Scholars Journal of Applied Medical Sciences
Abbreviated Key Title: Sch J App Med Sci
ISSN 2347-954X (Print) | ISSN 2320-6691 (Online)
Journal homepage: https://saspublisher.com/sjams/

OPEN ACCESS

General Medicine

Study of Effect of Anaemia on Hba1c in Diabetic Patients: A Case Control Study

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| **Received:** 02.04.2019 | **Accepted:** 10.04.2019 | **Published:** 30.04.2019
DOI: 10.21276/sjams.2019.7.4.15

Abstract | **Original Research Article**

Background and objectives: HbA1c is recommended for diagnosis of diabetes, is the most common means of guiding management of diabetes, and is the primary predictor of complications. It is well established that HbA1c can be affected by haemolytic anaemia, blood loss and iron deficiency. There is need for more evidence, especially in identifying the types and degrees of anaemia likely to have significant impact on HbA1c in diabetics. Objectives of our study were to study the correlation between HbA1c and blood glucose levels in anaemic diabetics, to assess the influence of anaemia on HbA1c and to study the effect of severity and type of anaemia on HbA1c. ***Methods:*** This was a case control study of 100 cases and 100 controls. Anemic diabetics and non-anemic diabetic controls were chosen, and patients with CKD and haemolytic anemia were excluded. Hemoglobin, erythrocyte indices, peripheral smear, vitamin B12 level and iron studies were done in cases. Mean of FBS, PPBS and post lunch blood glucose was taken as Mean blood Glucose and compared with HbA1c in cases and controls. Various statistical tests were applied to assess the effect of anemia on HbA1c. ***Results:*** Mean HbA1c in anemic individuals was 9.91±3.13 which is lower than that of controls i.e 10.51±2.90, though not significant. When compared with mean HbA1c in controls, mean HbA1c of Iron deficiency anemia was higher, whereas of vitamin B12 deficiency and anemia of chronic disease was lower. MeanHbA1c in iron sufficient individuals was 8.67±2.55, whereas in iron deficient individuals was 10.23±3.20, with a significant P value of 0.042. Severe anemia leads to statistically significant reduction in HbA1c than controls. ***Conclusion:*** Anemia especially when severe, leads to significant reduction in HbA1c. Iron deficiency leads to spurious increase in HbA1c. Hence larger studies are needed to identify definite association of anemia with HbA1c and also to comment on the utility of HbA1c as a reliable marker of glycemic control in anemic diabetes.

Keywords: HbA1c; Anemia; Diabetes mellitus.

INTRODUCTION

Diabetes is one of the largest global health emergencies of the 21st century. About 415 million adults are estimated to currently have diabetes worldwide [1]. India has the second largest population of diabetics in the world [2]. HbA1c is being increasingly recommended over fasting blood glucose for use in population-based settings by international expert committee [3]. The advantages of using HbA1c are that it requires a single blood sample and being able to test in the non-fasting state. The day to day intra-individual variability of HbA1c is also smaller than that of fasting glucose during periods of stress and illness. HbA1c measurement is also the most common means of guiding management of diabetes and adjusting treatment [4]. HbA1c is the primary predictor of complications [5, 6] as glycation is the first step which leads to advanced glycation end products implicated in various complications. It is well established that HbA1c levels can be affected by conditions unrelated to diabetes including haemolytic anaemia, blood loss and iron deficiency [7]. Anaemia is common in diabetes with an incidence of 18-34% [8-10] in various studies. There is need for more evidence, especially in identifying the types and degrees of anaemia likely to have significant impact on the reliability of HbA1c in diabetics. The study was conducted with the objective of studying the correlation between HbA1c and blood glucose levels in anaemic diabetics in order to assess the influence of anaemia on HbA1c and study the effect of severity and type of anaemia on HbA1c and thus assess the utility of HbA1c as a marker of glycaemic control in anaemic diabetics.

MATERIALS AND METHODS

This study was a prospective case control study conducted from November 2015 to November 2017 on patients presenting to Victoria hospital and Bowring and Lady Curzon hospital, BMCRI and included 100 cases and 100 controls.

