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RESEARCH ARTICLE

Association of increased levels of Glycated hemoglobin with variations in Red blood cell parameters in Diabetes mellitus.

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Abstract

Background and Aim: Hyperglycaemia has multiple effects on the red blood cell (RBC), including glycation of haemoglobin, reduced deformability and reduced lifespan. Red cell distribution width (RDW) is a measure of erythrocyte variability and heterogeneity. The aim of this study was to explore the relationships between HbA1c and red blood cell parameters in patients of diabetes mellitus.

Methods: This cross-sectional study was conducted on 204 diabetic patients in KMC Hospital Manipal. HbA1c, FBG, HbA1c, Hb, MCV, MCH, MCHC and RDW were measured in these patients. A Pearson product-moment correlation coefficient was computed to assess the relationship between HbA1c and red blood cell parameters.

Results: RDW significantly correlated inversely with HbA1c. There was no significant correlation between HbA1c and other red blood cell parameters.

Conclusion: In contrast to the observations of previous studies, this study showed that HbA1c was inversely correlated with RDW and there was no significant correlation between RDW and MCV, MCH and MCHC.This could be attributed to decreased lifespan of red blood cells and/or certain unknown variables. Further studies are warranted.

INTRODUCTION

Diabetes mellitus is one of the most common and complex metabolic disorders. Incidence of diabetes mellitus continues to rise with increase in elderly population, sedentary lifestyle and obesity .Diabetes mellitus is a chronic illness characterized by increased risk of macrovascular and microvascular complications. These complications of diabetes mellitus are responsible for the majority of morbidity and mortality associated with the disease. The risk of these complications is related with the degree and duration of hyperglycemia .When plasma glucose is episodically elevated over time, small amount of hemoglobin A are nonenzymatically glycated to form glycated hemoglobin – HbA1c. HbA1c has a glucose attached to the terminal valine in each β chain. The concentration of HbA1cdepends on concentration of glucose in the plasma and the duration of hyperglycemia. HbA1c concentration is measured clinically as an index of diabetic control for a period over past 8 to 12 weeks. High levels of Glycated hemoglobin have been shown to impair endothelium mediated vasoactive responses, which can lead to hypertension and vascular diseases in diabetic patients (1). Microangiopathy is a result of changes in the microcirculation which is caused by changes in blood viscosity and red blood cell deformability (2). One of the factors determining the cell deformability is the red blood cell cytoplasmic viscosity. Cytoplasmic viscosity is principally determined by the properties and the concentration of hemoglobin in the red blood cells (3). Hermann and Muller (4) attributed the increased intracellular viscosity of diabetic erythrocytes to higher levels of glycated hemoglobin, which would lead to the reduction in red blood cell deformability. While Watala et al. (5, 6) attributed an increase in the erythrocyte internal viscosity to glycation-derived structural alterations in hemoglobin molecules.

The quantitative and qualitative analysis of red cell parameters as measured by the red blood cell count, Hematocrit (Hct), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC) gives the indication of red cell deformability and the hemorheological state .The red blood cell distribution width (RDW) is a measurement of the size variation among circulating red cells and is calculated as part of the routine complete blood count. The RDW, along with mean cell volume (MCV), is useful in the differential diagnosis of the causes of anemia (7). RDW is shown to be significantly associated with diabetic nephropathy in a type 2 diabetic population with advanced proliferative retinopathy independent of traditional risk factors, including diabetes duration and glycaemic control (8).Increased RDW is associated with decreased red cell deformability in metabolic diseases although the mechanism is not clear (9,10).Slow glycosylation and consistent elevation of HbA1c can be associated with functional and structural change in Hb molecule and cytoplasmic viscosity within each red blood cell, along with osmotic disturbances within the cell. These changes may be reflected in any one or all the red cell analytic parameters such as Hct, MCV, MCH, and MCHC. Since the microvascular complications of diabetes mellitus are attributed to increase in HbA1c, changes in red cell deformability and other hemorheological alterations, and no in depth study has been done in this direction, this study was planned to show the association of increased HbA1c with the variations in red cell analytic parameters in patients of diabetes mellitus.

