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ORIGINAL ARTICLE

Effect of Total Hemoglobin on Glycated Hemoglobin (HbA1c) in Type 2 Diabetes Mellitus

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Abstract

Background: Glycated hemoglobin (HbA1c) is a pivotal marker in the diagnosis, prognosis, and therapeutic monitoring of diabetes mellitus. Given the involvement of hemoglobin in non-enzymatic glycation reactions, it is hypothesized that total hemoglobin concentration might impact HbA1c levels alongside blood glucose concentrations. **Objectives:** This study aimed to estimate and compare plasma fasting glucose level and total hemoglobin level in type 2 diabetes mellitus (T2DM) patients with and without anemia. The study also aimed to estimate HbA1C and compare with calculated HbA1C using formula in T2DM patients with and without anemia and to correlate between total hemoglobin levels with directly measured HbA1c in T2DM patients with and without anemia. **Methodology:** This cross-sectional study, employing purposive sampling, investigated 30 anemic and 30 non-anemic Type 2 Diabetes Mellitus (T2DM) patients at the Central Diagnostic Laboratory. Total Hemoglobin, HbA1C, and Fasting Plasma Glucose were measured for the patients and the data was analyzed. **Results:** Independent 't' tests revealed significant differences ($p < 0.0001$) in estimated HbA1c, fasting blood sugar, calculated HbA1c, and Hb levels between the two groups. Subgroup analyses exhibited significant differences in estimated HbA1c for both groups individually ($p = 0.003$ for T2DM without anemia and $p<0.0001$ for T2DM with anemia). Pearson's correlation analysis indicated no significant correlation between HbA1c and hemoglobin in either subgroup. Similarly linear regression analysis treating HbA1c as the dependent variable yielded a non-significant p-value, suggesting that hemoglobin levels do not significantly influence HbA1c. **Conclusion:** In conclusion, regardless of anemia status, our findings suggest that hemoglobin is not a significant predictor of HbA1c levels in T2DM patients, offering valuable insights into factors influencing HbA1c variability in diabetic populations.

Keywords: glycated hemoglobin, type 2 Diabetes Mellitus, anemia in diabetes

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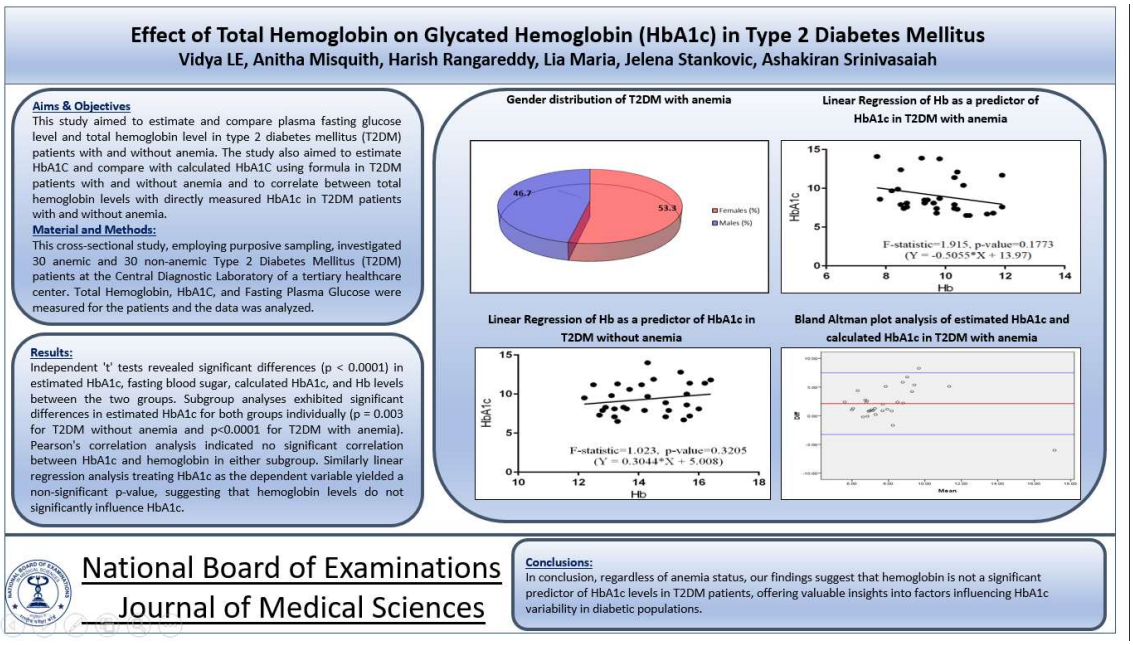
Graphical Abstract