Adult patients of diabetes mellitus (either on treatment or diagnosed newly) with anemia defined as Hb <12g/dl in adult non pregnant women and <13g/dl in adult men, were taken as cases. Diabetic patients with chronic kidney disease (GFR <60 mL/min/1.73 m2 for 3 months), pregnant women, patients with hemolytic anemia and acute blood loss were excluded from the study.

Adult patients of diabetes mellitus (either on treatment or diagnosed newly) without anemia were taken as controls. Informed consent was taken from all patients. Clinical examination was done to identify signs and possible etiology of anemia. Haemoglobin percentage, Peripheral smear, Erythrocyte indices (MCV, MCH and MCHC), iron profile, Vitamin B12 level, Fasting blood glucose (FBS), Postprandial blood glucose (PPBS), post lunch blood glucose (as it shows the strongest correlation with HbA1c) [11], HbA1c level, Blood urea and S. creatinine were done at presentation to the hospital for all cases.

Hemoglobin percentage, FBS, PPBS, Post lunch blood sugar and HbA1c were done for all controls. Further sampling or follow up was not done in both the groups.

Data was analyzed to look for the variation of HbA1c with anemia with special reference to type and severity of anemia. Severity of anemia was graded according to WHO criteria as follows- Mild (Female 11-11.9 g/dl Male 11-12.9 g/dl), Moderate (8-10.9 g/dl), Severe (Lower Than 8g/dl). Mean blood glucose was calculated by taking average of FBS, PPBS, and post lunch blood glucose. Cases and controls were matched with respect to age, sex and mean blood glucose. Estimated average glucose was calculated from HbA1c using the formula EAG mg/dl=28.7×HbA1c -46.7. Mean blood glucose and estimated average glucose have been compared to find the association between blood sugars and HbA1c among cases and controls.

Statistical analysis: Proportions were compared using Chi-square test of significance. The student't' test was used to determine whether there was a statistical difference between the groups in the parameters measured. Pearson correlation coefficients were calculated to determine whether there was any correlation between the HbA1c data and the Serum Iron and Vitamin B12.

RESULTS

We studied 100 cases and 100 controls and the results were as follows. The mean age group in the control group was 54±13.4 years and mean age in the study group was 53.4±13.6 years. The two groups were age matched with a P value of 0.739. Out of the 100 controls, 50 were males and 50 were females. Where as in the study group, 49 patients were male and 51 patients were female. The two groups were sex matched with a P value of 0.88.

The mean haemoglobin level in controls was 13.8±1.2 g/dl, whereas in cases was 9.41±2.18 g/dl. Mean PCV in controls was 41.5±4.4 and in cases was 28.7±6.73. Mean MCV in controls was 84.02±5.6 and cases were 79.89±10.2. Mean MCH in controls was 28.14±2.31 and in cases was 25.7±4.48. Mean MCHC in controls was 33.05 ±1.80 and in cases was 32.4±5.44.

The mean of the Mean blood glucose (MBG) in controls was 280.9±82.4 mg/dl, whereas in cases was 267.35±127.01. The mean of estimated average glucose (EAG) in controls was 254.57±83.41, whereas in cases was 237.24±90.02. The mean HbA1c in controls was 10.51±2.90, whereas in cases was 9.91±3.13 as depicted in the figure below.