Methods:

After taking the Institutional Ethics Committee clearance, the study was conducted on patients diagnosed with diabetes mellitus, from KMC Hospital, Manipal. Patients diagnosed with diabetes mellitus were according to ADA: HbA1c $\geq$ 6.5% or FBG $\geq$ 126 mg/dl or 2-hour plasma glucose $\geq$ 200 mg/dl during an OGTT. The patients with any anemia or other red cell or hemoglobin disorders or patients having hemolytic disorders including infections like malaria were excluded from the study.

The following data was collected from the patients: Fasting Blood Glucose, Red blood cell count, Hemoglobin level, Hematocrit, Glycated Hemoglobin level (HbA1c), MCV, MCH, MCHC and RDW. The blood parameters were analyzed using by Automated Hematology Analyzer – Beckmon Coulter 750 using impedance method. FBG was analyzed by Hexokinase method on Roche Cobas 6000 analyzer. HbA1c was analyzed by automated hemoglobin analyzer-Biorad Variant II using cation exchange HPLC method.

Statistical Analysis: Analysis of data was performed using SPSS 16 (Statistical Package for Scientific Studies) for Windows. Data were statistically described in terms of mean and standard deviation (SD). Correlation between various variables was done using Pearson moment correlation equation for linear relation in normally distributed variables. p value less than 0.05 was considered statistically significant.

Results

A Total of 204 diabetic patients were taken in the study.66.2% were males and 33.8% were females. The mean age of the study population was 56.25 years. Mean FBG was 185.78 mg/dl. The highest value was 623 mg/dl and the lowest was 66 mg/dl. Mean HbA1c was 8.76 %. The highest value was 16.4% and the lowest was 6.5%. Mean MCV was 87.12 fl. Mean MCH was 28.88 pg. Mean MCHC was 33.14 mg/dl.A Pearson product-moment correlation coefficient was computed to assess the relationship between FBG, HbA1c, RBC count, Hb, PCV, MCV, MCH, MCHC and RDW. (Table 1)RDW was significantly correlated with HbA1c, however this inverse correlation itself was relatively mild ($r = -0.235$, $p = 0.001$) (Figure 1)There was a no significant correlation between HbA1c and RBC count, Hb, PCV, MCV, MCH, and MCHC. (Figure 2 to 4)FBG was highly significantly correlated with HbA1c ($r = 0.563$ $p < 0.001$). But there was no significant correlation between FBG and RDW. (Table 2). RDW correlated inversely with hemoglobin ($r = –0.223$, $p = 0.001$), hematocrit ($r = –0.150$, $p = 0.033$), MCV ($r = –0.088$, $p = 0.209$), MCH ($r = –0.187$, $p = 0.007$) and MCHC ($r = –0.340$, $p < 0.001$).Additionally, significant correlations were found between RBC count, Hb, PCV, MCV, MCH and MCHC. (Table 1)

Table 1 Correlation between FBG, HbA1c, RBC count, Hb, PCV, MCV, MCH, MCHC and RDW **Correlations**

		FBG	HbA1c	RBC COUNT	Hb	Haematocrit	MCV	MCH	MCHC	RDW
FBG	Pearson Correlation	1	.563**	.143*	.172*	.176*	.043	.034	-.004	-.135
	Sig. (2-tailed)		.000	.041	.014	.012	.540	.633	.954	.055
	N	204	204	204	204	204	204	204	204	204
HbA1c	Pearson Correlation	.563**	1	.122	.159*	.138*	.019	.051	.104	-.235**
	Sig. (2-tailed)	.000		.083	.023	.049	.783	.470	.140	.001
	N	204	204	204	204	204	204	204	204	204
RBC COUNT	Pearson Correlation	.143*	.122	1	.732**	.793**	-.408**	-.407**	-.172*	-.073
	Sig. (2-tailed)	.041	.083		.000	.000	.000	.000	.014	.301
	N	204	204	204	204	204	204	204	204	204
Hb	Pearson Correlation	.172*	.159*	.732**	1	.970**	.277**	.315**	.228**	-.223**
	Sig. (2-tailed)	.014	.023	.000		.000	.000	.000	.001	.001
	N	204	204	204	204	204	204	204	204	204
Haematocrit	Pearson Correlation	.176*	.138*	.793**	.970**	1	.224**	.194**	-.010	-.150*
	Sig. (2-tailed)	.012	.049	.000	.000		.001	.005	.885	.033
	N	204	204	204	204	204	204	204	204	204
MCV	Pearson Correlation	.043	.019	-.408**	.277**	.224**	1	.950**	.253**	-.088
	Sig. (2-tailed)	.540	.783	.000	.000	.001		.000	.000	.209
	N	204	204	204	204	204	204	204	204	204
MCH	Pearson Correlation	.034	.051	-.407**	.315**	.194**	.950**	1	.536**	-.187**
	Sig. (2-tailed)	.633	.470	.000	.000	.005	.000		.000	.007
	N	204	204	204	204	204	204	204	204	204
MCHC	Pearson Correlation	-.004	.104	-.172*	.228**	-.010	.253**	.536**	1	-.340**
	Sig. (2-tailed)	.954	.140	.014	.001	.885	.000	.000		.000
	N	204	204	204	204	204	204	204	204	204
RDW	Pearson Correlation	-.135	-.235**	-.073	-.223**	-.150*	-.088	-.187**	-.340**	1
	Sig. (2-tailed)	.055	.001	.301	.001	.033	.209	.007	.000	
	N	204	204	204	204	204	204	204	204	204

