

### **Influence of anaemia on glycosylated haemoglobin levels in diabetic individuals**

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**Conflicts of Interest:** Nil

**Background:** Glycosylated hemoglobin level is diagnostic test used for diabetes and to determine the developing diabetic complications. The level of HbA1C is affected by factors such as the Hemoglobin, RBC count and the Hb glycation rate. The aim of this retrospective cross-sectional study is to assess the relationship of HbA1c levels with Hb count and RBC indices

**Material and Method:** A total of 70 diabetic individuals confirmed by previous records, their sugar, HbA1c level and CBC were noted for the study. 35 diabetic cases with Hb less than 13 gm% in males and less than 12 gm % in females and 35 controls with hemoglobin more than 13 gm % for males and more than 12 gm % females were considered as cases and control respectively with HbA1c levels more than 6.5%. Hemoglobin concentration and RBC indices were

calculated on ADVIA analyzer. Sugar and HbA1c levels were measured by VITROS 5600 analyzer

**Observation:** Present case-control retrospective descriptive study was performed on 70 diabetic subjects of both genders. HbA1c, Hb, RBS and RBC count were performed in all. Hb  $\leq$  10 gm % were taken as cases (N=35) and Hb  $>$  10 gm % were taken as controls (N=35). Result so obtained consist of female cases with mean age (49 $\pm$ 12.8) years had Hb levels of 9.2 $\pm$ 1.1 gm %, HbA1c levels of 7.9 $\pm$ 1.9 % and female controls with age (52 $\pm$ 14) years had Hb levels of 12.3 $\pm$  0.5 gm % and HbA1c levels of 8.2 $\pm$ 1.2%. Male cases with age (55 $\pm$ 14.2) years with HbA1c levels of 7.2 $\pm$ 2.5% and Hb levels of 7.3 $\pm$ 1.2 gm %. Male controls of age (40 $\pm$ 20) years had HbA1c levels of 6.7 $\pm$ 1.3% and Hb 14.2 $\pm$ 1.1 gm %. Mean RBC count in cases 3.2 $\pm$ 0.7 and controls are 4.8 $\pm$ 0.5. Female cases under the age of 50 showed

HbA1c levels of  $7.3 \pm 1$  and above 50 showed  $8.5 \pm 2.1\%$ . Female controls below 50 years of age showed HbA1c levels of  $7.5 \pm 0.7$  and above 50 showed HbA1c levels of  $8.2 \pm 1.3\%$ . Male cases below 50 years showed HbA1c levels  $7.9 \pm 1.7$  and above 50 showed HbA1c levels  $9.7 \pm 2.6$ . Male controls below 50 years showed HbA1c levels  $6.7 \pm 0.6\%$  and above 50 showed HbA1c levels  $7.3 \pm 1.6$

### Conclusion

1. HbA1c levels are increase in male anaemic diabetic patients
2. Anemic diabetic females and males more than 50 years of age have raised HbA1c levels.
3. HbA1c levels are increased in patients with low RBC count.
4. While interoperating HbA1c levels anaemia should be taken in to consideration.

**Keywords:** Anaemia, diabetes, HbA1c-Glycosylated haemoglobin, Hb - hemoglobin, RBC count.

### Introduction

Glycated haemoglobin including HbA1c as well as other haemoglobins constitute the fraction of adult haemoglobin (HbA). HbA1c is the predominant haemoglobin found in HbA1 fractions.

Glycosylated haemoglobin level is diagnostic test used for diabetes and to determine the developing diabetic complications<sup>1</sup>.

According to the American Diabetes Association Guidelines published (2007) HbA1c levels should be maintained below 7% in all diabetic patients in order to prevent development of microvascular complications<sup>2</sup>.

