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# Association of Glycosylated Haemoglobin to Red Blood Corpuscles Indices

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## ABSTRACT

Variations in Red Blood Corpuscles indices survival can cause clinically important differences in Glycosylated Haemoglobin levels. Many previous studies have shown that Glycosylated Haemoglobin levels did not correlate with the degree of anemia for a given mean plasma glucose and hence Glycosylated Haemoglobin level in patients with iron deficiency anemia should be interpreted with caution. Glycosylated Haemoglobin level is not affected by degree of anemia or glucose level alone but red blood corpuscles indices, notably Mean Corpuscular Haemoglobin, Mean Corpuscular Haemoglobin Concentration, Platelet Distribution Width, Red Cell Distribution Width & White Blood Cells. fractions may affect. This study has proved that all the above indices are significantly correlated to Glycosylated Haemoglobin levels, thus giving an understanding that Glycosylated Haemoglobin levels in Diabetes with iron deficiency anemia could be interpreted with Red Blood Corpuscles indices.

**Keywords:** Anemia, Glycosylated Haemoglobin, Red Blood Corpuscles , Platelet Distribution Width, White Blood Corpuscles.

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## INTRODUCTION

HbA1c concentrations in diabetic patients with Iron Deficiency Anemia (IDA) should be interpreted with caution. Impaired Red Blood Corpuscles (RBC) in Type 1 diabetes (Type I DM) may at least partly be attributed to the elevated ionised calcium ($Ca^{++}$) and poor glycaemic control. Modest vitamin E supplementation (100 IU/day) can significantly lower blood HbA1c and Triglycerides (TG) levels and does not have any effect on red cell indices in Type I DM patients. Administration of high dose erythropoietin (rEPO) in Hemodialysis patients (HDP) increased glycated Albumin (GA)/Glycosylated Haemoglobin(HbA1c) ratio, suggesting that rEPO administration may influence RBC life span. Thus, GA is more suitable index than HbA1c for the evaluation of diabetic control in diabetic HDP with high dose rEPO. Glycation of the membrane proteins rather than increase in membrane cholesterol content alters the enzyme function. Among Platelet Indices Mean Platelet Volume (MPV) and Platelet distribution width (PDW) were found to be significantly increased in diabetic patients as compared to control group. Recently, HbA1c level of 6.5% has been determined to be a criterion for diabetes mellitus (DM), and it is a widely used marker for the diagnosis of DM. The specificity of HbA1c for diagnosis of DM was significantly lower in the anemic subgroup ($P<0.05$). These results suggest that the diagnostic significance of HbA1c might be limited in anemic patients. HbA1c is not affected by the blood glucose levels alone, and there are various confounding factors when HbA1c is measured, especially that of iron deficiency, which is the commonest of the deficiency diseases worldwide. Like blood glucose levels, HbA1c levels are also affected by the presence of variant hemoglobins, hemolytic anemias, nutritional anemias, uremia, pregnancy, and acute blood loss. HbA1c levels and absolute HbA1c levels increased with treatment of iron deficiency anemia. This could be attributable to nutritional deficiency and/or certain unknown variables.

**Review of Literature**

The mean age of circulating Red Blood Corpuscles range from 39 to 56 days in diabetic subjects and 38 to 60 days in non-diabetic controls. Glycated Haemoglobin (HbA1c) synthesis was found to be linear and correlated with mean whole blood HbA1c ($R^2 = 0.91$). The observed variation in RBC survival was large enough to cause clinically important differences in HbA1c for a given mean blood glucose.[1] A positive correlation between iron deficiency anemia and increased HbA1C levels, especially in the controlled diabetic women and individuals having Fasting Plasma Glucose (FPG) between 100-126 mg/dL and before altering the treatment regimen for

diabetic patient, presence of iron deficiency anemia should be considered.[2] Consideration should be given for performing glucose testing in patients with Iron deficiency Anemia (IDA) to ascertain the reliability of HbA1c in the diagnosis of diabetes. HbA1c concentrations in diabetic patients with IDA should be interpreted with caution.[3] Erythrocyte indices are associated with HbA1c, independently of plasma glucose levels, in pre-menopausal women even when they are not anaemic. This should be appreciated when interpreting HbA1c in pre-menopausal patients with diabetes.[4]

