Original article:

Relationship between the levels of glycosylated haemoglobin red cell width and haemoglobin levels in patients with type 2 diabetes mellitus

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ABSTRACT

INTRODUCTION: While some studies have shown that HbA1c levels were increased in anaemia, there were no differences between HbA1c levels of anaemia patients and controls in some other studies.

AIM OF THE STUDY: Since anaemia is common in our population and the effect of anaemia or RDW on HbA1c levels has not been studied, we thought that it would be prudent to assess the impact of these confounding factors on the HbA1c levels in patients with type 2 diabetes mellitus.

RESULTS: Serum haemoglobin levelswere significantly lower in the study group of patients when compared with reference group. In the study group, a negative correlation was found between the levels of HbA1c and Hb and a positive correlation between the levels of HbA1c and RDW. However, the relationship between the variables was weak.

CONCLUSION: The relatively high prevalence of anaemia in ourpopulation of patients with diabetes suggests theneed for screening for anaemia inthe diabetes out-patient clinic.

KEY WORDS : Type 2 Diabetes Mellitus- Glycosylated Haemoglobin- Red cell width- Haemoglobin

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is an unremitting progressive disease, and there is a need for strict glycaemic control because of the link between glucose levels and the high risk for microvascular and microvascular and other complications[1, 2]. India has emerged as one of the epicentres of global diabetes mellitus pandemic [3]. Indians have been shown to exhibit clinical and biochemical characteristics that predispose them to T2DM, such as increased insulin resistance and abdominal obesity despite having lower body weight and body mass index (BMI). These characteristics are collectively referred to as the "Asian Indian Phenotype". Patients with T2DM in India generally develop the disease at a younger age than those in other parts of the world (i.e., 45–64 years vs. ≥65 years in developed countries [4].

Glycated haemoglobin or glycosylated haemoglobin (HbA1c) is a form of haemoglobin that is quantified mainly to ascertain the average plasma glucose concentration over extended periods of time. Levels of HbA1c represent the mean blood glucose levels over the preceding 120 days [5].Sincedetermination of glycaemic controlis of paramount importance in the diabetic population

and as improved glycaemic control reduces micro- and macro-vascular complicationsin patients with type-1 and type-2 diabetesmellitusHbA1c measurement has emerged as a cornerstone inthe evaluation of diabetic patients[6]. Both WHO and theADA have been advocating the use of HbA1c for diagnosingtype 2 diabetes, at a value of 6.5% (48 mmol/mol).HbA1c may be affected by a variety of genetic, physiological, haematological and illness-related factors such as haemolyticanaemia, hemoglobinopathies, acute and chronic blood loss, pregnancy and uraemia[7].There are also ethnic and racial differences in HbA1c [8, 9]. Anaemia is frequently found in patients with T2DM [10]. Different cross-sectional studies have reported that prevalence of anaemia in patients with diabetes mellitus range between 14-23% and that it mostly occurs who also have renal insufficiency[11]. It had been claimedthat haemolytic anaemia can lead to decreased HbA1c values due to reduced erythrocyte lifespan, and iron deficiency anaemia may result in increased HbA1c values due to an elongation of the erythrocyte lifespan [12, 13]. However, it is not known to what degree alterations in erythrocyte indices affect HbA1c values.Anaemia had been shown as an independent risk factor for the development of diabetic retinopathy in patients with diabetes and that individuals with anaemia were 1.80 times more likely to develop diabetic retinopathy than individuals with no anaemia [14].

The red cell distribution width (RDW) is an automatedmeasure performed as part of a complete blood cellcount, and it indicates the heterogeneity of red bloodcell sizes, that is, anisocytosis and is known to be associated with HbA1c. It has been used in the past to assist in the identification of type of anaemia.Cumulative evidence from recent studies suggest that RDW can be used to predict outcomesin cardiovascular disease[15].

