



Red Blood Cells Fragility among Sudanese Patients with Type 2 Diabetic Mellitus

Abdelgadir Ahmed, Amged Hussein* and Salma Haider

Department of Hematology and Immunohematology, Omdurman Islamic university / Sudan

ABSTRACT

Background: Diabetes mellitus (DM) is a metabolic disorder characterized by hyperglycemia and has become a major public health problem globally. Diabetes is the risk factor for osmotic fragility.

Objective: The aim of the present study is to describe erythrocyte osmotic fragility changes in type II diabetes. The osmotic fragility test is a measure of the resistance of erythrocytes to hemolysis by osmotic stress.

Material and method: In this study 50 diabetic type 2 patients were informed about the study and parameters selected for the study are : median corpuscular fragility (MCF), hemoglobin(HB) , hemoglobin A1c(HA1c) , Fasting blood glucose (FBG) .

Result: The analyzed results showed: Patients with type 2 diabetes have a significantly higher erythrocyte mechanical fragility. And that fasting blood glucose and anemia are also strongest correlate of increased mechanical fragility of erythrocytes.

Conclusion: The result of FBG, HBA1c and OFT were significantly increased in diabetic's type 2 patient (p value < 0.05), furthermore hemoglobin (HB) estimation was significantly decreased in diabetic's type 2 patient (p value <0.05). The correlation between OFT and duration of type 2 DM were positive significant correlation (p value < 0.05).

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KEYWORDS

Sudanese, DM, Red Blood Cell Fragility

List of abbreviations

RBC: Red blood cell

ATP: Adenosine triphosphate

EDTA: Ethylene di amine tetra acetic acid

FL: Femto litter

EPO: Erythropoietin

CVD: Cardio vascular disease

DM: Diabetes mellitus

HG: Hyperglycemia

ROS: Reactive oxygen species

AGEs: Advanced glycosylated end products

WHO: World health organization

RES: Reticule endothelial system

MDA: Malondialdehyde

NOS: Nitrogen oxygen species

OFT: Osmatic fragility test

SD: Standard deviation

Introduction

Mature red blood cell is typically 7–8µm, No nucleus is present in mature RBCs. On a Wright-stained blood film, it appears as a salmon-pink stained cell with a central pale area that corresponds to the concavity. The central pallor is about one-third the diameter of the cell. Mature RBCs remain active in the circulation for approximately 120 days. Aging leads to their removal by the spleen as described subsequently (Rodak et al, 2016). RBC deformability is primarily determined via following three factors: deformability of the RBC membrane itself, intracellular viscosity and the surface area per volume ratio (Tomaiuolo, 2014). Decrease in RBC deformability caused by genetic abnormalities, age of the cell, aging, hypertension, diabetes mellitus, dyslipidemia or any other factor increases blood viscosity and consequently impairs the proper tissue perfusion. This results in adverse outcomes in CVD (Fornal et al. 2010, Tomaiuolo 2014, Toth et al. 2014). The ATP-dependent cation pumps Na⁺ ATPase and K⁺ ATPase regulate the concentrations of Na⁺ and K⁺, maintaining intracellular-to-extracellular ratios of 1:12 and 25:1, respectively. Ca²⁺ ATPase expels calcium from the cell, maintaining low intracellular levels of 30 to 60 NM compared with 1.8 mm in the plasma.

Contact Amged Hussein Abdelrhman ✉ amgedhussen66@gmail.com 📧 Department of Hematology and Ammunohematology, Omdurman Islamic University, Sudan.

