



Impact of Iron Deficiency Anemia on Glycated Hemoglobin (HbA_{1c}) Levels in Diabetics with Controlled Plasma Glucose Levels

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ABSTRACT

Background: Glycated hemoglobin (HbA_{1c}) is used as a gold standard for monitoring glycemic control. American Diabetes Association (ADA) has certified HbA_{1c} $\geq 6.5\%$ as a diagnostic criterion for diabetes mellitus (DM). Recent studies suggest that conditions affecting erythrocyte turnover like Iron deficiency anemia (IDA) alters HbA_{1c} levels but their results are conflicting. However the effect of IDA on HbA_{1c} is rarely reported.

To determine the impact of IDA on HbA_{1c} levels among controlled diabetics [Fasting plasma glucose (FPG) $<126\text{mg/dl}$ since last 6 months] independent of blood glucose concentration and its variation according to the degree of anemia.

Methods: This cross-sectional study carried out in SRM Medical College Hospital and Research Centre, Chennai includes totally 300 controlled diabetic patients –Type 2 DM (150 with IDA and 150 without IDA). Medical history recorded. HbA_{1c}, complete hemogram and FPG were tested.

Result: The mean HbA_{1c} among controlled diabetics with IDA ($7.86 \pm 0.11\%$) was significantly higher than those without IDA ($5.45 \pm 0.038\%$) ($P < 0.05$). HbA_{1c} results were higher with the reduction of total hemoglobin ($p < 0.05$)

Conclusion: IDA spuriously elevates HbA_{1c} levels independent of blood glucose concentration in controlled-diabetics. HbA_{1c} increases significantly as severity of anemia worsens. Thereby this study insists on the utter importance to exclude IDA and to correct it before any diagnostic or therapeutic decision is made based solely on HbA_{1c} level.

Keywords: HbA_{1c}, Iron deficiency Anemia, Diabetes

Introduction

Glycated hemoglobin (HbA_{1c}) is a form of hemoglobin that is measured to estimate the three-month average blood glucose concentration. It is widely used as a gold standard for monitoring glycemic control over the previous three months as this is the life span of red blood cell. HbA_{1c} also serves as a predictor of complications of diabetes. Recently American Diabetes Association (ADA) has certified HbA_{1c} $\geq 6.5\%$ as a diagnostic criterion for diabetes mellitus.^[1] However HbA_{1c} levels can be influenced by a variety of other factors affecting erythrocyte turnover and glucose homeostasis.^[2-5] One such condition affecting erythrocyte turnover is anemia. Anemia may be associated with rapid erythrocyte turnover conditions like acute or chronic blood loss, hemolytic anemia, sickle cell anemia, vitamin B12 deficiency, pregnancy that lower HbA_{1c} levels (or) with slower erythrocytes turnover conditions like Iron deficiency anemia (IDA), alcoholism that increases HbA_{1c} levels. However some but not all studies suggest that iron depletion is associated with increased glycation of hemoglobin leading to falsely high values of HbA_{1c} independent of glycaemia.^[6,7] To shed additional light on

this, in the present study we aimed to analyze the effect of IDA on HbA_{1c} levels in controlled diabetics (fasting plasma glucose levels $\leq 126\text{ mg/dl}$ since last 6 months).

Materials and Methods

This is a cross-sectional study carried out in SRM Medical College Hospital and Research Centre, Chennai (February 2016 to September 2016) after obtaining approval from our institutional ethical committee. All diabetic patients (Type 2 DM) from both outpatient and inpatient departments of our hospital were included in the study evaluation.

Totally 300 controlled diabetics whose FPG level is $<126\text{ mg/dl}$ since last 6 months (150 with IDA and 150 without IDA) were included in this study. These two groups were matched for age, sex and plasma glucose levels. Their blood samples were tested for HbA_{1c}, hemoglobin (Hb), hematocrit (Hct), Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC), Peripheral smear, Serum iron, Ferritin and fasting plasma glucose (FPG) levels. Medical history was recorded.

