ORIGINAL ARTICLE

Glycaemic Control In Type 2 Diabetics And The Mean Corpuscular Fragility

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ABSTRACT

Background: *The osmotic effects of hyperglycaemia and glycosylation of haemoglobin and erythrocyte membrane proteins may play important role in the deformability of RBC in the diabetic state. These effects may be exaggerated in poorly controlled diabetes. The study aimed to determine the fasting blood sugar levels (FBS), glycated haemoglobin (HbA1c) and osmotic fragility of red cells (MCF) in diabetics and non-diabetics.*

Methods: *Fasting blood sugar, glycated haemoglobin and red cell osmotic fragility were determined in seventy-two diabetic subjects aged between 35-70 years and thirty age matched non-diabetic subjects in Calabar, Nigeria using colorimetric methods.*

Results: *The FBS, HbA1c and MCF were significantly ($p<0.01$) higher in diabetics than in non-diabetic subjects. The MCF of diabetics with FBS levels > 7.00mmol/l was significantly higher than those with FBS levels < 7.00mmol/l. No significant difference was observed in the MCF between diabetics with poor glycaemic control (HbA1c > 8.0%) and those with good glycaemic control (HbA1c < 8.0%). The MCF of patients who has been suffering from diabetes for less than five years were significantly ($p<0.05$) higher than those who have had the disease for more than five years. A positive correlation ($p < 0.003$, $r = 0.340$) was observed between the FBS and HbA1c in diabetic subjects. No significant association was seen between the MCF and HbA1c of diabetic subjects of the study.*

Conclusion: *Hyperglycaemia alters the membrane properties of the red cells leading to increase osmotic fragility of the red cells.*

KEYWORDS: *Osmotic fragility; Glycaemic control; Diabetes.*

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INTRODUCTION

Diabetes mellitus is one of the most common endocrine diseases characterized by hyperglycaemia, which results from deficient, or ineffective action of insulin [1,2] is estimated to afflict 170 million people world wide [3] and this represent about 2% of the worlds population [4]. The prevalence rate in Nigeria is about 1-7% of the population, with over 90% of these being non-insulin dependent [5]. The presence of excess glucose circulating in the blood results in enzymatic or non-enzymatic interaction or covalent bonding of blood glucose to other molecules present in the blood, including amino acids, fatty acids, proteins and lipids, referred to as glycosylation [6, 7]. The most frequently glycosylated protein in the body is haemoglobin A (HbA) chains designated as HbA1c [8], which is produced at a rate proportional to the prevailing glucose concentration in the preceding two months. It is the most widely used measure of long term glycaemic control in diabetes [9]. The effect this has on various protein structures has been implicated in the mechanism of several diabetic complications [8]. Glycosylation of haemoglobin and erythrocyte membrane proteins have been reported to mediate changes in the osmotic fragility of erythrocytes. Increased levels of glycosylated haemoglobin as well as glycosylated membrane proteins have a role in altering the membrane permeability of the red cell resulting in increase osmotic fragility of erythrocytes in diabetes [10]. The osmotic effects of hyperglycaemia on red cell membrane permeability properties may play important role in the deformability of RBC in the diabetic state.

This work assesses the effects of glycaemic control on the red cell osmotic fragility in diabetics and non-diabetics.

PATIENTS AND METHODS

The subjects recruited for the study were seventy-two diabetic subjects aged between 35-70 years attending the diabetic clinic of University of Calabar Teaching Hospital. The control subjects comprise of thirty age matched non diabetic healthy volunteers. Informed consent was sought and obtained from each of the subjects before recruitment into the study.

Five millilitres of fasting venous blood samples were collected aseptically via venepuncture for fasting blood sugar estimations, osmotic fragility and glycated haemoglobin estimations. The body mass index (BMI) of all subjects was also determined. Fasting blood sugar was determined using the copper reduction method of Nelson [11], glycated haemoglobin was determined using the column chromatography with cation exchange resin method of Trivelli [12] and osmotic fragility was determined using the Parpart method [13]. A graph of percentage lysis against NaCl concentration was

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plotted and the mean corpuscular fragility (MCF) was extrapolated from the graph. MCF is the concentration of NaCl at which 50% lysis takes place.

Results were analyzed using the student t- test analysis.

RESULTS

Table I. Mean fasting blood sugar (FBS), glycated haemoglobin (HbA1c) and osmotic fragility (MCF) in diabetics and non-diabetic subjects.

Subjects	FBS (mmol/l)	HbA1c (%)	MCF g/dl
Diabetics n = 72	8.70± 3.20	8.20 ± 1.40	0.47± 0.05
Non diabetics n = 30	3.95± 0.89	6.60 ± 1.30	0.44 0.04
p-value	p<0.01	p<0.01	p<0.01

Table II. The mean osmotic fragility (MCF) in diabetics with good glycaemic control and those with poor glycaemic control.