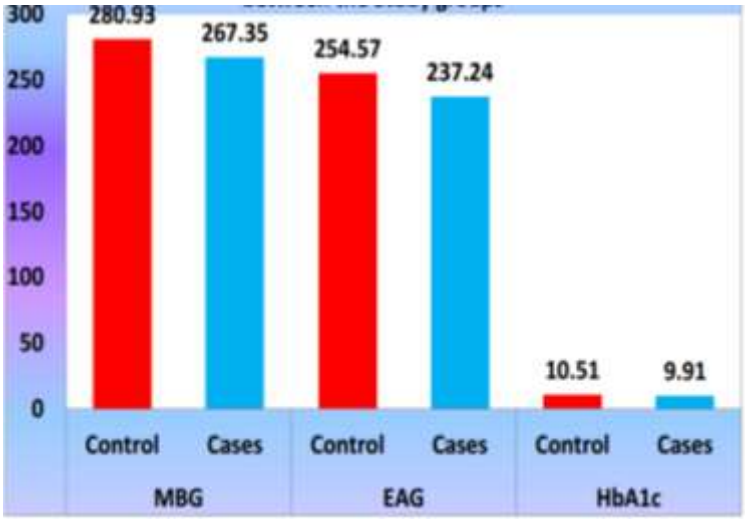

Fig-1: Comparison of Mean values of MBG, EAG and HbA1c between the study groups. The difference between MBG and EAG in controls is 26.36±5.30 mg/dl with a significant P value of <0.001 and in cases is 30.11±10.27 mg/dl with a significant P value of 0.004

Table-1: Correlation between MBG and HbA1c in cases and controls

Group			MBG
Control	HbA1c	Correlation Coefficient	0.796
		pvalue	<0.001
Cases	HbA1c	Correlation Coefficient	0.599
		pvalue	<0.001

The correlation coefficient of MBG vs HbA1c in controls is 0.796 which indicates a very strong positive relationship. Whereas in cases, the correlation coefficient is 0.599 which indicates a lesser degree of positive correlation than controls.

Out of the 100 cases 51 patients had iron deficiency anemia, 40 patients have anemia of chronic disease, and 9 patients have vitamin B12 deficiency.

Mean HbA1c in controls is 10.51, in iron deficiency anemia is 10.58, in anemia of chronic disease is 9.23, vitamin B12 deficiency is 9.11.

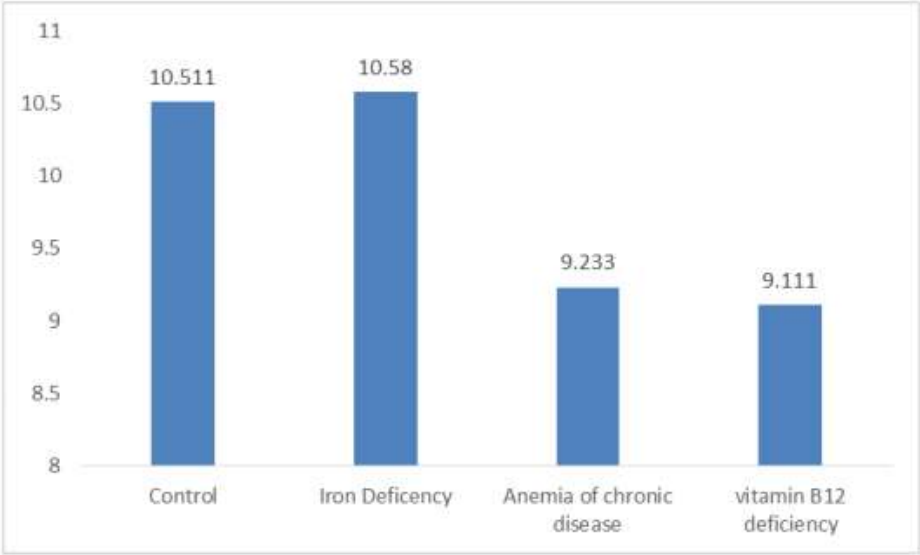

Fig-2: Mean HbA1c of different types of anemia when compared to controls

Among the cases, 79% have a serum iron of <45µg/dl, and 21% have serum iron of >45µg/dl. Mean HbA1c in iron deficient individuals is 10.23, whereas in iron sufficient individuals is 8.67, with a significant p value of 0.042. Among the cases, 83% had a vitamin B12 level of >185 pg/ml, whereas 17% had a level of <185 pg/ml. Mean HbA1c in vitamin B12 deficiency is 9.61 whereas in vitamin B12 sufficient individuals is 9.96, though P value was not significant.

Table-2: Correlation between HbA1c vs Serum Iron and Vitamin B12

	S. Iron	Vitamin B12
Correlation Coefficient	-0.118	-0.059
P value	0.244	0.562

Both S. iron and vitamin B 12 have a negative correlation with HbA1c, though relationship is very insignificant. Among the cases, 31% have mild anemia, 46.7% have moderate anemia, and 21.9% have severe anemia.