Impaired Red blood corpuscles deformability in Type 1 diabetes (Type I DM) may at least partly be attributed to the elevated ionized calcium ($Ca^{++}$) and poor glycaemic control. In addition, favorable effects of a ($Ca^{++}$)channel blocker on and RBC-df have been demonstrated.[5] Modest vitamin E supplementation (100 IU/day) can significantly lower blood HbA1c and Triglycerides (TG) levels and does not have any effect on red cell indices in Type I DM patients.[6] Measurement of HbA1c provides an excellent measure of glycaemic control for diabetic patients. However, haemoglobin (Hb) variants are known to interfere with HbA1c analysis have been studied in detail. Influence of HbE trait on HbA1c analysis have been studied in detail.[7]Administration of high dose erythropoietin (rEPO) in Hemodialysis patients (HDP) increased glycated Albumin (GA)/HbA1c ratio, suggesting that rEPO administration may influence RBC life span. Thus, GA is more suitable index than HbA1c for the evaluation of diabetic control in diabetic HDP with high dose rEPO.[8]

Liable glycated Haemoglobin (LHbA1c) is closely associated with contemporaneous intracellular and extracellular glucose but not stable HbA1c. Since LHbA1c is not predictive of Hemoglobin Glycation Index(HGI) or glycation status, this finding suggests that biological variation in stable HbA1c is related to haemoglobin glycation events that occur after the formation of LHbA1c.[9] Erythrocyte membrane Na+-K+ATPase activity is significantly related to glycemic status rather than to the lipidemic status of individuals in the Asian Indian population. Glycation of the membrane proteins rather than increase in membrane cholesterol content alters the enzyme function[10] The mean HbA1c and absolute HbA1c levels in anemic on diabetic patients were significantly lower than that in the control group. 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 / prediabetics.[11] There have been tremendous effort devoted to identifying the heritable basis of types 1 and 2 diabetes; however, studies on the heritable contributors to these mediators of glucose effect on complications are only beginning. New evidence for normal biologic variation in the distribution of glucose into the RBC

intracellular compartments and RBC lifespan in people with and without diabetes represent candidates for heritable mechanisms and contributors to the rise in HbA1c with age. Taken as a whole, genetic and mechanistic evidence suggests new potential targets for complications prevention and improvement in complications risk estimation. These observations could help tilt the risk—benefit balance in glycemic control toward a more beneficial outcome.[12] Among Platelet Indices, Mean Platelet Volume (MPV) and Platelet distribution width (PDW) were found to be significantly increased in diabetic patients as compared to control group. It is considered that alteration in platelet morphology and functions are associated with pathological processes and increased risk of vascular complications in patients with diabetes.[13]

Recently, a hemoglobinA1c (HbA1c) level of 6.5% has been determined to be a criterion for diabetes mellitus (DM), and it is a widely used marker for the diagnosis of DM. However, HbA1c may be influenced by a number of factors. Anemia is one of the most prevalent diseases with an influence on HbA1c; however, its effect on HbA1c varies based on the variable pathophysiology of anemia. Clinical characteristics were found to be similar between each subgroup. Also, when glucose levels were within the normal range, the difference in mean HbA1c was not significant (P=0.580). However, when plasma glucose levels were above the diagnostic cutoff for pre-diabetes and DM, the mean HbA1c of the anemic subgroup was modestly higher than in the non anemic group. The specificity of HbA1c for diagnosis of DM was significantly lower in the anemic subgroup (P<0.05).These results suggest that the diagnostic significance of HbA1c might be limited in anemic patients.[14]

HbA1c is not affected by the blood glucose levels alone, and there are various confounding factors when HbA1c is measured, especially that of iron deficiency, which is the commonest of the deficiency diseases worldwide. It is hence prudent to rule out IDA before making a therapeutic decision, based on the HbA1c levels[15]

Like Blood glucose levels, HbA1c levels are also affected by the presence of variant hemoglobins, hemolytic anemias, nutritional anemias, uremia, pregnancy and acute blood loss and absolute HbA1c levels increased with treatment of iron deficiency anemia and it could be attributable to nutritional deficiency and/or certain unknown variables.[16]

## MATERIALS AND METHODS

110 patients in the age group of 22-85 years consisting of 68 males (age group 22-71 years) and 42 females (age group 26-85 years) were selected for this study. All 110 patients attended the

master Health Check up. The study is mainly to evaluate the association of HbA1c to RBC indices, and hence laboratory data available for these patients were used for this study.

