Correlation of Glycated Hemoglobin with Oxidative Stress and Erythrocyte Fragility in Type-2 Diabetes Mellitus

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ABSTRACT

Introduction: Diabetes Mellitus, a frequent chronic disease, is a group of metabolic disorders characterized by chronic hyperglycaemia that results from absolute or relative deficiency of insulin. The aim of our study was to evaluate the effects of free radical generation in the form of lipid peroxides on erythrocytes fragility in Diabetic Patients.

Material and Methods: The present study included 120 subjects of age group 45-60 years, out of which 60 were type 2 diabetic patients and 60 were normal healthy individuals. Serum FBS and Glycated Hb were measured with the help of fully auto analyzer (CPC Turbochem 100). Kie Satoh method was used to measure serum MDA. The erythrocyte fragility was measured by the method described by Dacie and Lewis.

Results: In the present study, there was increase in erythrocyte fragility in type- 2 diabetic patients. As the level of glycated Hb is increased, oxidative stress as well as erythrocyte fragility is increased in type -2 Diabetic Patients.

Conclusion: Increased erythrocyte fragility may be associated with increased generation of oxygen free radicals and decreased levels of antioxidants in type 2 diabetes.

Keywords: Glycated Hb, Erythrocyte Fragility, Oxidative Stress, Free Radicals

INTRODUCTION

Absolute or relative deficiency of insulin produces diabetes mellitus.[1] A large number, approx 382 million people worldwide or 8.3% of adults are estimated to be suffering from Diabetes. About 80% of them are living in the low and middle income countries. Continuing trends may result in one adult in ten having diabtetes. This equates to approximately three new cases every 10 seconds or almost 10 million per year. The largest increases will take place in the regions where developing economies are predominant.[2]

Hyperglycaemia generates reactive oxygen species (ROS), which in turn cause damage to the cells in many ways. Damage to the cells ultimately results in secondary complications in DM.[3] Oxidative stress plays a pivotal role in cellular injury from hyperglycaemia. High glucose level can stimulate free radical production. Free radical production can accelerate free radical generation and ROS. This enhanced ROS generation may not be counteracted by the weakened/weak immune system of the body and results in a condition called oxidative stress.[4]

The measure of erythrocyte strength and its ablity to withstand varying osmotic gradients is called its osmotic fragility. Development of complications in diabetes is associated with the ability of various tissues to withstand oxidative stress, which in turn is critically dependent on the level of antioxidant enzymes.

Intrinsic antioxidant defences are lowest in beta cells and they are particularly affected.[5,6] The activity of a protein is decreased by its glycation and this can indicate the level of oxidative stress.[7] Hence the aim of the present study was to correlate glycated haemoglobin with lipid peroxidation and erythrocyte membrane fragility in Type -2 Diabetes.

MATERIAL AND METHODS

This Study was done in the Department of Biochemistry, Muzaffarnagar Medical College & Hospital Muzaffarnagar from December 2016 to June 2017. The study was approved by institutional ethical committee. Informed consent was taken from all subjects. A total number of 120 subjects of both sex groups were included in this study. Out of 120 subjects, 60 were Diabetic Patients and 60 were normal healthy individuals.

Exclusion criteria

The individuals suffering from hepatic disease, cardiovascular disease, any chronic or acute inflammatory illness, and all types of cancer, pulmonary tuberculosis, alcoholics, smokers and prolonged illness were excluded from the study.

Sample collection and analysis

About 10 ml of blood was drawn after an overnight fast under aseptic condition from clinically diagnosed Type 2 diabetes mellitus and controls and divided into 3 tubes, marked as 1, 2 and 3.

(a) 3 ml of blood was taken in test tube 1. There was no anticoagulant added. The sample was allowed to clot and the serum was separated. Serum was used for measurement of blood sugar and MDA.

(b) Test tube 2 contains 6 ml of blood with heparin anticoagulant, which was used for measurement of

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erythrocyte fragility.

(c) Test tube 3 contains 1 ml of blood with anticoagulant (EDTA) and was used for estimation of glycated haemoglobin.

(d) Test tube 4 contains 2ml of blood, with no anticoagulant after 2 hours of meals, which was used for estimation of post prandial blood sugar.

Sample Analysis

1. FBS, PPBS and Glycated haemoglobin were measured with the help of automated biochemistry analyzer (CPC Turbochem 100).
2. The method described by Kei and Satoh was used to measure Serum MDA[8]
3. Dacie and Lewis method was used to measure erythrocyte fragility. The lysis of the erythrocytes was observed in varying concentrations of buffered hypotonic solution and optical density was measured at 540nm[9]

STATISTICAL ANALYSIS

Statistical analysis was performed by using Graph Pad Quick Cals t-test calculator'. Student's t-test was used to assess the significance of difference between the groups. All results are presented as mean ± S.D. A *'p'* value of less than 0.05 was considered significant.

RESULTS

Out of 100 subjects, 50 subjects were diabetic and 50 were normal healthy subjects. In diabetic patients, the mean levels of FBS, PPBS, MDA and HbA1c increased significantly (<0.0001) when compared to normal healthy smokers (Table-1). The 50% mean erythrocyte fragility in g/100 ml of saline increased significantly (< 0.0001) in diabetic patients as compared to normal healthy control (Table-1). Among diabetic patients, the glycated Hb positively correlated with lipid peroxidation (r =0.247, Figure-1) and percent erythrocyte fragility (r = 0.401, Figure-2) and also the oxidative stress (MDA) was positively correlated with mean erythrocyte fragility (r= 0.125, Figure-3).