HbA1c is synthesized from glycation of terminal unit of the  $\beta$  - chain of haemoglobin. Its percentage is determined by plasma glucose level in last six to eight weeks<sup>3</sup>. It is also used to evaluate level of metabolic

control and especially measuring the quality of diabetes care<sup>4</sup>. Iron deficiency anaemia is the most common form of anaemia in India<sup>5</sup>. Anaemia is one of the common elements that affect HbA1c values where decreased Red Blood Cells count (RBCs)

Increases glycation rate of the Hb which contributes to the high value of HbA1c found in splenectomy or iron deficiency anaemia<sup>6</sup> whereas increase RBC count decreases HbA1c levels found in blood loss, haemolysis, haemoglobinopathies, red cell disorders, myelodysplastic disease and haemolytic anaemia. These suggest HbA1c as a poor marker for diabetic patient with haemolytic anaemia<sup>7,8,9,10</sup>. Previous studies on same topic suggest that iron deficiency anaemia causes an increase of HbA1c levels and reduced after iron therapy<sup>11,12</sup>.

The level of HbA1c is affected by factors such as the Hemoglobin, RBC count and the Hb glycation rate<sup>13,14</sup>. The aim of this retrospective study is to assess the relationship of HbA1c levels with Hb count and RBC indices

### Material and Method

Present retrospective case-control descriptive study was performed between the duration February 2022 to May 2022. Institutional ethics committee permission was taken prior to commencement of study. A total of 70 diabetic individuals confirmed by previous records, their sugar levels, HbA1c level and CBC were noted for the study. 35 diabetic cases with Hb less than 13 gm % in males and less than 12 gm % in females and 35 controls with hemoglobin more than 13 gm % males and more than 12 gm % females were considered as cases and control respectively with HbA1c more than 6.5. For calculation of sample size, G. Power software was used.

Alpha = 0.05, Power = 0.95, large effect size was considered = 0.8. using G\* power sample sized of each group was found to be 35 samples/ cases. Hemoglobin concentration and RBC in dices were calculated on ADVIA analyzer. Sugar and hba1c levels were measured by VITROS 5600 analyzer Statistical analysis was performed using SPSS software. Data are expressed as mean ± SD. Unpaired t-test were used for comparing the two groups. Statistical sign if I cance was assumed if P value less than 0.05.

**Result**

Table 1 shows females cases with mean age (49± 12.8) years had Hb levels of 9.2±1.1 gm %, HbA1c levels of 7.9 ± 1.9 % and females controls with age (52±14) years

had Hb levels of 12.3 ± 0.5 gm % and HbA1c levels of 8.2±1.2%. Males cases with age (55 ±14.2) years with HbA1c levels of 7.2±2.5 % and Hb levels of 7.3±1.2 gm %. Male controls of age (40±20) years had HbA1c levels of 6.7±1.3 % and Hb 14.2±1.1 gm %. Mean RBC count in cases 3.2±0.7 and control sare 4.8±0.5. Table 2 shows female cases under the age of 50 showed Hba1c levels of 7.3±1 and above 50 showed 8.5±2.1%. Female controls below 50 years of age showed hba1c levels of 7.5±0.7 and above 50 showed hba1c levels of 8.2 ±1.3 %. Male cases below 50 years showed Hba1c levels 7.9±1.7 and above 50 showed Hba1c levels 9.7±2.6. Male controls below 50 years showed Hba1c levels 6.7 ± 0.6 % and above 50 showed Hba1c levels 7.3± 1.6.

Table 1: Distribution of Haemoglobin profile.

Sr No	Variable	Cases35(50%)		Controls35(50%)		T Value	P. Value
		Male14(20%)	Female21(30%)	Male14 (30%)	Female21(20%)		
1	Age (Mean±SD)	55± 14.2	49±12.8	40±20	52± 14	Male -1.846 Female 0.47	Male 0.08 Female 0.63
2	Hb (gm%)	7.3±1.2	9.2+1.1	14.2±1.1	12.3±0.5	Male13.49 Female6.983	Male<0.0001 Female<0.0001
3	HbA1c (%)	8.785±2.24		7.845±1.42		-1.585	0.121
		7.2+2.5	9	3	2	Male-0.606 Female 0.374	Male 0.55 Female0.71
4	RBC Count (Million/ mm <sup>3</sup> )	3.2±0.7		4.8±0.5		8.318	<0.0001

Graph 1: Distribution of HbA1c as per RBC.