Background

Glycated hemoglobins are hemoglobins that have a sugar moiety attached to them, forming the HbA1 fraction within the adult hemoglobin HbA [1]. The HbA1c fraction, in particular, serves as a predominant component of HbA1 and provides an estimate of an individual's blood sugar levels over the previous three months [2]. Studies have indicated that maintaining an HbA1c level below seven percent can decrease microvascular complications in diabetic patients [3,4]. Glycated hemoglobin (HbA1c) is a pivotal marker in the diagnosis, prognosis, and therapeutic monitoring of diabetes mellitus. However, it's crucial to note that HbA1c is influenced by factors beyond just blood sugar levels. Other variables also play a role in affecting HbA1c measurements [5]. HbA1c is dependent on the interaction between the concentration of blood glucose and the lifespan of the erythrocyte. Given the involvement of hemoglobin in non-enzymatic glycation reactions, it is hypothesized that total hemoglobin concentration might impact HbA1c levels alongside blood glucose concentrations [2]. This study aimed to estimate and compare plasma fasting glucose level and total hemoglobin level in type 2 diabetes mellitus (T2DM) patients with and without anemia. The study also aimed to estimate HbA1C and compare with calculated HbA1C using formula in T2DM patients with and without anemia and to correlate between total hemoglobin levels with directly measured HbA1c in T2DM patients with and without anemia.

Methodology

Study design

This cross-sectional investigation was conducted within the department of Biochemistry, employing purposive sampling to select previously diagnosed cases of Type 2 Diabetes Mellitus (T2DM) patients attending follow-up visits. The Institutional Ethics Committee approved

the study protocol and access to the database was strictly limited to analytical purposes, with personal information remaining inaccessible. Laboratory reports of T2DM patients from the Central Diagnostic Laboratory were gathered, ensuring data anonymization procedures were rigorously followed.

Sample size was calculated considering the mean differences of HbA1c measured by the National Glycohemoglobin Standardization Program (NGSP) certified immunoturbidimetric method in a study by Silva et al. [6]

$$n = (Z_{\alpha/2}+Z_{\beta})^2 * 2*\sigma^2 / d^2$$

where $Z_{\alpha/2}$ is the critical value of the Normal distribution at α/2 (for a confidence level of 95%, α is 0.05 and the critical value is 1.96), Z_{β} is the critical value of the Normal distribution at β (for a power of 80%, β is 0.2 and the critical value is 0.84), σ^2 is the population variance, and d is the mean difference. Sample size was estimated to be 26.

The study population comprised of already diagnosed 30 T2DM patients both with anemia and 30 T2DM patients without anemia, who were recruited from individuals visiting the Central Diagnostic Laboratory, after obtaining written consent. Exclusion criteria encompassed hemolysis, hemoglobinopathies, renal disorders, severe anemia (defined as hemoglobin levels <7g/dL), inadequate sample volume, mislabeling, illegible slips, and erroneous sample collection techniques.

Biochemical analyses were conducted under aseptic conditions, involving the collection of approximately 3 mL of blood into EDTA and sodium fluoride-containing vacutainers, followed by processing for Hemoglobin, HbA1C, and Fasting Plasma Glucose. Hemoglobin levels were assessed using the Automated Hemato Analyzer SYSMEX XNL 550, while HbA1C was measured via National Glycohemoglobin Standardization Program (NGSP) certified turbidometric inhibition immune assay [7]. Fasting plasma glucose (FBS) levels were determined using the Glucose Oxidase-Peroxidase method on VITROS 5600 [8]. Calculated HbA1c values were derived using the formula: HbA1c = 2.6 + 0.03 × Fasting Blood Sugar (mg/dL) [9].

Statistical analysis

Data was tabulated and entered in Microsoft excel. Kolmogorov-Smirnov test revealed that the data was normally distributed. Independent ‘t’ test was applied for HbA1c values in Type 2 Diabetes Mellitus Patients with anemia and checked for significant difference in comparison to the data obtained from Type 2 Diabetes Mellitus Patients without anemia. Pearson’s correlation analysis was performed to assess the relationship between HbA1c and total hemoglobin. Linear regression analysis was done with HbA1c as a dependent variable and total hemoglobin as a predictor. Statistical analysis was performed using SPSS v16, and significance was set at $p < 0.05$.