AIM OF THE STUDY

While some studies have shown that HbA1c levels were increased in anaemia, there were no differences between HbA1c levels of anaemia patients and controls in some other studies and since anaemia is common in our population and the effect of anaemia or erythrocyte indices on HbA1c levels has not been studied, we thought that it would be prudent to assess the impact of these confounding factors on the HbA1c levels in patients with type 2 diabetes mellitus. Since RDW has been found to be associated with endothelialdysfunction that leads to adverse impact in patients with diabetes mellitus, the aim of the present study was therefore to assess whether a correlation exists between the levels of haemoglobin or red cell width and HbA1c inSouth Indian patients with type 2 diabetes mellitus.

PATIENTS AND METHODS

Study design

This study was a prospective study of case records undertaken at a rural tertiary care hospital in southern India, between June 2015 and July 2016. Studies were performed at no extra cost to the participants and patients were not compensated.

Patients

The electronic records of type 2 diabetes patients presenting to the institution as part of the routine care of our centre were reviewed. Patients with type 2 diabetes formed the study group, whereas those who attended the diabetes clinic for screening but were not found to be diabetic (HbA1c <6.5%) served as the reference group. The inclusion was as broad as possible in order to maximize generalization and to reflect the 'real-world' conditions. The study was approved by the Institutional Ethics Committee and written informed consent was obtained from all participants.

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The following data were extracted using a standardized data extraction form: Age, Sex, Body weight, Height, Body Mass Index (BMI), Waist circumference, HbA1c, Haemoglobin, Red cell , distribution width (RDW) and plasma glucose levels (fasting and post prandial).

Measurements

HbA1c was estimated by high performance liquid chromatography using a Variant machine (Bio-Rad, Hercules, CA). Haemoglobin and RDW estimation were carried out by an automated counter.Haemoglobin concentrations suggested by World Health Organisation [16]were used for the diagnosis of anaemia and assessment of its severity.

STATISTICAL ANALYSIS

Continuous variables were expressed as mean ± SD and categorical variables as the number and percentage. Student's t-test and chi square-test were used for comparisons between sex and groups. Analysis of variance (ANOVA) was applied for comparisons. The correlations among variables were determined using Spearman correlations. Multiple linear regression analysis was used to test the dependence of haemoglobin levels on HbA1c levels. A p value of <0.05 was considered as statistically significant.

RESULTS

The demographic and clinical characteristics of theReference and Study groups are shown in Table 1. Both the groups were comparable and there were nosignificant differences with respect to age, sex, weight,and BMI or waist circumference except for duration ofdiabetes.

Table 1

Demographic and Clinical Characteristics

Characteristics (n=28) Mean ± SD	Reference group (n=91) Mean ± SD	Study group
Age (years)	54.8 ± 8.8	58.7 ± 9.5
Women (%)	16 (57)	49 (53)
Body weight (kg)	67.2 ± 9.2	71.3 ± 10.6
Body mass index (kg/m^2)	24.7 ± 2.9	26.6 ± 4.1
Waist Circumference (cm)	91.1 ± 8.7	94.0 ± 12.7
Duration of diabetes (years)		
Less than 5 years	--	46
More than 5 years	--	50
A1C, haemoglobin FPG and PPG		

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The biochemical characteristics of study and reference group are depicted in Table 2. Serum haemoglobin levelswere significantly lower in the study group of patients when compared with control group. A negative correlation was found between the levels of HbA1c and Hb and a positive correlation between the levels of HbA1c and RDW, the relationship between the variables was weak (r= (-) 0.1051 and + 0.1274) and was not statistically significant. The value of R^2 the co-efficient determination was 0.011 and the results were not significant. Multiple regression also showed no significant correlation (r=0.297, 0.04 and -0.07 respectively).