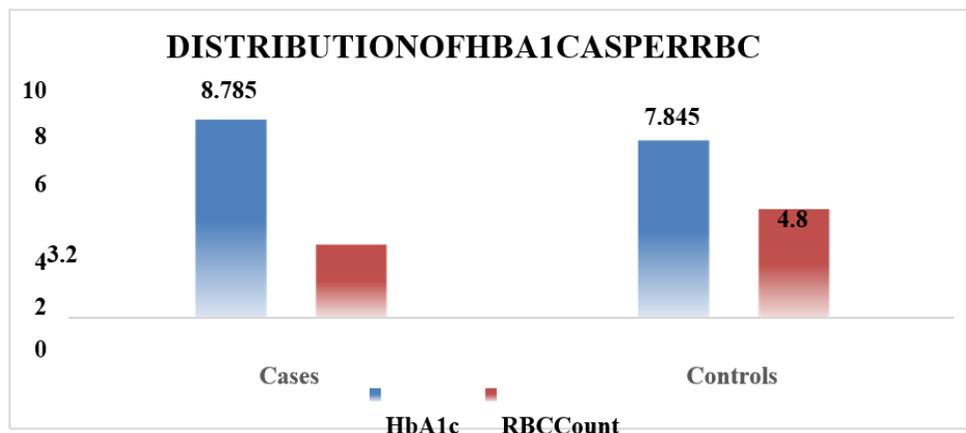
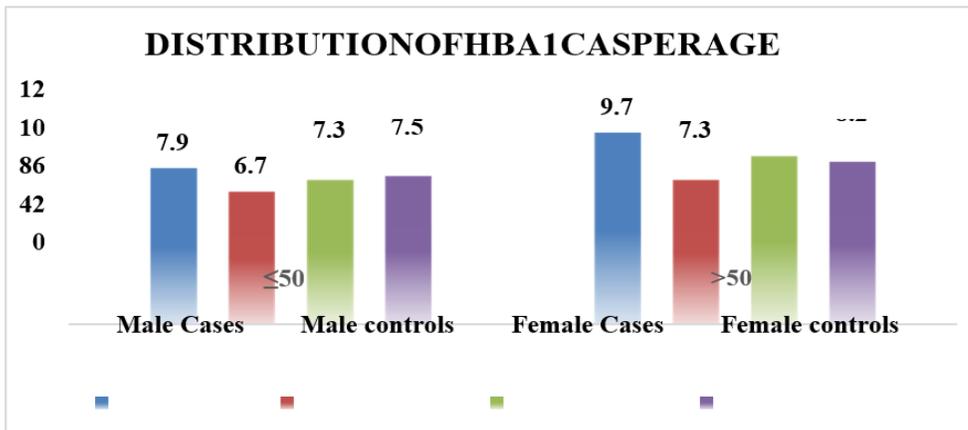


Table 2: Distribution of HbA1c as per age.

Sr. No	Age group (Years)	Cases 35 (50%)		Controls 35 (50%)		T Value	P Value
		Male 14(20%)	Female 21(30%)	Male 21(30%)	Female 14(20%)		
1	≤50	7.9±1.7	7.3±1	6.7±0.6	7.5±0.7	Male-2.349 Female-0.464	Male-0.02 Female-0.64
2	>50	9.7±2.6	8.5±2.1	7.3±1.6	8.2±1.3	Male-2.635 Female-0.340	Male-0.01 Female-0.73

Graph 2: Distribution of HbA1c as per age.



**Discussion**

Present cross sectional retrospective descriptive study was performed on 70 diabetic subjects of both genders. HbA1c, Hb, RBS and RBC count were performed in all. Hb ≤ 10 gm % were taken as cases (N=35) and Hb > 10 gm % were taken as controls (N=35). Results obtained consist of female cases with mean age (49±12.8) years had Hb levels of 9.2±1.1 gm %, HbA1c levels of 7.9±1.9 % and female controls with age (52±14) years had Hb levels of 12.3 ± 0.5 gm % and HbA1c levels of 8.2±1.2%. Male cases with age (55±14.2) years with HbA1c levels of 7.2±2.5 % and Hb levels of 7.3±1.2 gm %. Male controls of age (40±20) years had HbA1c levels of 6.7±1.3 % and Hb 14.2±1.1 gm %. Mean RBC count in cases 3.2±0.7 and control sare 4.8±0.5.