Results

The T2DM with anemia patients included 53.3% females and 46.7% males as shown in Figure 1. Independent 't' tests were employed to compare HbA1c, fasting blood sugar (FBS), calculated HbA1c, and hemoglobin concentrations between the two groups. The results revealed statistically significant differences in

estimated HbA1c, FBS, calculated HbA1c, and Hb levels ($p < 0.0001$) between T2DM patients with and without anemia as depicted in Table 1. Further subgroup analyses demonstrated significant differences in estimated HbA1c for both groups individually ($p = 0.003$ for T2DM without anemia and $p<0.0001$ for T2DM with anemia). Pearson's correlation analysis indicated no significant correlation between HbA1c and hemoglobin in either subgroup as depicted in Table 2. Linear regression analysis, with Hb as the predictor in T2DM with anemia yielded an F-statistic of 1.915 ($Y = -0.5055*X + 13.97$) with a non-significant p-value of 0.1773 as shown in Figure 2. Linear regression analysis, with Hb as the predictor in T2DM without anemia yielded an F-statistic of 1.023 ($Y = 0.3044*X + 5.008$) with a non-significant p-value of 0.3205 as shown in Figure 3.This suggests that hemoglobin levels do not significantly influence HbA1c. Calculated HbA1c relies on FBS levels and Bland Altman plot analysis was done in T2DM patients with anemia to check there was proportional bias between the means of estimated HbA1c and calculated HbA1c considering the possibility of decreased Hb in anemia to affect the estimated HbA1c values. However, the β coefficient was -0.172 and $p=0.364$ indicating that there was no proportional bias as shown in Figure 4. This further implies that though anemia may be having an impact on the HbA1c it may not be substantial.

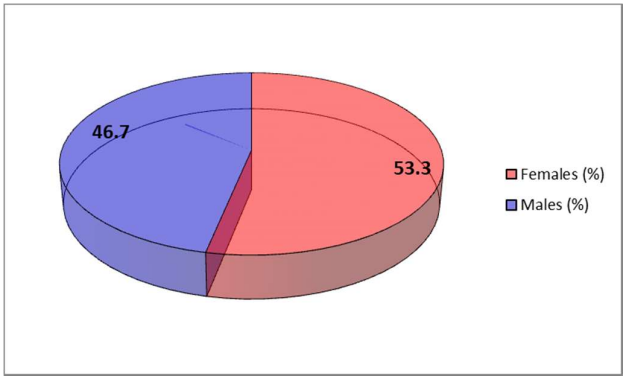


Figure 1. Gender distribution of T2DM with anemia

Table 1. Comparison of estimated HbA1C, FBS, Calculated HbA1C and Hb among type 2 diabetes mellitus with and without anemia

Parameters	T2DM without anemia, n=30 (Mean±SD)	T2DM with anemia, n=30 (Mean±SD)	p value
Estimated HbA1C (%)	9.3±1.2	9.1±2.3	<0.0001
FBS (mg/dL)	182.43±74.08	144.53±91.09	<0.0001
Calculated HbA1C (%)	8.1±2.2	6.9±2.7	<0.0001
Hb (g/dL)	14.3±1.2	9.7±1.2	<0.0001

p<0.05 statistically significant

Table 2. Pearson's correlation analysis of estimated HbA1c with Hb

	r	p value
T2DM without anemia n=30	0.188	0.321
T2DM with anemia n=30	-0.253	0.177