The anemic patients were selected based on their hemoglobin levels (Hb $<13\text{ gm\%}$ in males and <12



gm% in females) based on definition of World Health Organization (WHO).^[8] And those with predominantly microcytic red cell indices (MCV<76 fl), hypochromic red cell indices (MCH<27 pg/cell and MCHC<32 g/dl) and on their peripheral smear (microcytic hypochromic) were considered to have IDA which was confirmed by low serum iron (<59 µg/dl in males and <37 µg/dl in females) & low serum ferritin (<15 ng/ml in males and <9 ng/ml in females).

Subjects having FPG >126 mg/dl (or) RPG >200 mg/dl (or) 2 hour post prandial plasma glucose >200 mg/dl and patients with hypothyroidism, vitamin B12 deficiency, pregnancy and those having abnormal renal function tests (serum urea, creatinine), hemolytic anemia were excluded from the study.

HbA_{1c} was measured by HPLC method using Bio-Rad analyzer. Hb, MCV, MCH, MCHC were estimated by sysmex XT-1800 analyzer. FPG estimated by glucose oxidase/peroxidase method. Serum ferritin (Bio-Rad Quanimune Ferrin IRMA, Bio-Rad lab) & Serum iron (TPTZ method).

Statistical analysis: The data are presented as mean ± S.D for continuous variables. A student's t- test was applied for comparison of group means. Pearson's co-efficient of correlation was calculated to determine correlation between two variables. P value <0.05 was considered statistically significant.

Result

Our study results show that mean HbA_{1c} levels in controlled diabetics with IDA patients was 7.86±0.11% while that in controlled diabetic patients without IDA was 5.45±0.03%. The HbA_{1c} levels were significantly higher in IDA patients than those without IDA (p<0.05). We also

observed a statistically significant difference (p<0.05) in mean Hb levels in controlled diabetics with and without IDA (10.33±0.15gm/dl and 14.06±0.10gm/dl respectively) These data are presented in [Table-1].

The mean Hct, MCV, MCH, MCHC, serum iron and ferritin levels in controlled diabetics with IDA and without IDA were 34.20 ± 0.44, 72.67 ± 1.39, 25.52 ± 0.32, 30.20 ± 0.209, 32.68±0.71, 10.06±0.79 and 41.90 ± 0.32, 86.79 ± 0.39, 29.39 ± 0.12, 33.11 ± 0.08, 75.26±0.79, 45.19±1.11 respectively.

These data show that Hct, MCV, MCH and MCHC levels were lower in controlled diabetic patients with IDA than in those without IDA and the observed difference was statistically significant as shown in [Table-2].

Additionally when patients were classified according to the degree of anemia, 55 presented with mild anemia, 70 presented with moderate anemia and 25 presented with severe anemia. HbA_{1c} results were higher with the reduction of total hemoglobin (p<0.05) [Fig-1].

Additionally we classified study subjects into well controlled (FPG <100mg/dl) and controlled diabetics (FPG 100 – 126mg/dl) and compared HbA_{1c} levels between anemic and not anemic groups. However HbA_{1c} level was found to be negatively correlated with IDA in study subjects and positively correlated in control subjects (p<0.05) [Table-3].

The baseline characteristics of the study subjects were analyzed. Among 150 controlled diabetics with IDA, 89 were females (59%) and 61 were males (41%) and among those without IDA 92 were females (61%) and 58 were males (39%) as shown in [Fig-2]. The mean age of the IDA patients was 50.24±1.55 and those without IDA was 54.79±1.38.

Table 1: comparison of HbA_{1c}% between anemic and not anemic controlled diabetics.

Parameters	IDA	No anemia	T test	P value
Hb (g/dl)	10.33±0.15	14.06±0.10	-20.276	0.0001
HbA _{1c} %	7.86 ± 0.11	5.45 ± 0.03	20.842	0.0001

