

Original article:

Effect of Total Hemoglobin level on HbA1c value in Type 2 Diabetes Mellitus patients

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Abstract:

Background & objectives: Glycated hemoglobin level (HbA1c) is widely used for the diagnosis, prognosis and monitoring of therapy in diabetes mellitus patients. The hemoglobin molecule is a part of the non- enzymatic glycation reaction, so it is assumed that the total hemoglobin concentration may have an effect on the HbA1c value along with blood glucose concentrations. Hence this study was done to evaluate the effect of total hemoglobin level on HbA1c value in type 2 diabetes mellitus patients and to find out whether reduced total hemoglobin level is reflected on the HbA1c level giving an erroneous impression on the glycaemic status of the patient. **Methods:** 100 diagnosed Type 2 Diabetes Mellitus patients were evaluated for their Fasting Blood Sugar (FBS), Total Hemoglobin level and HbA1c level and assessed for any effect of reduced total hemoglobin level on the HbA1c value. **Results:** It was observed that HbA1c value correlated significantly only with FBS level and total Hemoglobin level was not found to have any effect on the HbA1c value in Type 2 Diabetes Mellitus patients. **Interpretation :** The factor significantly affecting the HbA1c value is Fasting Blood Sugar level. Hemoglobin level doesn't seem to be affecting the HbA1c indicating that Hemoglobin is not a significant predictor of HbA1c. **Conclusions:** This study documented a significant positive relationship between FBS and HbA1c level only. No correlation was found between total Hemoglobin level and HbA1c. Patients with mild to moderate anemia still revealed a high HbA1c value correlating only with the blood sugar status.

Keywords: HbA1c, Fasting Blood Sugar, Hemoglobin, Anemia

Bangladesh Journal of Medical Science Vol. 19 No. 01 January'20. Page :110-113
DOI: <https://doi.org/10.3329/bjms.v19i1.43882>

Introduction

Hemoglobin (Hb) gets non-enzymatically glycated when glucose gets added on to the N-terminal valine residue of the beta chain of Hb forming HbA1c. The level of HbA1c correlates with the blood sugar level during the preceding 1 -3 months period¹⁻⁵. The effect of reduced level of hemoglobin on the HbA1c value is hitherto not well examined despite the high prevalence of anemia in India. Few studies revealed a relationship between iron deficiency anemia and HbA1c levels [6-13]. Possible mechanism behind the alteration in HbA1c level in Iron deficiency

anemia as proposed by various authors was 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 [7]. Another explanation for the elevated HbA1c level was proposed by Sluiter et al [8] that hemoglobin glycation being an irreversible process, HbA1c levels in erythrocyte is likely to be elevated with increased cell age which happens in iron deficiency anemia.

In this scenario, it thus becomes essential to know the effect of reduced level of hemoglobin on the HbA1c

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value which may lead to an erroneous impression of the glycaemic status of the patient.

Hence this study was done to evaluate the effect of total hemoglobin level on HbA1c value in type 2 diabetes mellitus patients.

Material and Methods

This was a cross sectional study carried out in the department of Biochemistry, All India Institute of Medical Sciences, Bhubaneswar during 16 July 2016 to 18 September 2016 after approval by The Institutional Ethics Committee (IEC). This study included 100 diagnosed Type 2 Diabetes mellitus cases, in the age group of 30-65 years. Selected cases coming to outpatient department of All India Institute of Medical Sciences, Bhubaneswar were recruited into study after examining them with a questionnaire and taking their informed consent. Cases on any type of oral supplementation of iron and hematopoietic vitamins, recent infections, cases with acute complications of diabetes, recent history of surgery, accidents and trauma leading to acute blood loss (within 2 weeks), cases with known haemoglobinopathies, transfusion and pregnancy were excluded from the study. 5ml of blood sample was collected from the recruited patients and were assessed for HbA1c, Complete Blood Count, Fasting Blood Sugar. HbA1c was estimated by latex agglutination inhibition assay and fasting blood sugar levels was done by GOD-POD (Glucose oxidase-peroxidase) method in Beckman Coulter Fully automated analyzer RU5800. CBC was done by automated hematoanalyzer.

The cases were divided into three groups according to total hemoglobin level (Total Hb):

1. Total Hb->12gm/dl in females, >13gm/dl in males (normal level)
2. Total Hb-8-11.9gm/dl in females, 8-12.9gm/dl in males (mild to moderate anemia)
3. Total Hb-<8gm/dl in both.