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Figure 1. Correlation between HbA1c and RDW (r = -0.235, p = 0.001)

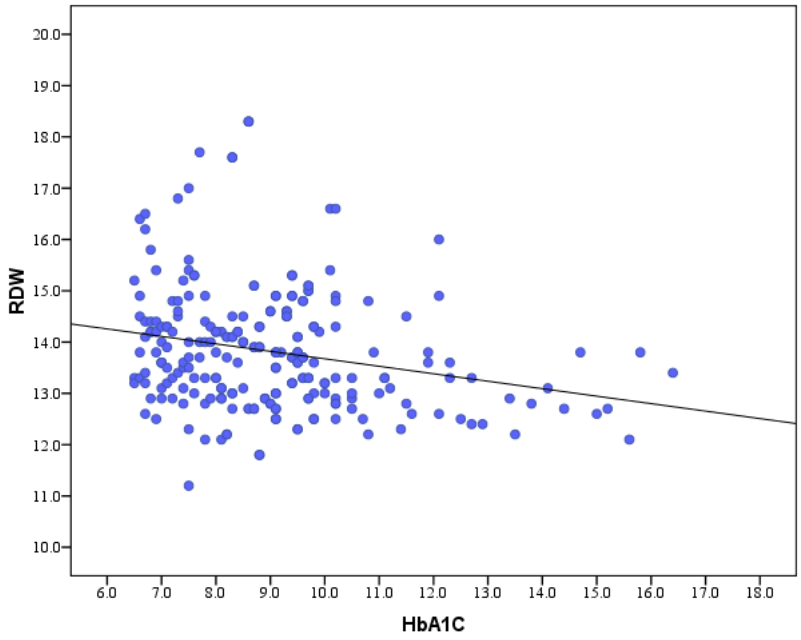


Figure 2. Correlation between HbA1c and MCV (r = 0.019, p = 0.783)

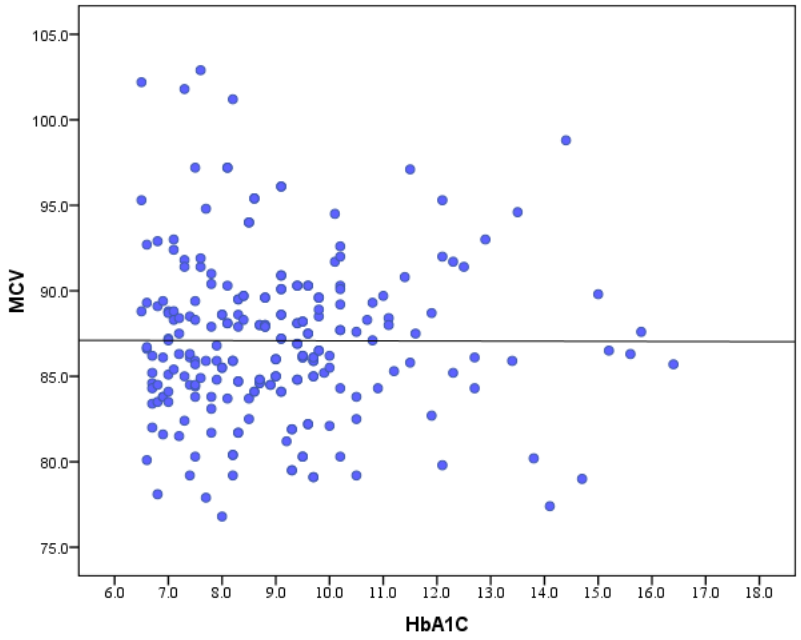


Figure 3. Correlation between HbA1c and MCH (r = 0.051, p = 0.470)