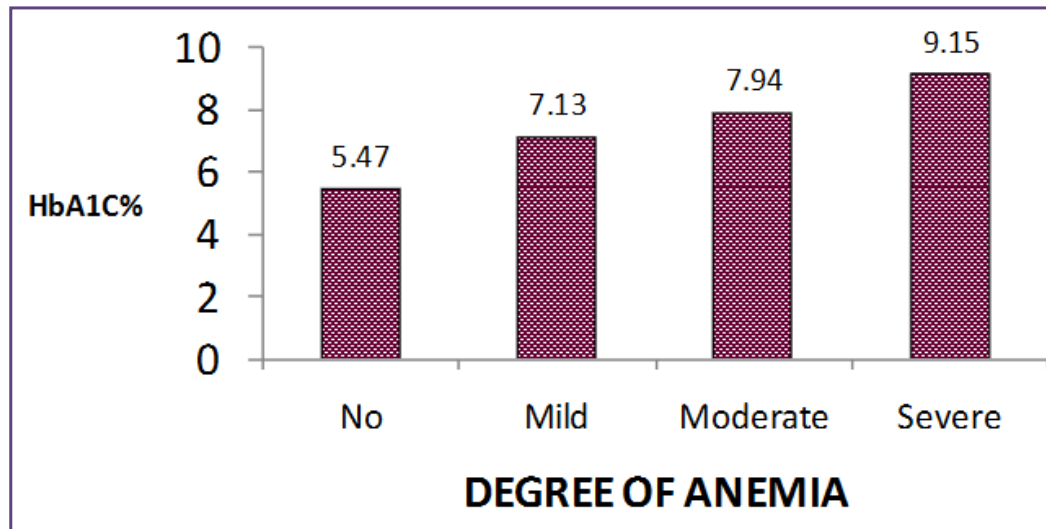
Table 2: comparison of red cell indices.

Parameters	IDA	Not anemic	T test	P value
Hct (%)	34.20 ± 0.44	41.90 ± 0.32	-13.922	0.0001
MCV (fl)	72.67 ± 1.39	86.79 ± 0.39	-9.718	0.0001
MCH (pg/cell)	25.52 ± 0.32	29.39 ± 0.12	-10.930	0.0001
MCHC (gm/dl)	30.20 ± 0.20	33.11 ± 0.08	-13.358	0.0001

Table 3: MEAN HbA_{1c}% GENDERWISE IN WELL CONTROLLED AND CONTROLLED DIABETICS.

	Females		Males		Total	
	IDA (n=89)	NA (n=58)	IDA (n=61)	NA (n=92)	IDA (n=150)	NA (n=150)
HbA _{1c} in FPG < 100	7.88 ± 0.17	5.45 ± 0.06	7.62 ± 0.25	5.33 ± 0.06	7.77 ± 0.15	5.38 ± 0.04
HbA _{1c} in FPG 100 - 126	7.93 ± 0.22	5.78 ± 0.04	7.95 ± 0.22	5.47 ± 0.06	7.94 ± 0.16	5.57 ± 0.05
T test	-0.183	-3.560	-0.983	-1.568	-0.775	-2.749
P value	0.855	0.001	0.330	0.120	0.439	0.007

Fig. 1: HbA_{1c} VARIATION WITH DEGREE OF ANEMIA.



