ERYTHROCYTE FRAGILITY INCREASES WITH THE DURATION OF DIABETES IN INDIAN POPULATION

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ABSTRACT

Anemia is one of the common complications in patients with type 2 diabetes; one of the reasons could be due to alteration of the erythrocyte membrane. This study was under taken to study osmotic fragility of the erythrocyte with duration of disease in patients with diabetes mellitus type 2 in Indian population. 120 subjects were randomly selected for this study; of which 60 were type 2 diabetic subjects and 60 were non-diabetic subjects. Among the diabetic subjects; 30 patients were with type 2 diabetes mellitus with duration less than 5 years and 30 patients were with type 2 diabetes mellitus with duration more than 5 years. All subjects were analyzed for blood glucose, glycosylated hemoglobin and erythrocyte osmotic fragility tests. Initial hemolysis of erythrocyte (p < 0.001), median corpuscular fragility (p < 0.001) and complete hemolysis (p < 0.001), were significantly higher for diabetes than non-diabetic. The initial hemolysis of erythrocyte (p < 0.001), median corpuscular fragility (p < 0.001) and complete hemolysis (p< 0.001), were significantly higher of patients with duration of diabetes more than 5 years when compared with of patients with duration of diabetes less than 5 years. The study demonstrated that osmotic fragility of erythrocyte was greater in type 2 diabetic subjects when compared to non-diabetic controls and erythrocyte fragility was higher in diabetic patients with duration more than 5 years. Hence, it is necessary to emphasize increasing investigations of pathogenic mechanisms exacerbated by red cell fragility to prevent complications of diabetes mellitus.

Key Words: Diabetes, Glycosylated Hemoglobin, HbA1c, fasting blood glucose, postprandial blood glucose

INTRODUCTION

Diabetes is one of most prevalent non-communicable disease in world and India is home for majority of the patient with diabetes, in a recent study conducted in 2011 by the ICMR-INDIAB, it was reported that India is home to 62.4 million people with type 2 diabetes (T2DM) and 77 million people with prediabetes (Anjana *et al.*, 2011) and a global projections shows that it will increase to 87 million by 2030 (Whiting *et al.*, 2011). The non-nucleated erythrocyte is unique among human cells, an important and distinguishing feature of the discoid human red cell is its ability to undergo large passive deformations during repeated passage through the narrow capillaries of the microvasculature, with cross-sections one-third its own diameter, throughout its 120-day life span. Therefore cellular deformability and membrane integrity plays a key role for in regulating red cell function and survival (Narla *et al.*, 2008).

Anemia is more common in patients with diabetes than without diabetes (Thomas *et al.*, 2003; Bosman *et al.*, 2001), a recent Epidemiological study conducted by Padmaja Kumari Rani *et al.*, (2010), estimated the prevalence of anemia to be 12.3% in individuals with type 2 diabetes mellitus, above the age of 40 years in Indian population (Padmaja *et al.*, 2010), and one of the proposed mechanisms for anemia is the formation of abnormal erythrocyte cell membranes in the diabetic environment, which leads to reduced surface area-to-volume. Reduced surface area-to-volume ratio might result in decreased deformability and thereby influence splenic sequestration of erythrocytes (Giuseppe *et al.*, 2012) leading to increased destruction and anemia.

Though there studies done other countries which has reported that, there is increased erythrocyte osmotic fragility in type 2 diabetic patients (Ibanga *et al.*, 2005; Osuntokl *et al.*, 2007; Kung *et al.*, 2009), there is hardly any study done in Indian population.

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MATERIALS AND METHODS

This study was conducted in Vydehi Institute of Medical Sciences & Research Center, Bangalore India. The institutional ethical committee gave the clearance for this study.120 subjects aged between 30 to 70 years patients attending the outpatient division of medicine and allied department, were selected for this study; of which 60 were type 2 diabetic subjects and 60 were non-diabetic subjects.

Diabetic subjects were divided into group 1 and group 2; Group 1 included 30 patients with type 2 diabetes mellitus with duration less than 5 years and Group 2 included 30 patients with type 2 diabetes mellitus with duration more than 5 years.

All the Biochemical tests were done in the central lab of Vydehi Institute of Medical Sciences and Research Center, Bangalore. Hb estimation (Niederau *et al.*, 1998), HbA_{1c} percentage (Niederau *et al.*, 1998), FBS (Stein, 1965) and PPBS (Stein, 1965) was measured using Unicell dxc 600 and a synchron 5 pro clinical chemistry system from Beckman coulter.

The osmotic fragility test was done by Dacie's method (Turgeon, 2005) on the subjects within 30 minutes of collection of blood. The study was carried out in accordance with the Declaration of Helsinki and under the terms of all relevant local legislation.

Statistical Analysis

The data was entered and analyzed using SPSS statistical package version 21.Data are presented as mean \pm SD. Independent sample 't' test was used to compare the mean values of, Osmotic fragility between diabetic and non-diabetic controls, Osmotic fragility between diabetic with duration of diabetes less than 5 years and more than 5 years. p≤0.05 was considered as statistically significant.

RESULTS AND DISCUSSION

Results

The demographic and laboratory data of participants are summarized in Table 1.