Control State	MCF g/dl
HbA1c (< 8.0%) n = 25	0.48± 0.06
HbA1c (> 8.0%) n = 47	0.47± 0.04
p-value	p>0.05

Table III. The mean osmotic fragility (MCF) in diabetics with FBS level > 7.00mmol/l and those with FBS < 7.00mmol/l.

FBS level mmol/l	MCF g/dl
> 7.00	0.47± 0.04
< 7.00	0.44± 0.03
p-value	p<0.05

Table I shows the mean FBS, HbA1c and MCF of diabetics and non-diabetic subjects. The FBS, HbA1c and MCF were significantly higher in diabetics than non-diabetic subjects.

Table II shows the mean osmotic fragility (MCF) in diabetics with good glycaemic control and those with poor glycaemic control. No significant differences were observed in the MCF between diabetics with poor glycaemic control and those with good glycaemic control.

Table III shows the mean MCF in diabetics with FBS levels > 7.00mmol/l and those with FBS levels < 7.00mmol/l. The MCF of diabetics with FBS levels > 7.00mmol/l was significantly ($p<0.05$) higher than those with FBS levels < 7.00mmol/l.

The MCF of patients who has been suffering from diabetes for < 5 years were significantly ($p<0.05$) higher than those who have had the disease for > 5 years. No significant differences were observed in the MCF between the males and females, and between the young and the elderly subjects.

DISCUSSION

The ability of red cell to resist haemolysis in buffered hypotonic saline solution is a measure of the integrity of its cell membrane and is a useful indirect measure of the surface to volume ratio of the red cells [14]. The effects of glycaemic control on the osmotic fragility of red cells were determined in the present study.

The osmotic fragility and glycated haemoglobin levels of diabetics were observed to be significantly higher than those of the non-diabetic population of this study. In diabetes, insulin facilitated diffusion of glucose into cells is impaired; the resultant hyperglycaemia results in increase osmotic gradient between the intracellular fluid (ICF) and extracellular fluid (ECF). The increase osmotic pressure in the ECF causes osmotic transfer of water out of the cells. This in association with osmotic diuresis of glycosuria leads to generalized dehydration, leading to reduction in cell size and shape. The ability of these abnormal red cells to accommodate fluid when subjected to hypotonic solution of Nacl is greatly reduced thereby leading to susceptibility of these cells to lysis hence increased osmotic fragility. The osmotic fragility in diabetics with FBS values greater than 7.00 mmol/l were significantly higher than those with FBS values less than 7.00 mmol/l this may be attributed to glucose induced membrane lipid peroxidation of erythrocytes associated with hyperglycaemia. Jain[15] also reported a higher osmotic fragility in diabetics with high blood glucose levels. The exact mechanism that leads to glucose induced membrane lipid peroxidation in human red

blood cells has been attributed to increased free radical activity seen in diabetes. Hyperglycaemia and the resultant increase in protein glycosylations and the associated alterations in membrane lipid protein interactions in diabetes might result in altered visco-elastic property of erythrocytes membrane [16]. Significantly higher mean osmotic fragility and glycated haemoglobin was also demonstrated in diabetic pregnant women than non-diabetic pregnant women by Ramana *et al*[10]. Chronic hyperglycaemias in diabetes have been reported to increase cell membrane anisotropy[17].

Duration of diabetes seem to exert a significant effect on the osmotic fragility of the red cells in the diabetic population of the study. Diabetic patients who have been suffering from the disease for five years or less have significantly higher osmotic fragility than those who have had the disease for more than five years. This may be attributed to the fact that those who have been with the disease for a long time have a better knowledge of how to control their blood glucose level. Age and sex does not seem to have any effect on the osmotic fragility of the red cells. The reason for this is not known.

No significant correlation was observed between the HbA1c and osmotic fragility in this study. Osmotic fragility depends on the prevailing glucose concentration at the time of analysis; therefore the preceding glucose concentration of the past weeks may not have any significant effects on the osmotic fragility of the red cells. This agrees with works of Kamada *et al* [18], who also reported higher levels of HbA1c in diabetic than non-diabetic subjects but no correlation between HbA1c and membrane fluidity in either group. A positive correlation was also observed between fasting blood sugar levels and glycated haemoglobin levels of diabetics. The rate of protein glycosylation is proportional to the glucose concentration in the surrounding medium.

From our observations, we therefore conclude that hyperglycaemia alters the membrane property of red cells leading to increased osmotic fragility of red cells in the diabetic state.

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