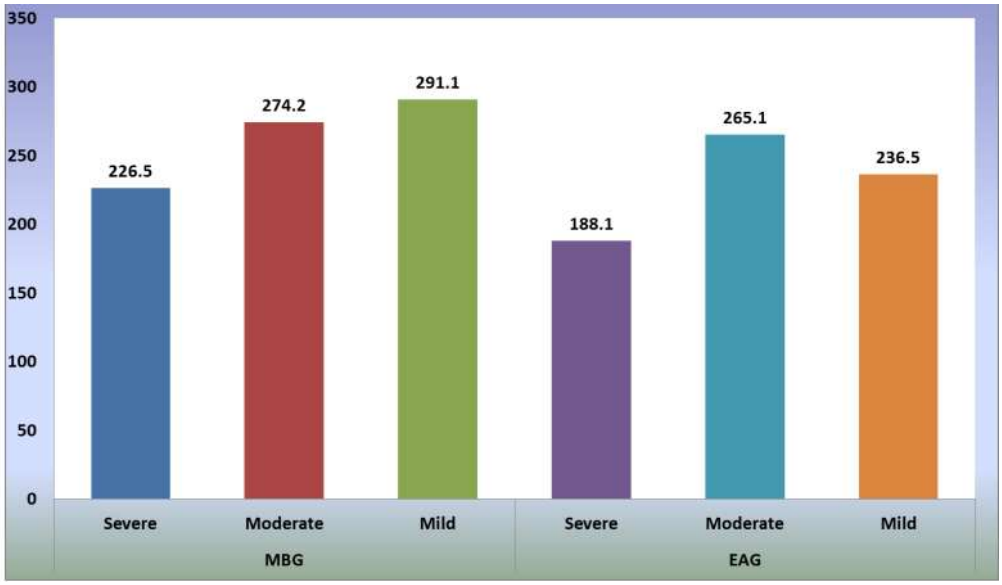

Fig-3: Comparison of Mean values of mean blood glucose, Estimated average glucose according to severity of anemia.

Table-3: P value of Comparison of mean HbA1c of controls vs mean HbA1c of varying severity of anemia

Control vs Severe anemia	Control vs Moderate anemia	Control vs Mild anemia
0.004	0.886	0.494

Mean HbA1c in severe anemia is 8.17, in moderate anemia is 10.88, and in mild anemia is 9.62.

Severe anemia had a significantly lower HbA1c when compared to controls, which was statistically significant with a P value of 0.004.

DISCUSSION

This study was done to assess the influence of anaemia on HbA1c and also to study the effect of severity and type of anaemia on HbA1c.This was a case control study of 100 cases and 100 controls.

In our study, the mean of the mean blood glucose in controls was 280.9±82.4 mg/dl, whereas in cases was 267.35±127.01. The mean of estimated average glucose in controls was 254.57±83.41, whereas in cases was 237.24±90.02. Both these parameters did not have a statistically significant difference between the two groups.

Correlation between blood sugars and HbA1c and effect of anemia on HbA1c

The mean HbA1c in controls was 10.51±2.90, whereas in cases was 9.91±3.13. The difference between the two groups is not significant, with a P value of 0.161. Here we note that mean HbA1c in anemic individuals was lower than in controls, though not significant. This is in contrast to a study conducted by Son JI *et al*. in korea in 2013 wherein they found that in diabetics, mean HbA1c was 7.8%±1.6% in the anemic group and 7.2%±1.4% in the nonanemic group, a difference with borderline significance (P=0.059) [12]. Hence they opined that the specificity of HbA1c for diagnosis of DM was significantly lower in the anemic subgroup (P<0.05). However, reason for this contrasting result cannot be explained. Due to paucity of studies which have studied the effect of anemia (without characterising type of anemia) on HbA1c, no further conclusions can be drawn.

In order to assess the correlation between blood sugars and HbA1c, the difference between MBG and EAG (derived from HbA1c) was analysed. In the controls, the difference was 26.36±5.30 mg/dl with a significant P value of < 0.001 and in cases was 30.11±10.27 mg/dl with a significant p value of 0.004. Hence we can infer that both in cases and controls, MBG was higher than EAG. The difference of MBG and EAG i.e (MBG-EAG) in the two groups was compared by student paired t test. Though the mean difference was higher in cases i.e anemic diabetics by 3.7 mg/dl, it was not statistically significant, with a P value of 0.746.