The data was analyzed with the help of Epi Info version 7.2. The results obtained were analyzed with relevant statistical methodologies like Pearson correlation analysis and Linear regression analysis.

Results and Discussion

Total 36 participants were found to be anemic which included 12 males and 24 females. All the anemic cases were moderately anemic in nature (Figure I, Table I and II).

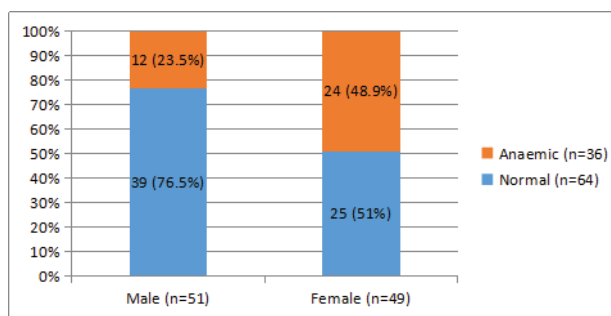


Figure I: Sex wise distribution of anemia in participants.

Table I: Means of different study variables in the male and female participants

	Male	Female	P-Value
Age (years)	52±11	48±10.33	0.1388
Hemoglobin (g/dl)	13.7±1.6	11.9±1.5	0.0000*
HbA1c (%)	7.7±2.25	8.11±2.5	0.3686
Fasting Blood Sugar (mg/dl)	126±59.5	133.6±56	0.5120
MCV	80.31±6	79.7±8	0.6672
MCH	26.54±6.5	26.16±6.5	0.5111
MCHC	32.7±3.6	32.43±3.1	0.4646

*p<0.05

Table II: Distribution of anemia among Different HbA1c status groups

Anemic status	HbA1c Status		Total
	<7	>7	
Normal	24(63.16%)	40(64.52%)	64
Anemic	14(36.84%)	22(35.48%)	36
Total	38	62	100

As per FBS values, 69% cases had their sugar level under control whereas as per HbA1c cutoffs only 38% cases were found to have good control in their glycaemic status (Table III).

Table III: HbA1c and FBS level in the study participants

FBS Status mg/dl	HbA1c Status		Total
	<7.0%	>7.0%	
<130	38(100%)	32(51.61%)	69
>130	0(0%)	30(48.39%)	31
Total	38	62	100

A positive correlation was observed between Fasting blood sugar and glycated hemoglobin using Pearson's

correlation analysis. No significant correlation was found between Hemoglobin values and HbA1c (Figure II and III).

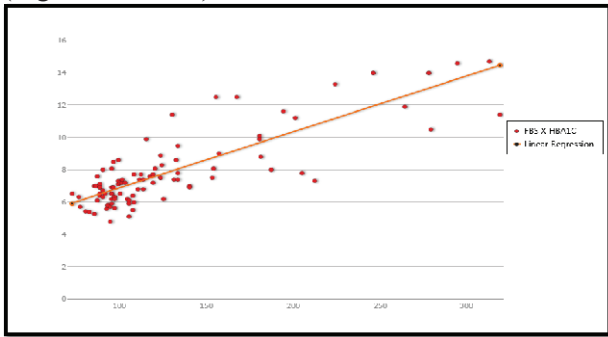


Figure II: Scatter plot between FBS and HbA1c ($y = \text{HbA1c}$, $x = \text{FBS}$)

Pearson’s correlation analysis, the correlation coefficient of HbA1c and FBS levels was 0.847 and the regression equation was $y = (0.0346)x + 3.4118$, R-square value is 0.71.

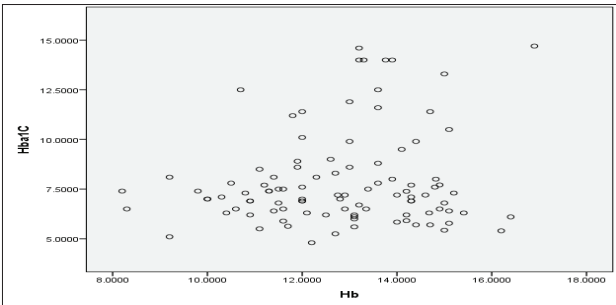


Figure III: Scatter plot between Hemoglobin and HbA1c Values

Pearson’s correlation analysis, the correlation coefficient of HbA1c and Haemoglobin levels was 0.148 and the regression equation was $y = (0.1957)x + 5.3955$. R-square value is 0.021

Pearson’s correlation co-efficient analysis of HbA1c and FBS in the anemic and non-anemic patients documented a significant association between Fasting Blood Sugar and HbA1c values in both the groups. (Figure IV & V)