Calmodulin, a cytoplasmic Ca²⁺ binding protein, controls the function of Ca²⁺ ATPase. These pumps, in addition to aquaporin, maintain osmotic balance in the RBC. The cation pumps consume a significant portion of RBC ATP production. ATP loss or pump damage permits Ca²⁺ and Na⁺ influx, with water following osmotically. The cell swells, becomes spheroid, and eventually ruptures. This phenomenon is called colloid osmotic hemolysis (Rodak et al, 2016). Type 2 diabetes is a chronic disease in which there is a high level of glucose in the blood, type 2 diabetes is the most common form of diabetes. Insulin is a hormone produced in pancreas by beta cells. Insulin is needed to move blood glucose into cells. Inside the cells glucose is stored and later used for energy. In type 2 diabetes fat, liver and muscle cell do not respond correctly to insulin this is called insulin resistance as a result blood glucose does not get into cells, high level of glucose in the blood this is called hyperglycemia. Family history and genes play a role in type 2 diabetes, low activity level, poor diet and excess body weight increase risk factor of type 2 diabetes. (David pyke et al, 2013). According to WHO blood hemoglobin (Hb) less than 13 mg / dl and 12 mg /dl respectively for an adult male and female are diagnosed as anemia (Priyadarshini et al., 2015). Anemia in chronic disease like Diabetes mellitus is normocytic normochromic type, although in a few cases, microcytic hypochromic also occur (Prakash, 2013). Hemolysis is one of the important causes for anemia in diabetes which may bedue to increased osmotic fragility of RBC (Prakash, 2013; Priyadarshini et al., 2015; Ibrahim et al., 2012). Erythrocyte fragility was more in diabetic than non-diabetic. It was supposed that reduced surface area-to volume ratio of RBC might result in decreased RBC deformability leading to increased destruction and anemia (Prakash, 2013; Lippi et al., 2012). HbA1c is a more reliable method of monitoring long-term diabetes control than random plasma glucose. Normal values range from 4.5 to 8. It is also important to remember that two factors determine the glycosylated hemoglobin levels: the average glucose concentration and the red blood cell life span. If the red blood cell life span is decreased because of another disease state such as hemoglobinopathies, the hemoglobin will have less time to become glycosylated and the glycosylated hemoglobin level will be lower (bishop et al, 2016). Anemia in chronic disease like Diabetes mellitus is normocytic normochromic type, although in a few cases, microcytic hypochromic also occur (Prakash, 2013). Hemolysis is one of the important causes for anemia in diabetes which may bedue to increased osmotic fragility of RBC (Prakash, 2013; Priyadarshini et al., 2015; Ibrahim et al., 2012). Erythrocyte fragility was more in diabetic than non-diabetic. It was supposed that reduced surface area-to volume ratio of RBC might result in decreased RBC deformability leading to increased destruction and anemia (Prakash, 2013; Lippi et al., 2012). HbA1c is a more reliable method of monitoring long-term diabetes control than random plasma glucose. Normal values range from 4.5 to 8. It is also important to remember that two factors determine the glycosylated hemoglobin levels: the average glucose concentration and the red blood cell life span. If the red blood cell life span is decreased because of another disease state such as hemoglobinopathies, the hemoglobin will have less time to become glycosylated and the glycosylated hemoglobin level will be lower (bishop et al, 2016).

Materials and Methods

Study population: Diabetic patient (Type 2 diabetes mellitus), both male and female, age from 30 to 65 years.

Inclusion criteria: Diabetic patient and both male + female

Exclusion criteria: Diabetic patient with hereditary spherocytosis, chronic renal disease, pregnant, hypertension, leukemia and any other chronic disease were excluded from the study.

Data collection: A predesigned questionnaire was used for collecting the required data from both male + female diabetic. The variable in this questionnaire included: age, sex, duration of DM, FBG, HB, OFT, HbA1C. The questionnaire was administered by the researcher.

Blood sampling: About 2ml of venous blood was collected from diabetic patient using sterile disposable syringe into sterile Ethylene Di-amine Tetra-acetic Acid (EDTA) containers and 2ml into sterile heparin containers. Then each sample was mixed thoroughly and gently. All samples were tested within less than 6 hours of collection.

Methods

Photoelectric Colorimeter: used to Hb estimation , Osmatic Fragility test and Glucose analysis .

I Chroma for HbA1c: Non-enzymatic reaction between glucose and amino groups on protein. HbA1c is a clinically useful index of mean glycemia during the preceding 120 days, the average life span of erythrocytes. HbA1c is considered as a more reliable parameter in monitoring glycemia Over the glycemic reading with the conventional glucometer.

Statistical Analysis

Statistical assessment was carried out with statistical package for social sciences (SPSS) version 17.0 for windows statistical software.

Results

The study populations included 50 individuals with diabetic type 2 collected from zeenam specialized hospital in Khartoum state in order to assess red cell fragility (OFT), hemoglobin (HB), fasting blood glucose (FBG) and hemoglobin A1c (HbA1C), the volunteers their ages ranged between (30—65)years old. The study populations have showed the frequency of the disease as 28 (percent 65%) in male and 22 (percent 44%) in females (figure 1). According to duration of type 2 DM classified study population into two groups, group1 duration (1—9) years and group2 duration (> 9 years). The (table 3) displayed that OFT; FBG and HBA1c were significantly increased in diabetic's type 2 patient (p value < 0.05), furthermore hemoglobin (HB) estimation was significantly decreased in diabetic's type 2 patient (p value <0.05). The correlation (table 4) between OFT and duration of type 2 DM were postive significant correlation (p value < 0.05). Also the correlations (table 5) of OFT with parameters, HB were insignificant correlation (p value = 0.07). FBG were insignificant correlation (p value = 0.05). HBA1c were insignificant correlation (p value= 0.8).