**Inclusion Criteria**

Laboratory results from patients who attended routine master health check up and who were investigated for the analytes used in this study were included.

**Exclusion Criteria**

Results from patients who showed abnormal lipid profile were excluded.

**Statistical Analysis**

For statistical analysis of data, a software downloaded from the website http://www.vassarstats.net was used to calculate correlation coefficient (r), students 't' distribution (t) and probability (p) between HbA1c and RBC indices.

**Statistical Parameter r, t & p for all patients (N=110)**

| S.no | Group | Pairs Compared | r | t | p |
|---|---|---|---|---|---|
| 1 | All patients | HbA1c Vs MCHC | -0.1760 | -1.858 | <0.05 |
| | (n=110) | HbA1c Vs PDW | 0.2500 | 2.683 | <0.01 |
| 2 | Males (n=68) | HbA1c Vs MONO | -0.2065 | -1.715 | <0.05 |
| | | HbA1c Vs PDW | 0.2339 | 1.954 | <0.05 |
| 3 | Females (n=42) | HbA1c Vs HGB | -0.3091 | -2.056 | <0.05 |
| | | HbA1c Vs MCV | -0.3283 | -2.198 | <0.05 |
| | | HbA1c Vs MCH | -0.3954 | -2.723 | <0.01 |
| | | HbA1c Vs MCHC | -0.4318 | -3.028 | <0.01 |
| | | HbA1c Vs RDW | 0.3923 | 2.697 | <0.01 |
| | | HbA1c Vs NEUT | -0.3617 | -2.454 | <0.01 |
| | | HbA1c Vs LYMP | 0.3670 | 2.495 | <0.01 |
| | | HbA1c Vs PDW | 0.2690 | 1.766 | <0.05 |

Abbreviations used in the table

MCH- Mean Corpuscular Haemoglobin ; PDW – Platelet Distribution Width; MCHC – Mean Corpuscular Haemoglobin Concentration; Mono- Monocytes; HGB- Haemoglobin ; MCV-Mean Corpuscular Volume; RDW- Red Cell Distribution Width; NEUT-Neutrophil; LYMP-Lymphocyte.

## RESULTS AND DISCUSSION

The above table shows the statistical parameters r, t, and p obtained for all patients as well as for males and females. All 110 patients showed a significant negative association ($p<0.05$) of HbA1c to MCHC and a positive significant ($P<0.01$) to PDW. Among the 68 males, HbA1c showed a negative association ($P<0.05$) to monocytes and a positive association ($P<0.05$) to PDW. Among 42 female patients, while a feeble association ($p<0.05$) was found for HbA1c to HCB, MCV, PDW, a highly significant association of HbA1c to MCH, RDW, Neut, Lymp

(P<0.01) suggesting that female populations circulating HbA1c shows a good associations to the above RC indices mentioned. Any change in these indices will affect the level of circulating HbA1c concentrations. The outcome of this study are in consisting with earlier findings done partially on the association between HbA1c to RBC indices, [4] however this study has identified components of RBC indices with which HbA1c shows significant association. Previous studies have pointed out alterations/increase in platelet counts in Diabetic patients but they were not correlated to HbA1c [13].

## CONCLUSION

This study done on a reasonable number of 110 patients comprising of both men and women has clearly established that some key RBC indices shows good associations to HbA1c level giving the impression that in Diabetic patients HbA1c level correlates well with RBC indices rather than total haemoglobin or total WBC. The data shown in this study could be used as interpretive guide to justify the level of HbA1c in diabetic populations with any type of anemia. Further studies are required in this field to dynamically establish the clinical usefulness of RBC indices to validate HbA1c results in diabetic patients with anemia.

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