blood sugar levels alone but also any condition that shortens the life span of erythrocytes is likely to decrease the HbA1c level. Iron and Vitamin B12 deficiency, renal failure, chronic blood loss and bone marrow suppression in alcoholism inhibit erythropoiesis and increase the mean survival of erythrocytes, leading to increase in HbA1c level. However hemolytic anemia, and increased hemolysis from splenomegaly,

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chronic liver disease increase reticuloyte and decrease the mean age of erythrocyte, which can decrease HbA_{1c} level.[3] Since Vitamin B12 & Iron deficiency affects erythropoiesis and itself cause of anemia it is likely that HbA1c level will be affected in Vitamin B12 deficiency anemia.

METHODS:

The study was conducted in tertiary care hospital JLN Medical College Ajmer and patients admitted in the medicine wards and outdoor. It included total 180 participants out of them 60 patients having iron deficiency anemia, 60 patients with Vitamin B12 deficiency anemia and rest 60 patients belong to the control group. All the patients were screened for Fasting Blood Sugar, Hematological parameters, Renal Function and those with Fasting Plasma Glucose < 100 mg/dl were classified into groups vitamin B12 deficiency anemia, iron deficiency anemia, and controls without anemia. HbA1c was measured in each group and as compared using ANOVA and T test analysis.

The data was recorded on a predesigned proforma and analyzed. The data was presented as Mean±SD for continuous variables. A Student's t-test was applied for comparison of group means. Pearson's coefficient of correlation was calculated to determine the correlation between 2 variables. P value of less than 0.05 was considered statistically significant.

RESULTS:

During the period of study 180 patients were screened and out of which anemia, 60 patients of vitamin B12 deficiency anemia, 60 patients were Iron deficiency anemia and 60 were control. The mean age distribution of participants in Iron deficiency anemia was 36.27+7.282, Vitamin B 12 deficiency group was 37.23+6.218 and control group was 38.10+7.112.

Out of 60 participants in each group, 22 participants (36.67%) were male and 38 participants (63.335%) were female in iron deficiency group, 32 (53.33%) were male and 28 (46.67%) were female in vitamin B12 deficiency group, 36 (60%) were male and 24 (40%) were female in control group. The Mean Hb in Group 1(Iron deficiency Anemia) was 7.82+1.28, In Group 2 (Vitamin B 12 deficiency anemia) was 7.45+1.46, and in Group 3 (Control) 13.21+0.80. In Iron deficiency anemia 28 patients had moderate anemia and 32 patients had severe anemia. In Vitamin B 12 Deficiency group 18 patients belonged to moderate anemia and 42 patients belonged to severe anemia group.

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Table 1: Mean S. Iron, Ferritin, Vitamin B12 levels in study group

	Group	N	Mean	SD	ANOVA		'p' Value*		
					F	'p' Value	I-III	II-III	I-II
Serum Iron µgm/dl	I	60	19.73	6.01	355.87	<0.001	<0.001	<0.001	<0.001
	II	60	110.33	19.97					
	III	60	81.24	10.33					
Serum ferritin ng/ml	I	60	7.28	1.90	117.509	<0.001	<0.001	<0.001	<0.001
	II	60	72.03	26.54					
	III	60	53.47	11.99					
Vitamin B 12 pg/m	I	60	307.27	38.65	313.29	<0.001	0.760	<0.001	<0.001
	II	60	78.07	13.33					
	III	60	299.91	56.59					

*Post hoc Tukey HSD

The Mean S. Iron levels in iron deficiency group was 19.73+6.01 µgm/dl, 110.33+19.97 µgm/dl in Vitamin B 12 deficiency group and 81.24+10.33 µgm/dl in control group. The mean serum ferritin levels in Iron deficiency group was 7.28+1.90 ng/ml, 72.03+26.54 ng/ml in Vitamin B12 deficiency group and 53.47+11.99 ng/ml in control group. The mean serum vitamin B 12 levels in iron deficiency group was 307.27+38.65 pg/ml, 78.07+13.33 pg/ml in vitamin B 12 deficiency group and 299.91+56.59 pg/ml in control group. The mean of fasting blood sugars in iron deficiency anemia was 84.33+5.93 mg/dl, vitamin B12 deficiency anemia group was 88.83 +6.69 mg/dl and control group was 84.07+6.99 mg/dl.

The mean HbA1c levels in iron deficiency anemia group was 5.61+0.69, in Vitamin B12 deficiency was 5.50+0.39 and in control group was 5+0.32. The mean HBa1c value of both iron deficiency anemia and Vitamin B 12 deficiency anemia were more compared to control group and this difference was found to be statistically significant.

In Iron deficiency anemia 20 out of 60 participants (33.33%) had Hba_{1c} in the range of impaired glucose homeostasis(5.6 -6.5)[2] and in Vitamin B12 deficiency anemia 24 out of 60 participants (40%) had Hba_{1c} in the range of impaired glucose homeostasis. Interestingly 4 patients from iron deficiency anemia had Hba1c in diabetic range (more then 6.5)2. Our study reported significant negative trend when Hemoglobin was compared with HbA1c in patients with iron deficiency anemia, that is, patients with lower Hemoglobin had higher HbA1c.