Pearsons correlation coefficient was calculated for MBG vs HbA1c, and it has been found that the correlation coefficient of MBG vs HbA1c in controls was 0.796 which indicates a very strong positive relationship. Whereas in cases, the correlation

coefficient was 0.599 which indicates a lesser degree of positive correlation than controls. Hence we conclude that anemia does influence HbA1c, though not statistically significant. This result could be because; different types of anemia and severity of anemia which have varying effect on HbA1c have been taken as a whole.

Effect of type of anemia on HbA1c

Out of the 100 cases, 51 patients had iron deficiency anemia, 40 patients had anemia of chronic disease, and 9 patients had vitamin B12 deficiency. Mean Hb in iron deficiency anemia, anemia of chronic disease and vitamin B12 deficiency was 8.75±2.29 g/dl, 10.57±1.31 g/dl and 8±2.43 g/dl respectively. We can note that both iron deficiency anemia and vitamin B12 deficiency had severe degree of anemia compared to anemia of chronic disease.

Difference between MBG and EAG in iron deficiency anemia was 10.45±15.82 with a P value of 0.512, in anemia of chronic disease was 50.50±13.5 with a P value of 0.001and in vitamin B12 deficiency was 50.77±33.7 with a P value of 0.171. Here we can note that, with similar MBG levels between the three types of anemia, difference between MBG and EAG is higher in non-iron deficiency anemias than iron deficiency anemia. This is similar to the conclusion drawn by a review article published by English E et al which states that 'The difference between the non-IDA and control values was greater than was observed between IDA and controls, suggesting that HbA1c may be spuriously elevated in iron deficiency and spuriously depressed in non-IDAs' [13].

Mean HbA1c was 10.58±3.46 in iron deficiency anemia, 9.23±2.29 in anemia of chronic disease and 9.11±3.87 in vitamin B12 deficiency anemia. Here, mean HbA1c values in iron deficiency anemia were higher than other two types of anemia, though it was not statistically significant, with a P value of 0.090. When compared with mean HbA1c in controls i.e 10.51±2.90, mean HbA1c of Iron deficiency anemia was higher, whereas of vitamin B12 deficiency and anemia of chronic disease was lower. This is similar to the conclusion stated by English E *et al*. as mentioned above.

Iron deficiency and HbA1c

Among the cases, 21% had a serum iron of more than 45µg/dl and 79% had less than 45µg/dl. Mean HbA1c in iron sufficient individuals was 8.67±2.55, whereas in iron deficient individuals was 10.23±3.20. Here we can note that iron deficiency leads to a statistically significant increase in HbA1c with a significant P value of 0.042.

Pearsons correlation coefficient was calculated to determine the correlation between HbA1c and serum iron, and it was found to have negative correlation with a co efficient of -0.118, though not very significant. So with a lower serum iron, HbA1c would be higher. This is similar to the results obtained by various researchers as discussed below.

In the study done by Christy AL *et al*. to find the influence of iron deficiency anemia on HbA1c levels in diabetic individuals with controlled plasma glucose levels, they found a positive correlation between iron deficiency anemia and increased A1C levels, especially in the controlled diabetic women and individuals having FPG between 100-126 mg/dl. Mean HbA1c in iron deficiency anemia was 6.87 ± 1.4 and in non anemic individuals was 5.65 ± 0.69. Hence, they concluded that before altering the treatment regimen for diabetic patients, presence of iron deficiency anemia should be considered [14].

Tarim O *et al*. concluded that among type 1 DM patients with similar level of glycemia, iron deficiency anemia is associated with higher concentrations of HbA1c. In addition, iron replacement therapy leads to a drop in HbA1c in both diabetic and non-diabetic patients. HbA1c decreased from a mean of 10.1±2.7% to a mean of 8.2±3.1% (P<0.05) after therapy. The iron status of the patient must be considered during the interpretation of HbA1c concentrations in type 1 DM [15].