$p<0.05$ statistically significant

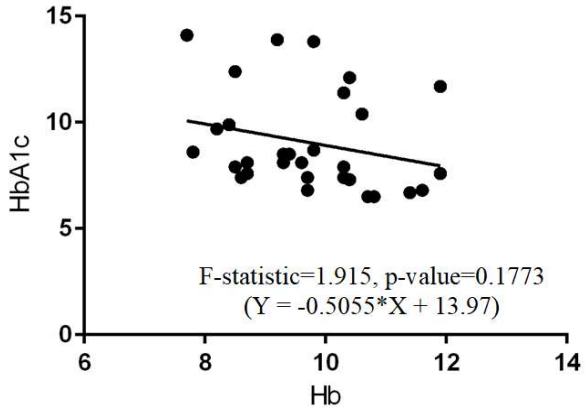


Figure 2. Linear Regression of Hb as a predictor of HbA1c in T2DM with anemia

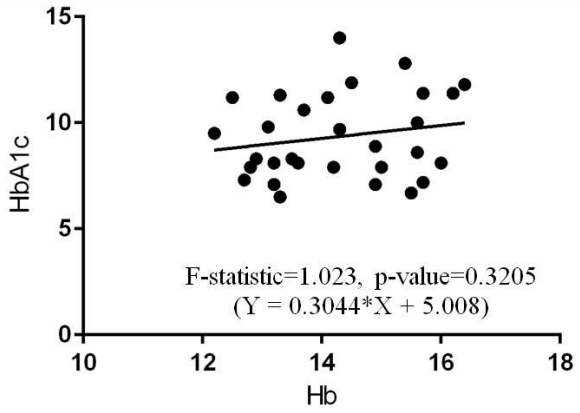


Figure 3. Linear Regression of Hb as a predictor of HbA1c in T2DM without anemia

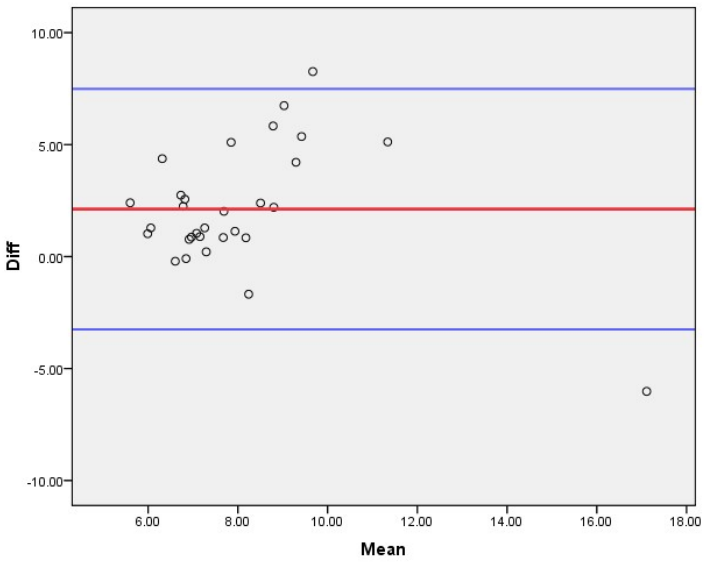


Figure 4. Bland Altman plot analysis of estimated HbA1c and calculated HbA1c in T2DM with anemia

Discussion

This study looked into how anemia affects glycated hemoglobin (HbA1c) levels in individuals with Type 2 Diabetes Mellitus (T2DM) and examined various factors that could influence HbA1c measurement. The study involved 30 patients with T2DM who had anemia and 30 who did not, with gender distribution of 53.3% females and 46.7% males in T2DM with anemia. The average age was 52.5±6.3 years in T2DM with anemia. This is similar to the prevalence study by Panda AK et al but in contrast in their study anemia was more common in males [10]. Anemia is recognized as a common condition that can have detrimental effects on their overall health. In a study by Hizomi Arani et al., it was found that a high number of patients with T2DM in northern Iran (around 22%) had anemia, which is linked to obesity, high levels of triglycerides, duration of T2DM, and chronic kidney disease [11]. Additionally, in a study by Sharif et al., observed that individuals with anemia tend to have comparatively shorter lifespan compared to individuals without anemia [12]. According to a research conducted by SC Thambiah et al., patients with anaemia showed elevated levels of serum urea, creatinine, and reduced FBS, estimated glomerular filtration rate (eGFR) when compared to patients without anaemia [13]. Patients with diabetes and nephropathy who were anaemic had a significantly lower level of haemoglobin than those without this complication ($p=0.022$). At a threshold eGFR value of 38.3 mL/min/1.73 m^2 with a maximum Youden index of 0.462, the accuracy to differentiate mild from moderate anemia was 66.7% for sensitivity and 79.5% for specificity. This research demonstrates that anemia is detected in T2DM patients when they first visit the specialist outpatient clinic and is closely linked to CKD [13]. However, in a prospective study by Mounika et al., found that diabetics even without kidney problems experienced a

high occurrence of anemia. Additionally, their research also indicated that inadequate blood sugar management and advanced age are linked to the development of anemia in diabetic individuals with unaffected kidney function [14].