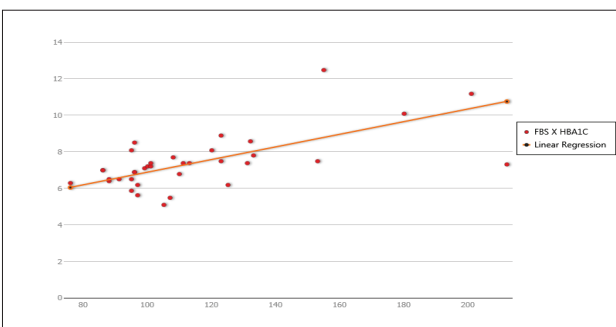


Figure IV: Scatter plot between Fasting Blood Sugar and HbA1c Value in Anemic cases.

Pearson’s correlation co-efficient of HbA1c and FBS is 0.61 and R square value is 0.381.

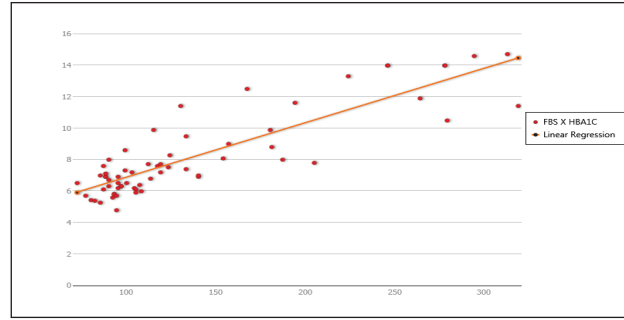


Figure V: Scatter plot between Fasting Blood Sugar and HbA1c Value in Non-anemic cases

Pearson’s correlation co-efficient of HbA1c and FBS is 0.87, and R square value is 0.76.

The linear regression model with HbA1c as the dependent variable and Age, Sex, Hemoglobin, FBS as the predictor variable exhibited that the only factor which significantly affects the dependent variable is Fasting Blood Sugar Value (FBS) depicting that Hemoglobin level doesn’t seem to be affecting the dependent variable HbA1c. Hemoglobin is not a significant predictor of HbA1c (Table IV).

Table IV: Linear regression Analysis with HbA1c as the dependent variable and Age, Sex, Hemoglobin, FBS as the predictor variable

Variable	Coefficient	95% Confidence	Limits	Std Error	F-Test	P-Value
Age	-0.009	-0.041	0.023	0.016	0.330	0.567
Sex	0.058	-0.816	0.932	0.437	0.0175	0.895
Hb	-0.143	-0.519	0.233	0.188	0.576	0.450
FBS	0.035	0.030	0.041	0.003	187.178	0.000
Constant	5.693	-0.163	11.55	2.927	3.784	0.056

From the results obtained it was revealed that HbA1c level was affected only by the Blood sugar value and the total hemoglobin level of the participants doesn’t seem to be affecting the relationship between fasting blood sugar and HbA1c levels. Our study is in accordance with that of Hansen et al [14], van Heyningen [15] Ju et al [16] and Mitchell et al [17] who did not find any correlation of anemia and total hemoglobin level with HbA1c value. They opined that Iron deficiency anemia and Iron therapy brought no change in the HbA1c value and that it is only the blood sugar level which affects the HbA1c assessment. Our observations were different from many other studies that depicted a change in HbA1c level in anemic cases. Refuting the explanations put forward in the studies who documented a change in HbA1c level due to anemia [6-13], Mitchell et

al [17] commented that red cell age is unlikely to be significantly increased in Iron deficiency states affecting the glycation of HbA1c. Hansen et al [14] opined that erythrocyte survival rate is normal in Iron deficiency anemia and though decreased in B12 deficiency anemia the hemolytic component is not significant enough to bring about a change in HbA1c value. Heyningen et al [15] attributed the differences in HbA1c value in anemia cases to different laboratory methodologies of HbA1c assessment.

Conclusion

This study on 100 Type 2 Diabetes mellitus patients whose FBS, HBA1c and CBC analysis was done,

documented a positive significant relationship between FBS and HbA1c level only. No correlation was found between total Hemoglobin level and HbA1c. Patients with mild to moderate anemia with decreased total Hemoglobin level still revealed a high HbA1c value correlating only with the blood sugar status.

Limitation of the study: This study was done in a short duration and with limited number of cases. The results may be different with a larger cohort which should be evaluated.

Acknowledgement : This study was done under ICMR-STs project .

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