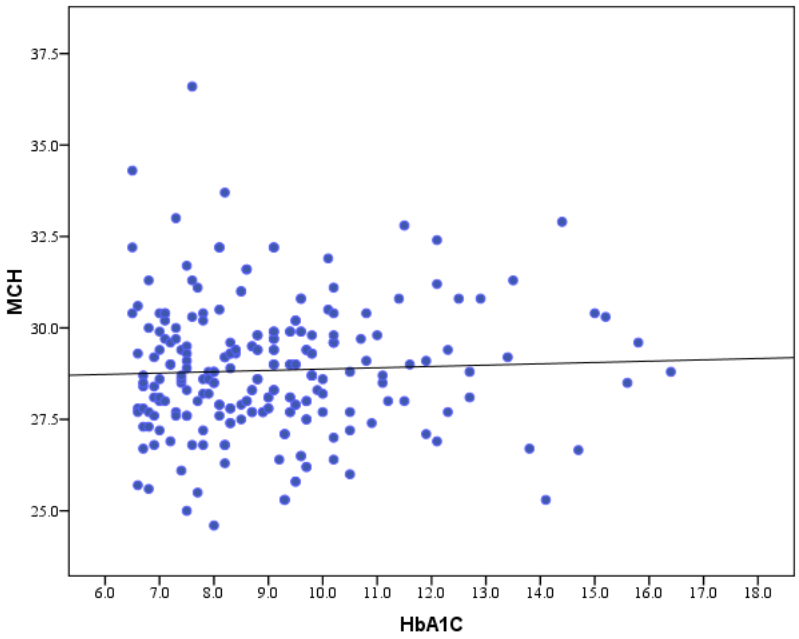
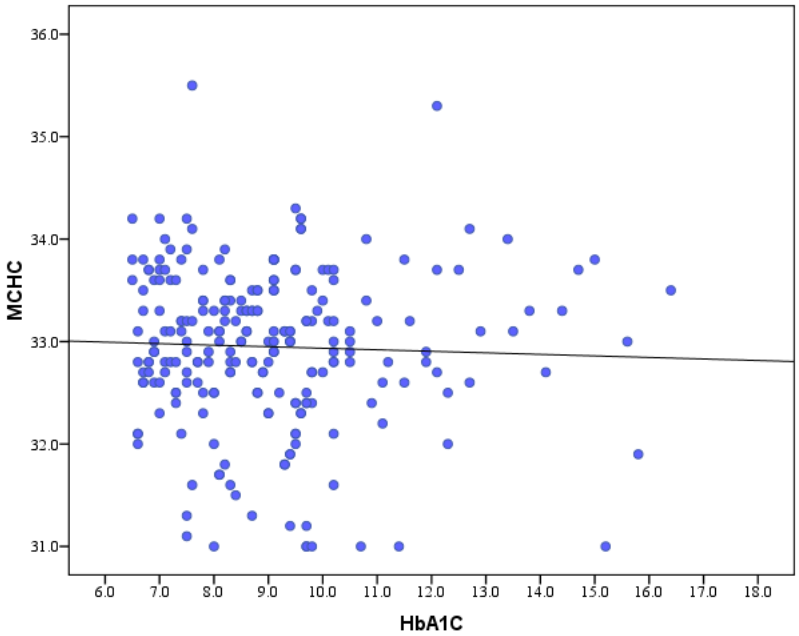


Figure 4. Correlation between HbA1c and MCHC (r = 0.104, p = 0.140)



Discussion

Diabetes mellitus is a disease associated with abnormal substrate metabolism, arising from insulin deficiency or decreased responsiveness of tissues to insulin. Insulin is the key hormone in substrate homeostasis and insulin deficiency results in wide variety of metabolic defects affecting carbohydrate, lipid and protein metabolism (11). Insulin deficiency and hyperglycemia affects the tissues as well, resulting in complications of diabetes. Hyperglycemia affects erythrocytes and the vascular endothelial cells, including the walls of capillaries which lead to vascular complications.