In a study by Çetinkaya Altuntaş et al., it was observed in iron deficiency anemia individuals otherwise healthy without any other illness had low HbA1c levels and following iron administration as therapy the HbA1c levels increased; the possibility of hemoglobin affecting the HbA1c levels was highlighted [15]. In our study we selected T2DM with anemia patients and T2DM without anemia as the comparator group showed significant variations in estimated HbA1c, FBS, calculated HbA1c, and hemoglobin levels between T2DM patients with and without anemia, suggesting that anemia could affect these parameters. Further analysis of subgroups confirmed notable disparities in estimated HbA1c and calculated HbA1c levels within each group, indicating the diversity in HbA1c levels among various patient profiles.

In a study by Son et al., anemic individuals (n=112), their age and gender matched controls (n=217) suspected of diabetes were included. They underwent glucose tolerance and HbA1c tests. Mean HbA1c levels were compared for sensitivity and specificity in diabetes diagnosis. Clinical traits were similar. In normal glucose, HbA1c didn't differ significantly (P=0.580). Yet, anemic subjects with higher glucose showed slightly higher HbA1c levels. Anemia lowered HbA1c specificity in diabetes diagnosis ($p<0.05$) [16]. Our study results align with the findings of Solomon et al, indicating that diabetic patients with iron deficiency anemia (IDA) tend to have lower HbA1c levels compared to non-IDA diabetic patients [17].

In a prospective interventional case-control study conducted by Kalairajan et al., a significant correlation was found between Hb and HbA1c levels, with a coefficient of correlation of 0.26 and a p-value of less than 0.01 [18]. Urrechaga et al., study highlighted a positive association between HbA1c levels and iron deficiency [19]. In a study by Madhu SV et al, significant increase in HbA1c among patients with iron deficiency anemia (IDA) with a p-value of less than 0.001 was observed, and they also noted a substantial enhancement in HbA1c levels following oral iron supplementation [20]. Esfahani et al., observed a noteworthy enhancement in HbA1c levels following iron therapy treatment in patients with Type II diabetes and iron-deficiency anemia (IDA) [21]. This indicates that using only HbA1c to monitor T2DM anemia individuals could provide inaccurate information. Hence, healthcare providers and physicians need to take this into consideration prior to making treatment decisions.

In a study by Alsayegh et al., revealed a significantly elevated prevalence of anemia among diabetic patients ($p < 0.001$). Additionally, they found a frequent correlation between anemia and diabetic peripheral neuropathy as well as diabetic foot conditions. Interestingly, no significant association was observed between HbA1c and Hb levels ($p = 0.887$) [22]. Similarly in our study Pearson's correlation analysis revealed no significant relationship between HbA1c and hemoglobin in either subgroup, indicating that hemoglobin levels may not have a substantial impact on HbA1c values in T2DM patients.

Linear regression was also carried out to evaluate the association between Hb levels and HbA1c. The results showed that the F-statistics and p-values were not significant (F-statistic = 1.915, p = 0.1773 for T2DM with anemia; F-statistic = 1.023, p = 0.3205 for T2DM without anemia), suggesting that hemoglobin levels do not have a significant effect on HbA1c.

Moreover, the Bland-Altman plot analysis indicated no significant proportional bias between the mean values of estimated HbA1c and calculated HbA1c, indicating that while anemia could affect HbA1c levels to some extent, it may not have a considerable impact. These results add to our knowledge of the variables influencing HbA1c measurement in T2DM individuals and underscore the necessity for more studies to clarify the intricate connection between HbA1c and hemoglobin levels when anemia is present.

Conclusions

The comparative analysis between T2DM patients with and without anemia reveals substantial differences in estimated HbA1c, fasting blood sugar (FBS), calculated HbA1c, and hemoglobin concentrations. These results emphasize the importance of considering anemia status when interpreting HbA1c levels in diabetic individuals. Moreover, the subgroup analyses for T2DM patients with and without anemia individually highlight distinct patterns, further refining our understanding of the factors influencing HbA1c variability within these subpopulations. The lack of significant correlation between HbA1c and hemoglobin in both subgroups challenges previous assumptions, suggesting a more nuanced relationship between these variables.

A larger sample size may facilitate the exploration of potential confounding variables or modifiers that might have been overlooked in a smaller cohort. This step could contribute to a more comprehensive understanding of the complex dynamics between hemoglobin and glycemic control in diabetes. In conclusion, while the current study provides valuable initial insights, advocating for further exploration with a larger sample size is a prudent suggestion to strengthen the scientific validity and widen the applicability of the findings.

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Conflicts of Interest

The authors declare no conflicts of interest.

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