Tuble 1. The demographic prome of the study subjects					
Variable	Type 2 DM	I	Controls		
	Mean	Range	Mean	Range	
Age(Yrs)	50.8	31-69	47.4	30-70	
Duration of diabetes (Yrs)	6.2	0.2-20.0	0	0	
Hb (gm/dl)	13.3	12.0-17.5	13.8	12.0-17.2	
HbA _{1c} (%)	8.7	4.9-13.3	4.6	3.5-6.0	
FBS (mg/dl)	188.25	64- 438	92.98	80-115	
PPBS (mg/dl)	293.38	110-568	108.55	90-133	

Table 1: The demographic profile of the study subjects

We found no statistically significant differences between type 2 diabetic subjects and non-diabetic controls in Age, Hb (gm/dl), HbA1c (%), FBS (mg/dl) and PPBS (mg/dl) p>0.05.

Osmotic Fragility of the Erythrocyte

The concentration of NaCl for initial hemolysis, complete hemolysis and MCF (% of NaCl) was 0.55 ± 0.06 and 0.49 ± 0.02 , 0.40 ± 0.07 and 0.29 ± 0.03 , 0.48 ± 0.05 and 0.41 ± 0.02 for Type II diabetes mellitus cases and non- diabetic controls respectively. It was statistically highly significant (p<0.001).

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	Diabetic		Non-Diabetic		
Variables	Mean	S.D	Mean	S.D	p value
Initial Hemolysis (% NaCl)	0.55	±0.06	0.49	±0.02	0.000
Complete Hemolysis (% NaCl)	0.40	±0.07	0.29	±0.03	0.000
MCF (% NaCl)	0.48	±0.05	0.41	±0.02	0.000

I able 2: The mean concentration of NaCl of OFT in Diabetic and non-diabetic subject	Table 2: The mean	concentration of	of NaCl of OFT i	in Diabetic and	non-diabetic subject
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Graph 1: The mean concentration of NaCl of OFT in Diabetic and non-diabetic subjects

Table 3: The mean concentration of NaCl of OFT in Group 1 and group 2

Variables	Group 1		Group 2		n voluo
	Mean	S.D	Mean	S.D	p value
Initial Hemolysis (Conc of NaCl)	0.51	±0.06	0.57	±0.06	0.000
Complete Hemolysis (Conc of NaCl)	0.37	±0.07	0.45	±0.06	0.000
MCF (Conc of NaCl)	0.45	±0.04	0.51	±0.05	0.000

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Graph 2: The mean concentration of NaCl of OFT in Group 1 and group 2

Osmotic Fragility of Erythrocyte in Type 2 DM Patients with Duration Less than 5 Years and more than 5 Years

The mean Initial Hemolysis (% of Nacl) was 0.51 ± 0.06 and 0.57 ± 0.06 (p<0.000), the mean Complete Hemolysis (% of Nacl) was 0.37 ± 0.07 and 0.45 ± 0.06 (p<0.000), the mean MCF (% of Nacl) was 0.45 ± 0.04 and 0.51 ± 0.05 (p<0.000) in type 2 DM patients with duration less than 5 years and more than 5 years respectively.

Discussion

The chronic hyperglycemia in diabetes mellitus leads to many hematological abnormalities, including alterations in erythrocyte membrane structure (Megha *et al.*, 2009). The deformability and fluidic nature of the RBC's is very important for the survival (Turgeon, 2012). The reasons for formation of abnormal erythrocyte cell membranes include alteration of the ratio of cholesterol to phospholipid in the core of the erythrocyte cell membranein diabetes (James and Sam *et al.*, 1998). Also, de novo oxidative damage, a result of increased glycosylation proteins namely, serum albumin, α -crystallin, collagen, low density lipoprotein and hemoglobin, could participate in the mechanism, whereby diabetic erythrocytes may acquire membrane abnormalities (Sherif *et al.*, 2007). In our study we found the increased osmotic fragility of erythrocyte in diabetes than non-diabetic controls.

In study conducted by Padmaja *et al.*, (2010) they showed that mean age for occurrence of anemia in diabetic male patient was 5 years. Based on this study, we have divided the diabetic subjects based on duration of diabetes into less than 5 years and more than 5 years duration. The osmotic fragility of the erythrocyte was compared between the two groups. Initial Hemolysis ($0.53\pm.05$ and $0.59\pm.05$), Complete Hemolysis (0.37 ± 0.06 and 0.46 ± 0.05), MCF (0.46 ± 0.05 and 0.52 ± 0.04) of less than 5 year and more than 5 years respectively. There is significant difference between the two groups (p<0.001). This finding was in line with study conducted by Ibanga (2005), where they found that the MCF was increased in patients with duration of diabetes more than 5 years than patients with duration less than 5 years.

Our study findings supports that indeed there is alteration of the erythrocyte membrane in diabetic patients in Indian population. The mechanism for increased fragility could include increased glycosylation of the membrane protein, alteration of the Na+/K+ ATPase on the erythrocyte membrane and there is a necessary to emphasize on further research to understand the mechanisms of exacerbated red cell fragility, in order to prevent the complications of diabetes mellitus.

Conclusion

In this study, that was conducted on 120 subjects. We studied the effect of the osmotic fragility of erythrocyte in type 2 diabetic subjects. As per the present study: There is increased osmotic fragility of erythrocyte in type 2 diabetic subjects has compared to non-diabetic subjects. There is increased osmotic fragility of erythrocyte in diabetes subject with duration of diabetes more than 5 years when compared subjects with duration of diabetes less than 5 years.

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The present study has shown that the vulnerability of the erythrocyte to the osmotic effect increases with increase in duration of diabetes. The result of this study adds to the importance of maintaining the blood glucose levels within the normal range in the type 2 diabetes mellitus subjects. Increasing the survival of the erythrocyte will improve the quality of life in diabetics and also help preventing the progression of microvascular complications. It also emphasis on need for further research on identifying ways to prevent the delirious effects of hyperglycemia on the erythrocyte membrane.

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