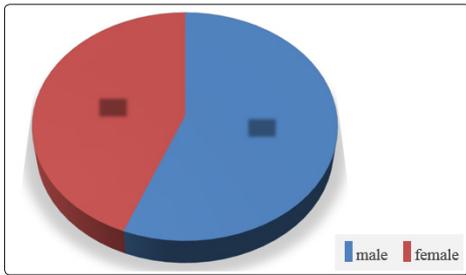


Figure 1: frequency of gender among study group

Table 1: gender distribution

Gender	Frequency	Percent
Male	28	56%
Female	22	44%

Table 2: statistic descriptive of parameters among study group

Parameters	Minimum	Maximum	Mean	SD
HB (g/dl)	9	13	10.6	1.36
FBG (mg/dl)	102	381	228	79.4
HbA1c (%)	5.6	13	8.6	1.48
OFT (%)	0.5	0.6	0.56	0.02

Table 3: compare means of parameters with mean of control

Parameters	Case	Control	P value
			Mean
OFT	0.5	0.3	0.001
HB	10.6	12.75	0.001
HbA1c	8.6	4.58	0.001
FBG (mg/dl)	228	90	0.000

Table 4: correlation of OFT with duration of type 2 DM

Parameters	Duration of DM	
	R value	P value
OFT	0.7	0.000

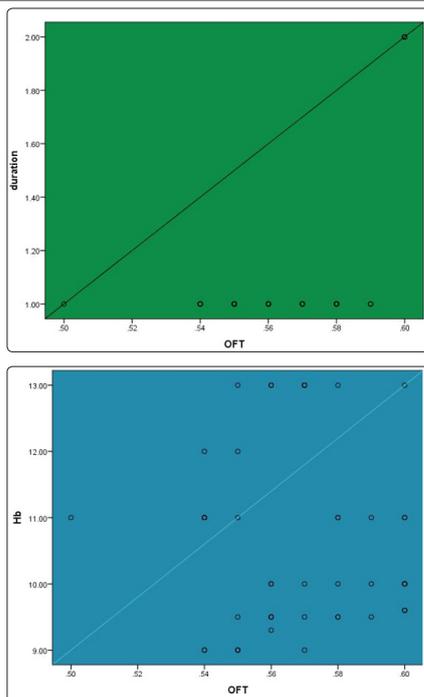


Figure 2: correlation of OFT with duration of type 2 DM

Table 5: correlation of OFT with Hb, HbA1c, FBG

Parameters	OFT	
	R value	P value
HB	-0.05	0.07
HbA1c	0.03	0.8
FBG	0.3	0.05

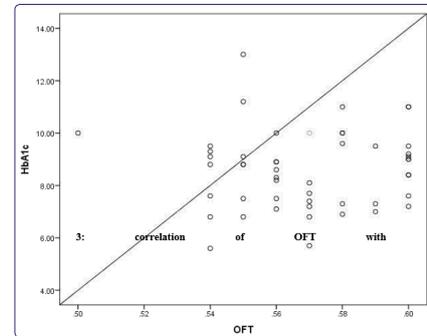


Figure 3: correlation of OFT with HB

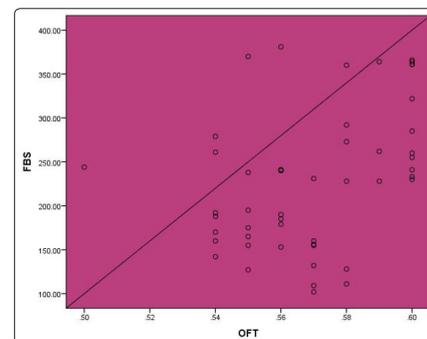


Figure 4: Correlation of OFT with FBS

Discussion

This study was a descriptive cross sectional study, carried out in Zeenam specialized hospital in Khartoum state from the period 20 march 2020 to 30 October 2020, aimed primarily to focus on the red cell fragility in diabetics type 2 patients, FBG, HBA1c and HB estimation. 50 samples was collected by standard questionnaire and involved in this study. In present study the current results revealed that patients with type 2 DM had statistically higher values of Red cell fragility (OFT). Our findings agreed with study done by (Parakash, 2013) the study showed osmotic fragility was increased in chronic type 2 DM patients. Our findings as same results with study by (Harika et al, 2013) the study showed osmotic fragility of erythrocyte was elevated in type 2 DM patients. Our findings similar with study done by (Ibrahim et al, 2013, Habbu Padmini and Kale Bhagwat, 2015) the study showed abnormal erythrocyte membranes formation in hyperglycemia might be associated with increased RBC fragility leading to anemia. Statistical data obtained from this study results revealed that OFT; FBG and HBA1c were significantly increased in diabetic's type 2 patient (p value < 0.05), Fasting blood glucose level increases osmotic fragility also increases Significantly. This is found due to oxidative modifications of membrane bound proteins in RBCs, the strength and functional properties of membrane may change. The RBCs membrane becomes weak and cannot withstand even mild hypotonic condition leading to increased osmotic fragility. Furthermore hemoglobin (HB) estimation was significantly decreased in diabetic's type 2 patient (p value < 0.05).

Conclusion

Regarding to our study we conducted that there is a significant increase of red blood cell fragility in type 2 diabetic patients. The study showed that OFT; FBG and HBA1c were significantly increased in diabetic's type 2 patient (p value < 0.05), furthermore hemoglobin (HB) estimation was significantly decreased in diabetic's type 2 patient (p value <0.05), compared with control group. The correlation between OFT and duration of type 2 DM were +ve significant correlation (p value < 0.05). The correlations of OFT with parameters: HB were insignificant correlation, FBG were insignificant correlation and HBA1c were insignificant correlation.

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