DISCUSSION

Free radical generation in diabetes is disproportionate and is by glucose autooxidation, polyol pathway and non-enzymatic glycation of proteins.[10] Glycosylation of proteins, such as hemoglobin results from chronic hyperglycaemia. This also leads to autoxidation of amadori products and generation of free radicals. Uncontrolled diabetes mellitus results in the production of large amounts of NADPH by pentose phosphate pathway which promotes lipid peroxidation in the presence of Cytochrome-P 450. In the presence of NADPH, Oxyhemoglobin in erythrocytes acts like Cytochrome P 450 and results in lipid peroxidation.[11]

Erythrocyte membrane proteins are damaged by increased oxidative stress which in turn may result from high glucose concentrations.[12] Enzymes are inactivated by peroxidation of membrane proteins. Cross linking of membrane lipids and proteins results in increased osmotic fragility and cell death. Glucose induced lipid per oxidative damage can cause changes in the properties of the RBC membrane.[13]

In present study, we found significant increased levels of MDA in diabetes patients as compared to the normal healthy individuals. The prolonged exposure to hyperglycemia also

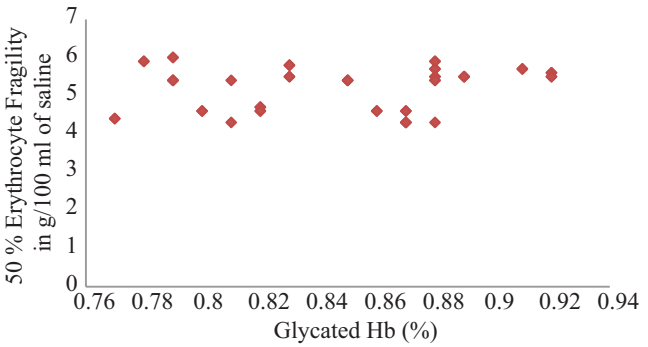

Figure-1: Correlation Between Erythrocyte Fragility and HbA1c in Type -2 Diabetes

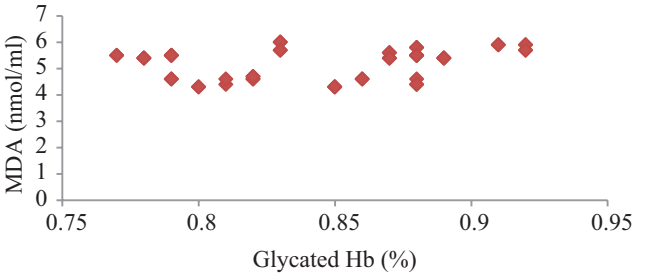

Figure-2: Correlation Between Glycated Hb and MDA in Type-2 Diabetes

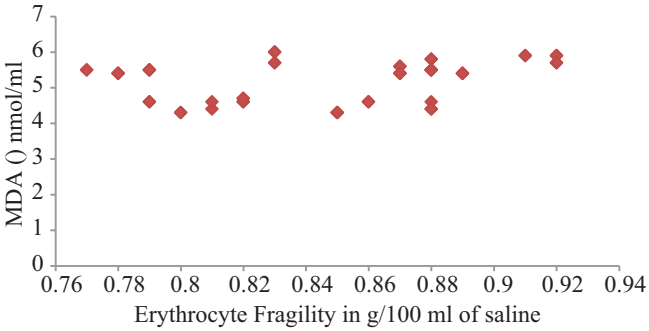

Figure-3: Correlation Between MDA and Erythrocyte Fragility in Type-2 Diabetes

Parameters	Diabetic (50)	Non-Diabetic (50)	p-Value
FBS (mg/dl)	154.28±18.13	92.50±7.64	<0.0001
PPBS (mg/dl)	246.46±50.23	116.08±14.78	<0.0001
MDA (nmol/ml)	5.200±0.57	2.9±0.55	<0.0001
HbA1c %	7.45±0.93	4.95±0.45	<0.0001
50% mean erythrocyte fragility in g/100 ml of saline	0.85±0.04	0.55±0.09	<0.0001

Table-1: Showed mean and standard deviation of variants in non-Diabetic and Diabetic Patients

leads to the increased oxidative stress. Similar findings were reported by many researchers.[11,14-17] In our study, we observed that the erythrocyte fragility was greater in diabetic patients as compared to normal healthy individuals and was statistically significant. Our results are in accordance with many previous studies.[18-20]

In diabetes, there is an increased glycation of a number of proteins including hemoglobin. Several studies have reported that Glycated hemoglobin (HbA1c) was found to be increased in patients with diabetes mellitus and the amount of increase is directly proportional to the fasting glucose levels. Glycated hemoglobin level is also considered as a marker of oxidative stress in Diabetes Mellitus.[21] In the present study, we found positive correlation between glycated haemoglobin and oxidative stress, glycated Hb and erythrocyte fragility and between oxidative stress and erythrocyte fragility in diabetic patients. As the glycated Hb was increased, MDA and erythrocyte fragility also increases. Many researcher in their study showed that MDA was positively correlated with HbA1c in diabetic patients.[22-24]

CONCLUSION

In our study, we found that, HbA1c positively correlated with MDA and erythrocyte fragility in diabetic patients. Elevated blood glucose levels leads to generation of oxygen free radicals and decreased levels of antioxidants which causes erythrocyte fragility in type 2 diabetes. Further studies with adequate sample size are needed to validate this suggestion.

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