Monitoring Glycosylated Haemoglobin and Osmotic Fragility with Respect to Blood Glucose Level in Type II Diabetes Mellitus

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ABSTRACT

Many complications of Diabetes mellitus could be due to free radical mediated damages. Very scanty work is done on cellular damages. This work was planned to study hyperglycemia and their effects on cells like RBCs and platelets. 50 diabetic patients and 50 controls were studied. Two groups of parameters were studied. The first group included blood glucose, glycated hemoglobin, to assess glycemic control at the time of sample collection and ninety days before that. The second group included osmotic fragility and platelet aggregation to assess the changes in cellular functions. The results indicate that the diabetic patients have poor glycemic control, increase osmotic fragility and platelet aggregation; leading to altered cellular properties. It is concluded that cellular effects play important role in diabetic complications through modifications of proteins.

Keywords: Diabetes mellitus, cellular effects, osmotic fragility, platelet aggregability.

INTRODUCTION

Diabetes mellitus is the most prevalent endocrine and metabolic disease. It is associated with high morbidity and mortality. The basic pathology involves hyperglycemia due to actual or potential deficiency of insulin. The prevalence of diabetes mellitus is increasing exponentially throughout the world including India. In India this disease strikes people in all strata irrespective of their socio-economical status, age, sex etc. (1)

Proteins play wide variety of functions. They act as receptors, enzymes, and transport proteins etc. Damages to them may affect their ability to serve these functions. This may adversely affect different structural and functional aspects of the cells, which may lead to various pathological conditions. (2,3) Thus, many of the complications in diabetes mellitus may be due to increased oxidative damages to proteins.

Hence, the present work was designed to study possible link between increased blood glucose level, and their effects on cells in diabetes. For these two groups biochemical parameters were done. The first group included blood glucose level, glycated haemoglobin, to access glycemic control at the time of sample collection and before 90 days. In second group, Osmotic
fragility and platelet aggregation to access changes in cellular functions of RBCs and platelet.

MATERIALS AND METHODS

The study was conducted in C.S.M Govt. Hospital and Medical College, Solapur over the period of 6 months after taking consent from the subjects. Ethical clearance was obtained from the institution. Total 150 individuals were studied including 75 individuals with type II diabetes mellitus and 75 sex and age matched healthy controls. Their fasting and post prandial blood samples were collected under aseptic conditions. These samples were used for estimation of blood glucose levels. Only fasting samples were used for estimation of other parameters. This is because preliminary work had shown that other parameters are not affected by the fasting or post prandial status of the patient.

Blood glucose level: The fasting glucose level was estimated by GOD-POD method using commercial kit on autoanalyzer at 520 nm. (4)

Glycated hemoglobin (HbA1c): Glycated hemoglobin was estimated by Johnson method. The glucose moiety of Glycated hemoglobin is converted to 5-hydroxymethyl furfural by heating with oxalic acid at 124ºc. The glycation reaction requires initial Schiff base formation between glucose and the amino group polypeptides in hemoglobin and subsequent stabilization in the ketoamine form via the Amadori rearrangement. In the presence of oxalic acid, which promotes the Amadori rearrangement, the equilibrium is driven to the ketoamine form, which after heating dehydrates and results in the liberation of 5-hydroxymethyl furfural (5 HMF). The 5-HMF can be detected colorimetrically as an adduct of nitroblue tetrazolium. (5)

Osmotic fragility: Osmotic fragility was estimated by O’ Dell method. For sake of convenience it is determined from 0.30 %, 0.35 %, 0.40 %, 0.45 %, 0.50 %, 0.55 %, 0.60 % and 0.65 % saline solution. Normal Red blood cells are remains suspended in isotonic solution (0.9% saline) for hours without rupturing. But when they are suspended in hypotonic solution, they absorb the fluid, swell and ultimately get lysed. This is known as osmotic fragility. The hypotonicity at which RBCs undergo lysis depends on the integrity of their membrane. The osmotic fragility increases as the membrane becomes weak. Thus, osmotic fragility is measure of the strength of RBC membrane. (6)

Statistical analysis:-

The data was analyzed by using students paired ‘t’ test and SPSS -17 software. The difference in mean values of various parameters was analyzed for significance and the values expressed in terms of p value.