In a study by Ford ES *et al*. Iron status did not significantly affect HbA1c concentrations in diabetic individuals (regression coefficient = 0.05762; SE = 0.32298; P =0.860). However they opined that caution should be used when diagnosing diabetes and prediabetes among people with high or low Hb when the HbA1c level is near 6.5% or 5.7%, respectively, as changes in erythrocyte turnover may alter the test result and also the trend for HbA1c to increase with iron deficiency does not appear to require screening for iron deficiency in ascertaining the reliability of HbA1c in the diagnosis of diabetes and prediabetes in a given individual [16].

The exception to this is the study by Sinha et al, which found low values of HbA1c in severe cases of IDA that increased with iron replacement therapy. But this study excluded proven diabetic patients. The authors did not discuss why their data conflicts with other studies but it may be due to the severity of anaemia as the participants in this study had low mean Hb levels (62 g/l) [17].

Various studies investigating iron deficiency anemia in non-diabetics have shown spuriously high HbA1c when compared to non-anemic controls.

Vitamin B12 deficiency anemia and anemia of chronic disease (non-iron deficiency anemias) and HbA1c

Among the cases, 17% had vitamin b12 deficiency and 83% had normal vitamin B12 levels. The mean HbA1c in vitamin B12 deficient individuals was 9.61±3.07 which is lower than the vitamin B12 sufficient individuals with mean HbA1c of 9.96±3.16, though this was not statistically significant. As mentioned earlier, mean HbA1c of non-iron deficiency anemias was lower than controls. This result is similar to the study done by Ford *et al*. wherein they found that compared with controls, those with a low Hb concentration and normal iron status had a significantly lower concentration of HbA1c (P = 0.001)[16].

Correlation coefficient calculated for HbA1c vs vitamin B12 was -0.059, indicating a negative correlation of doubtful significance. With anemia of chronic disease, mean HbA1c was 9.23±2.29, which was lesser compared to the mean HbA1c of controls i.e 10.51±2.91, though it is not statistically significant.

Ours is one of the first few studies which have looked into the association of non-iron deficiency anemias with HbA1c. Hence due to paucity of studies, further comparisons could not be drawn.

Effect of severity of anemia on HbA1c

Among the cases, mild anemia (Hb 11-12.9 g/dl in males and 11-11.9 g/dl in females) was 31.4%, moderate anemia (8-10.9 g/dl) was 46.7%, and severe anemia (<8 g/dl) was 21.9%. Mean of MBG in mild anemia was 291.1±145 mg/dl, moderate anemia was 274.2±103.7 mg/dl and severe anemia was 226.5±131.5 mg/dl.

Mean HbA1c in mild anemia was 9.62 in moderate anemia was 10.88 and severe anemia was 8.17. Here we can note that severe anemia leads to statistically significant decrease in HbA1c, whereas moderate and mild anemia might not affect HbA1c much. Due to paucity of studies on severity of anemia on HbA1c, we could not compare with other studies.

Though there are few studies studying the effect of anemia on HbA1c, there are varying results. Hence studies with large sample size targeting the diabetic population are needed.

Larger studies are needed to identify definite association of anemia with HbA1c and also to comment on the utility of HbA1c as a reliable marker of glycemic control in diabetes, though we can only conclude that caution has to be exercised in patients with iron deficiency anemia, as statistically significant results have been obtained in most studies.

CONCLUSION

Mean HbA1c in anemic individuals was lower than in controls, though not significant. By correlating MBG with HbA1c, we conclude that anemia does influence HbA1c, though not statistically significant. When compared with mean HbA1c in controls, mean HbA1c of Iron deficiency anemia was higher, whereas of vitamin B12 deficiency and anemia of chronic disease was lower. Serum iron has a negative correlation with HbA1c with a coefficient of -0.118, though not very significant. The mean HbA1c in vitamin B12 deficient individuals was lower than the vitamin B12 sufficient individuals, though not statistically significant. Mean HbA1c in anemia of chronic disease was lower than controls, though not statistically significant. Severe anemia leads to statistically significant reduction in HbA1c than controls.

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