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Table 2: Mean HbA_{1c} levels of different groups

Group	N	Mean HbA1c (%)	SD	ANOVA		'p' Value*		
				F	'p' Value	I-III	II-III	I-II
I	60	5.61	0.69	13.00	<0.001	<0.001	0.001	0.665
II	60	5.50	0.39					
III	60	5.00	0.32					

*Post hoc Tukey HSD

DISCUSSION:

Hemoglobin A_{1c} which has been included as a diagnostic tool for the diagnosis of Diabetes in American Diabetes Association criteria 2011 will now play an important role in diagnosis and treatment of Diabetes mellitus.[4] However several factors other than blood sugar can alter the HbA_{1c} levels are blood loss[5] hemolytic anemia[6], blood urea, pregnancy[7], Iron deficiency anemia and vitamin B 12 deficiency anemia. So we conducted a study to analyze the effects of iron deficiency anemia and Vitamin B12 deficiency anemia on HbA_{1c} levels.

We conducted a study on 120 patients of IDA and Vitamin B12 deficiency anemia along with 60 control to study the effect of anemia on HbA_{1c}. Of 60 patients of IDA, 38 (63.33%) were female and 22 (36.67%) were male. Among 60 patients of Vitamin B12 deficiency anemia, 28(46.67%) were female and 32(53.33%) were male. In the control group, 24 participants (40%) were female and 36 (60%) were male. This sample size was adequately matched with the studies conducted earlier. The mean age of the patients of IDA was 36.27 yr and that of Vitamin B12 deficiency anemia was 37.23 yr. The mean age of the control participants was 38.10 yr. In Iron deficiency anemia 28 patients had moderate anemia and 32 patients had severe anemia. In Vitamin B 12 Deficiency group 18 patients belonged to moderate anemia and 42 patients belonged to severe anemia group. We found no cases of mild anemia.

Our results suggested that IDA was associated with higher concentrations of HbA_{1c}. It was found that the mean values of HbA_{1c} in Iron deficiency group were significantly higher (5.61%) compared to that of control group where it was 5.00%. This difference was found to be significant ($P < 0.001$.) on statistical testing. Our study also revealed that the difference in mean HbA_{1c} levels of severely anemic patients (6.04%) and moderately anemic patients (5.11%) was statistically significant ($p<0.001$).

Although prolongation of erythrocyte survival in patients with IDA is known to elevate the HbA_{1c} level, some studies have shown normal or even shortened lifespans of erythrocytes in patients with IDA. In study by Sinha et al (2012)[8] the HbA_{1c} levels were found to be significantly lower in patients with iron deficiency anemia than in the controls. Moreover, the HbA_{1c} levels increased after treatment, which had not been reported in any previous study. The absolute HbA_{1c} levels, which were significantly lower in patients than in

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controls, were also found to increase significantly after iron treatment. The hemoglobin and HbA_{1c} levels were positively correlated in anemia patients before treatment, but no positive correlation was apparent at 2 months after treatment. The authors did not discuss why their data conflicts with other studies but Emma English et al.(2015)[9] in the review article postulated that it may be due to the severity of anemia as the participants in this study had low mean hemoglobin levels(62g/dl); the duration of anemia was not given. But in our study patients with severe anemia had significantly higher HbA_{1c} levels compared to patients with moderate anemia as well as controls.

The present study observed that 10 cases of Iron Deficiency Anemia and 12 cases of Vitamin B 12 deficiency anemia cases fell into impaired glucose homeostasis category and 2 patients of Iron Deficiency Anemia fell into diabetic category according to American Diabetes Association 2011 criteria even while their fasting sugars were normal. This agrees with Hardikar et al (2012)[10] who investigated a population where 34% were anemic, 37% were iron deficient, 40% were vitamin B_{12} deficient, and 22% were folate deficient. Only 20% of those diagnosed as prediabetic and diabetic by HbA_{1c} had prediabetes and diabetes according to the OGTT, and among the anemic, this figure was only 7%. In this young, apparently healthy, and non-diabetic group, 2-h glucose concentrations explained only 25.6% of the variance in HbA_{1c} concentrations and hematological parameters contributed up to 13.1%, leaving over half of the variance unexplained. They concluded that use of HbA_{1c} to diagnose prediabetes and diabetes in iron-deficient populations may lead to a spuriously exaggerated prevalence of prediabetes. This study has got a significant relevance because Iron and Vitamin B12 deficiency anemia is very highly prevalent in a tropical country like India. Iron and Vitamin B12 deficiency anemia being common variables, influences the HbA_{1c} levels when they are estimated by the most commonly employed methods like immunoturbidometry and so, they must be corrected before making any diagnostic or therapeutic decision based on the HbA_{1c} levels. HbA_{1c} is commonly used to assess the long-term blood glucose control in the patients with diabetes mellitus, because the HbA_{1c} value has been shown to predict the risk for the development of many of the chronic complications in diabetes.

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