RESULTS AND DISCUSSION

Diabetes mellitus is characterized by impaired glucose utilization and hyperglycemia due to actual or potential deficiency of insulin levels. Glycated hemoglobin is a form of hemoglobin which is measured primarily to identify the average plasma glucose concentration over prolonged periods of time. It is formed in a non-enzymatic glycation pathway by hemoglobin’s exposure to plasma glucose and binding to the N-terminal of valine of βchain of hemoglobin. Normal levels of glucose produce a normal amount of glycated hemoglobin. As the average amount of plasma glucose increases, the fraction of glycated hemoglobin increases in a predictable way. This serves as a marker for average blood glucose levels over the previous months prior to the measurement. (7-9) We found that as fasting sugar level increases glycosylation also increases significantly. (p< 0.001). (Table-1)
Many of the proteins are membrane bound and are functioning as receptors, enzymes etc. They control physico-chemical properties like fluidity, permeability of membrane. They also control physiological functions of membrane like cell-cell adhesion, aggregation etc. In this regard, we have determined RBC osmotic fragility from patients having different fasting glucose levels. We found that as fasting sugar level increases osmotic fragility also increases significantly. \( p < 0.001 \). (Table-2) 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. Due to this, diabetic patients with poor glycemic control may be at the risk of developing anemia and associated complications. (10-12)

<table>
<thead>
<tr>
<th>Biochemical Parameter</th>
<th>Control (Mean ± SD)</th>
<th>Type II diabetic patients (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting glucose level (mg/dl)</td>
<td>93.42±13.30</td>
<td>114.23±16.75</td>
</tr>
<tr>
<td>Glycosylated Hb in %</td>
<td>1.44±0.44</td>
<td>1.93±0.48</td>
</tr>
<tr>
<td>Osmotic fragility in % saline solution</td>
<td>0.35±0.50</td>
<td>0.40±0.50</td>
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| n | 75 | 09 | 13 | 18 | 19 | 16 |

CONCLUSION

The present study has attempted to find out an association between hyperglycemia, protein modification and their effects on cell functions with RBCs and platelets as model cells in diabetes mellitus. This study shows that diabetics with hyperglycemia have poor glycemic control. Among other things, this causes extensive damages to proteins which affect many aspects of cell membrane; and they may develop anemia and have more risk of development of intravenous thrombosis. In this regard, it would be interesting to know the effects of various antioxidants in preventing protein damages and the complications.

A study conducted by Waqar Azim et. al. pointed out that there is a direct correlation between glycated hemoglobin and random plasma glucose levels with no correlation between age of the patients and the glycated hemoglobin or the age and the random plasma glucose levels. In contrast to the above mentioned studies Ghazanfarri et. al. stated that fasting blood glucose is more reliable to separate diabetic from non-diabetic subjects than HbA1c. They found that the association of HbA1c with fasting blood glucose was relatively stronger particularly in diabetic subjects by determining the sensitivity, specificity and predictive values in detection of abnormal values. Similarly, Rajni Dawar Mahajan suggested that though HbA1c has advantages over fasting plasma blood glucose levels for diagnosing diabetes but its use as a single diagnostic agent is limited because of number of biochemical, clinical and economical factors. In developing countries and underdeveloped countries the laboratories are not standardized for HbA1c.
Also the clinician should consider the overall patient profile and a number of local variations and disorders as hemoglobinopathies or anemias. It is also not cost effective, so HbA1c cannot be used as a sole and independent test to diagnose diabetes mellitus. On other hand, osmotic fragility is significant test for the diagnosis of hemolytic anaemia associated with diabetes. However, it is time consuming test. Thus, if better standardized laboratories are available then both glycosylated hemoglobin and osmotic fragility tests will be better for diagnosis in future.

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REFERENCES

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