ANOVA – 202.613, P – 0.0001

No anemia to mild anemia, T test - -7.591, P – 0.0001

Mild anemia to Moderate anemia, T test - -3.306, P – 0.001

Moderate anemia to Severe anemia, T test - -5.805, P – 0.0001

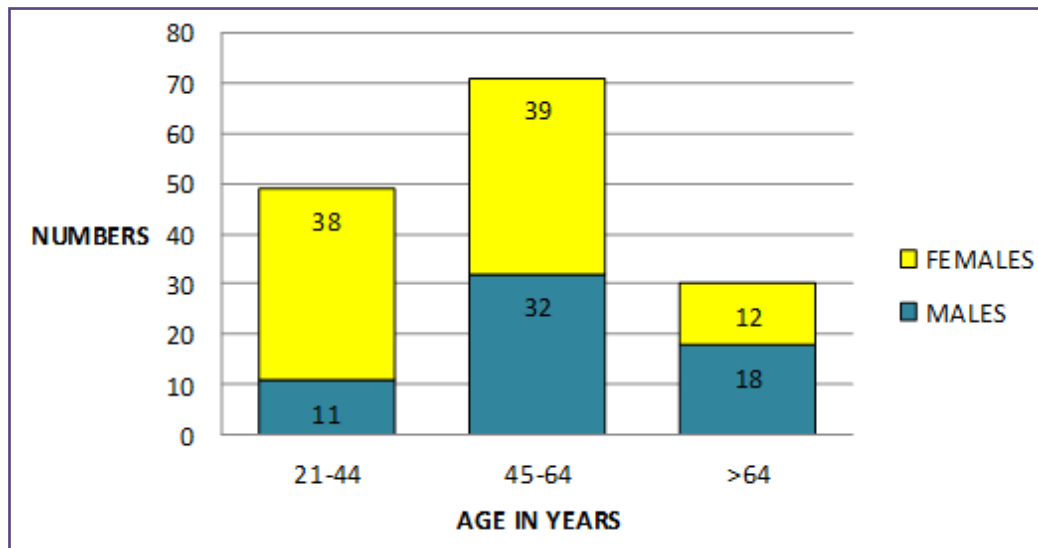


Fig. 2: AGE AND SEX DISTRIBUTION OF ANEMIA IN DIABETICS.

Discussion

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia and its incidence is sharply increasing worldwide with many long term macrovascular and microvascular complications.^[9] The HbA_{1c} test is commonly used to diagnose diabetes and also as a marker of glycemic status over previous 3 months. Many studies suggest that anemia is twice common in diabetics when compared with non-diabetics.^[10-12] Prevalence of anemia is estimated about 10-30% in patients with diabetes.^[13] The etiology of anemia in diabetics is multi-factorial and includes inflammation, nutritional deficiency, concomitant autoimmune diseases, drugs, hormonal changes in addition to kidney disease. Approximately one-third of patients with anemia exhibit iron deficiency.^[14] Though there are several studies on the role of anemia on HbA_{1c} levels, only few studies have reported on the effect of iron deficiency anemia on HbA_{1c} levels.

Our study result suggested that IDA elevates HbA_{1c} levels independent of plasma glucose concentration. This is in accordance with the study results of Brooks et al, Gram-Hansen et al and Coban et al who showed that iron therapy significantly reduces HbA_{1c} levels in non-diabetic population.^[6,7] Also our results positively correlates with results of Koga et al, Shanti et al who showed that HbA_{1c} levels in patients with iron deficiency anemia (IDA) were higher than those of subjects with normal iron levels.^[15,16] Our finding confirms the study results of Tarim et al, who reported that iron deficiency elevate HbA_{1c} levels in diabetics when compared with iron-sufficient controls when matched for FPG levels.^[1]

This elevation of HbA_{1c} in IDA may be explained by iron deficiency related changes in the quaternary structure of hemoglobin molecule increasing the glycation of globin chain.^[6] Some studies proposed that the glycation of hemoglobin is a permanent process and hence the HbA_{1c} levels in red blood cell will increase as the cell's age increases. They also found that after treatment of IDA in patients with normal blood glucose levels, HbA_{1c} concentration was reduced because of very young red cells. However if iron deficiency persists for a long time, production of red cells would fall, leading to a higher average age of circulating erythrocytes and therefore increased HbA_{1c} levels.^[16]

However our study results differs with study results of E.S.Ford et al, Van Heyningen et al and Saudek et al who reported that there is no significant difference in mean HbA_{1c} concentration according to IDA status.^[6,17,18]

Few studies by Horton BF & Huisman TH, Rau et al and Sinha et al reported that HbA_{1c} level decrease in IDA patients and this contradicts with our study result.^[6,19]

There are several strengths of this study. First we designed this study to reduce as much as possible confounding factors that could affect our HbA_{1c} results like renal insufficiency, pregnancy, alcohol etc. Second we also analyzed HbA_{1c} results in different degrees of anemia (mild, moderate and severe) and observed that HbA_{1c} level increases as severity of anemia worsens.

Limitations to this study were we couldn't follow up patients after iron supplementation which might have given a new dimension to our study and since this was a cross-sectional study and the mechanism by which anemia affects HbA_{1c} was not evaluated.

Conclusion

IDA spuriously elevates HbA_{1c} levels independent of plasma glucose concentration. HbA_{1c} level increases significantly with severity of anemia. Thereby this study insists on the utter importance to exclude IDA in diabetes and to correct it before any diagnostic or therapeutic decision is made based solely on HbA_{1c} level.

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