HbA1c measurement is a standard part of diabetes care including diagnosis and management. HbA1c is shown to be a predictor of complications of diabetes as well as mortality and morbidity. Red blood cell distribution width (RDW) is a measure of variation of the volume of red blood cells. The ‘‘width’’ referring to the width of the distribution curve and not the width of the cells. Increased RDW results from heterogeneity of erythrocyte size. Increased erythrocyte size heterogeneity may result from iron or folate deficiency, decreased erythrocyte life-span, or impaired erythropoiesis. Both HbA1c and RDW are shown to be as prognostic markers in diabetic as well as in nondiabetic patients.

This study shows a significant correlation between RDW and HbA1c. However this inverse correlation is relatively mild ($r = -0.235$, $p = 0.001$). In a study done by Sherif *et al* a positive correlation was shown between RDW and HbA1c, however it was not statistically significant ($p = 0.92$) (12).

HbA1c is formed by the nonenzymatic attachment of glucose to the N-terminal valine of the β-chain of hemoglobin. The concentration of HbA1c depends not only on the concentration of glucose in blood but also on the lifespan of the erythrocytes. Diabetes mellitus and the related metabolic syndrome are associated with chronic inflammation (13).Inflammation may influence erythropoiesis, erythrocyte circulatory half-life and erythrocyte deformability, promoting anisocytosis and thus increasing RDW levels (14). Thus as lifespan of erythrocytes is decreased there will be a decrease in HbA1c concentration as the average time during which erythrocytes are exposed to glucose is reduced. This probably may explain the negative correlation between RDW and HbA1c. A study by Virtue *et al* showed an inverse relationship between erythrocyte lifespan and HbA1c percentage and they suggested that there is a hyperglycemia related decrease in erythrocyte survival that results in a progressively greater underestimation of the severity of the hyperglycemia (15).

There were no significant correlation between RDW and FPG. This indicates that the correlation of RDW with HbA1c is independent of plasma glucose levels.

In the present study, no significant correlation was found between HbA1c and other red blood cell analytic parameters. In a study by Koga *et al* in nondiabetic premenopausal women it was shown that HbA1C levels were inversely associated with MCV ($r = -0.368$, $p < 0.0001$) and MCH ($r = -0.320$, $p < 0.0001$). But in postmenopausal women no such relation was observed between HbA1c and MCV ($r = -0.019$, $p = 0.771$) and MCH ($r = -0.104$, $p = 0.107$) (16). Also a study by Hardikar *et al* in nondiabetic population showed an inverse correlation between HbA1c and MCV ($r = -0.22$, $p < 0.05$), MCH ($r = -0.30$, $p < 0.05$) and MCHC ($r = -0.32$, $p < 0.05$) (17). It was suggested that in conditions with low MCH, a decrease in the haemoglobin concentration may lead to an increase in the glycation fraction (16). In a study, in diabetic women with HIV infection, by Glesby *et al* an inverse correlation was found between HbA1c and MCV. They suggested that higher MCV values are a marker of a greater proportion of younger erythrocytes that have had a shorter time to become glycated due to greater red blood cell turnover in the HIV-infected patients (18).Further studies are needed in diabetic population to explain how red blood cell parameters influence HbA1c levels.

Various studies have shown that RDW is inversely correlated with haemoglobin levels, hematocrit, MCV, MCH, MCHC(19,10).In the present study a similar inverse correlation between RDW and other red blood cell parameters was seen. Vayá *et al* suggested that metabolic diseases lead to perturbations of erythropoiesis which can lead to higher anisocytosis and probably lowering MCHC, which may explain the inverse relationship between RDW and MCHC (10).

Conclusion

The present observational study shows a statistically significant but mild inverse correlation between HbA1c and RDW. No significant correlation between HbA1c and other red blood cell analytic parameters was observed. However there are discrepancies between the correlation between HbA1c and Red blood cell parameters in various studies. More studies, using large sample size may help to explain the correlation between HbA1c and Red blood cell parameters. Once the correlation is established Red blood cell parameters may be considered while interpreting HbA1c results.

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