Table 2

Biochemical Characteristics

Characteristics	Reference group (n=28) Mean ± SD	Study group (n=91) Mean ± SD
Fasting Blood sugar (mmol/L)	05.21 ± 0.69	10.37 ± 3.91**
Postprandial Blood Sugar (mmol/L)	07.10 ± 1.25	15.28 ± 5.47**
HbA1c (%)	04.44 ± 1.12	09.49± 2.03**
Hb (g/dL)	14.62 ± 0.91	10.02 ± 1.87**
RDW (%)	16.39 ± 0.78	18.14 ± 1.32***

ANOVA F = 63.51

**P< 0.01 as compared to reference group

*** P< 0.001 as compared to the reference group

Anaemia was found in 5 persons in the reference group while fifty nine subjects (65%) were found to be anaemic in the study group which was statistically significant (Chi-square p 0.007947). There was a moderate negative correlation of -0.5002 between HbA1c and Hb in severely anaemic subjects but it was insignificant (Table 3). The mean RDW value was higher in the study group as compared to the reference group (p<0.01). Amongthe study group, the mean RDW value was lower among mild anaemic subjects as compared to severely anaemic subjects and there was a slight but albeit positive correlations between the RDW and HbA1c. In subjects with the high red cell width, HbA1c levels were higher and waist circumference tended to be more. Although red cell width was positively associated with the waist circumference (r=0.11), it did not reach levels of statistical significance.

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Table 3

Correlation between Haemoglobin Glycosylated Haemoglobin and Red cell Width

	HbA1c (%)	r	RDW (%)	r
Anaemia				
No (n=32)	09.08 ± 1.80	0.17	17.60 ±1.37	0.21
Mild (n=27)	09.37 ± 2.04	0.10	18.15 ±1.20	0.06
Moderate (n=23)	09.82 ± 2.32	-0.004	18.43 ± 1.29	0.04
Severe (n=9)	10.22 ± 2.01	-0.50	18.48 ± 1.30	0.43

DISCUSSION

There has been considerable interest in the use of HbA1c ever since it was recommendedfor the diagnosis of diabetes despite caveats to its use.Anaemia has been cited as a major confounder to its use; however,to what degree anaemiainfluences HbA1c levels in Indian population where anaemia is common is not known. The present study carried out to assess the relationship betweenHbA1c, haemoglobin and RCWin South Indian population indicated that Hb levels were low and RCW were higher in the population studied.

The relation between anaemia and HbA1c concentrations is not clear. While the study by Christy and co-workers suggested that iron-deficiency anaemiamay increase concentrations of HbA1c[17],the study by Sinha et al [18]suggested decrease in the concentration. Our study sheds some light on the association between level of HbA1c and anaemiain South Indian predominantly Tamil speaking population attending a rural tertiary referral hospital. Despite a negative correlation between the levels of HbA1c and Hb, the relationship between the variables was weak; hence more studies are warranted to identify the type and degree of anaemiabefore firm conclusions can be made.

RDW, a measure of the variation in circulatory erythrocyte size that is routinely reported as a part of complete blood cell counts at no additional cost, has recently been found to be a novel and independent predictor for mortality in the general population, in chronic as well as acute clinical settings [19]. Our study, revealed a slight but albeit positive correlation between the RDW and HbA1c levels as well as the waist circumference. One plausible explanation could be that increase in waist circumference could be associated with enhanced subcutaneous and visceral fat deposits which might enhance insulin resistance[20].

Ours is a single centre study and we did not exclude any patient from the study which may reflect the real clinical practice situation. We used a single assessment of haemoglobin as the basis for diagnosis of anaemia. Therefore, prevalence of anaemia may be overestimated in our sample. The cross-sectional design in which one cannot assess cause and effect relationship was another limitation to this study.We did not investigate the causes of elevated RDW values, such as iron or vitamin B12 deficiency, which could confound the association between the RDW values and adverse outcomes.

We do believe that the results of the present study were not affected by the absence of the causes of elevated RDW since iron deficiency is common in India and that ferritin estimation is not a routine clinical test.

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CONCLUSION

The relatively high prevalence of anaemia in ourpopulation of patients with diabetes suggests theneed for screening for anaemia inthe diabetes out-patient clinic. Our study shows that RDW and RPR may be used as promising predictors for the risk of significant liver fibrosis

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