Female cases under the age of 50 showed Hba1c levels of 7.3±1 and above 50 showed 8.5 ±2.1%. Female controls below 50 years of age showed Hba1c levels of

7.5±0.7 and above 50 showed Hba1c levels of 8.2 ±1.3%. Male cases below 50 years showed Hba1c levels 7.9±1.7 and above 50 showed Hba1c levels 9.7±2.6. Male controls below 50 years showed Hba1c levels 6.7 ±0.6 % and above 50 showed Hba1c levels 7.3±1.6 (Table1&2). In similar study by Rashed E Retal (2020)<sup>15</sup> they found that correlation of HbA1c and blood sugar levels with RBC parameter indicates positive correlation with RBC count and negative correlation with MCV and MCH. Comparison among diabetic, pre - diabetic and non-diabetic patients showed significantly higher mean of RBC count, Hb concentration and Hct in diabetic patients, and the mean MCV and MCH were significantly higher in non – diabetic compared with pre-diabetic and diabetic. They concluded that the low level of HbA1c has been found to shorten RBCs life span, which is affected by RBC parameters and decrease of RBC life span in hyper glycaemia patients. Nitin Sin

haetal (2012)<sup>16</sup> in their study found mean base line HbA1c level in anemic patients (4.6%) was significantly lower than that in the control group (5.5%,  $P < 0.01$ ). There was a significant difference between the base line values of patients and controls. HbA1c levels and absolute HbA1c levels increased with treatment of iron deficiency anemia which could be attributed to nutritional deficiency and/ or certain unknown variables. Sree dev Narayanan et al (2020)<sup>17</sup> in their study 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.

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. They concluded that a significant positive relationship found between FBS and HbA1c level only. No correlation was found between total Hemoglobin level and HbA1c. Patients with mild to moderate anemia revealed a high HbA1c value correlating only with the blood sugar status. Shaoying Ye et

Al (2016)<sup>18</sup> in their study explores the impact of HbA1c levels on the structure of hemoglobin (Hb) in patients with type 2 diabetes.

They concluded that high HbA1c levels might be a factor contributing to Hb structural modifications in diabetic patients.

FTIR spectral analysis can provide a novel way to investigate the pathogenesis of type 2 diabetes mellitus. Renuka Petal (2020)<sup>19</sup> in their study found a negative correlation between HbA1c and MCV, MCH and MCHC and positive correlation with RDW.

Hematological parameters like MCH, MCV and MCHC should be taken into account in interpreting HbA1c level in diagnosis and management of prediabetes and diabetes.

Increased HbA1c level indicating elevated hyperglycaemia may increase the  $\beta$ -sheet structure content of Hb, causing it to aggregate. These consequently decrease solubility of Hb in RBC which increases viscosity of the contents of RBC.

These changes may reduce the deformability of the erythrocytes in diabetic patients and impair erythrocyte flexibility, which further adversely affects the microcirculation and leads to diabetic complications by impeding their flow through capillaries<sup>20</sup>.

In addition to these structural changes in Hb might be induced by persistent hyperglycaemia due to decrease in peroxidase activity of Hb<sup>20</sup>.

In view of changes in RBC & Hb altering HbA1c values, diagnostic or management purpose in diabetics will require further investigation especially RBC indices which reflect the RBC life span.

Also nutritional influence of iron, vitamin B12 and folic acid on HbA1c needs to be evaluated.

### Conclusion

1. HbA1c level is increased in male anemic diabetic patients.
2. Anemic diabetic females and males more than 50 years of age have raised HbA1c levels.
3. HbA1c levels are increased in patients with low RBC count
4. While interpreting HbA1c level, type of anemia and RBC count should be taken into consideration.

### Abbreviations

HbA1c- Glycosylated hemoglobin, Hb- Hemoglobin, CBC- Complete blood count, RBC- Red blood cells.

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