Association of Decrease Na⁺/K⁺-ATPase activity with Diabetes mellitus Type 2

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ABSTRACT

Background: Prolonged uncontrolled diabetes mellitus type 2 can affect all protein channels in the cell membrane that can leads to diabetic complication. One of such channels is Na⁺/K⁺-ATPase whose disturbed activity can disturb the normal distribution of Na and K across the cell membrane and can contribute in the pathogenesis of diabetic polyneuropathy.

Methods: This was a case control study in which Na⁺/K⁺-ATPase activity in RBCs in patients suffering from diabetes type 2 was compared with normal subjects. Forty patients having diabetes type 2 were selected from diabetes center of Jinnah Hospital Lahore, and same number of normal controls were selected from the staff members and relatives of the patients of Jinnah Hospital.

Results: BMI and HbA1-c were higher in diabetics as compared to normal healthy individuals but the difference is not significant in case of BMI, as P-value (0.07) is more than 0.05, but this difference was significant in case of HbA1c as p-value (0.02) is less than 0.05. Moreover, erythrocytes Na⁺/K⁺-ATPase activity is lower in patients with diabetes type 2 than in normal healthy control. But difference between these two groups is not significant as P value (0.08) is greater than 0.05. Stepwise regression analysis revealed that hyperglycemia decreases the activity of Na⁺/K⁺-ATPase and increases the concentration of glycosylated hemoglobin.

Conclusions: In patients having Diabetes Mellitus type 2, HbA1-c was significantly higher, BMI was also higher but this increase is not significant. Na⁺/K⁺-ATPase activity was lower in patients suffering from diabetes mellitus type 2 as compared to normal healthy controls but it was not significant as P-value (0.08) is greater than 0.05.

Keywords: Diabetes mellitus, Na⁺/K⁺-ATPase, HbA1c.

INTRODUCTION

Diabetes mellitus is a syndrome characterized by disturbances in the metabolism of carbohydrates, Lipids and Protein¹. Patients need excellent control on blood glucose to prevent or delay the diabetic complications like Nephropathy, Retinopathy and Neuropathy². It is a major public health problem all over the world³. Different studies in humans indicate that uncontrolled diabetes mellitus causes change in membrane protein structure, its organization and also its function which play major role in the pathogenesis of diabetic complications²,³,⁴. Sodium Potassium pump which is also called as sodium-potassium adenosine triphosphatase (Na⁺/K⁺-ATPase) is a membrane enzyme (protein in nature) and is expressed in all most all eukaryotic cells⁵. It catalyzes the counter transport of sodium and potassium across the cell membrane by using ATP as energy source⁶. Sodium pump consist of a alpha, β beta and γ gamma subunits⁷,⁸. In diabetes mellitus along with other membrane proteins sodium Potassium pump is affected structurally as well as functionally⁹,¹⁰.

Different mechanisms like depletion of intracellular pool of myo-inositol, an increased flux through the aldose reductase pathway may play vital role in decreasing sodium pump activity¹⁰. Excessive production of oxygen free radicals, formation of glycated proteins and the disturbance in metabolism of nerve growth factor are also responsible for reduced sodium pump activity¹¹. Abnormal ratio between omega 6 and omega 3 due to abnormality in essential fatty acid metabolism also contribute in decreasing sodium pump activity¹². Diabetes induced by streptozotocin can decrease sodium pump activity in different tissues of the animals²,³,⁴. It is suggested that decrease sodium pump activity has a vital role in the pathogenesis of diabetic polyneuropathic complications¹³-¹⁷. As sodium pump has role in maintaining resting membrane potential so any decrease activity in this pump, as happened in diabetes mellitus, will be detrimental for the normal functioning of relevant tissues. Animal models were used to assess sodium pump activity by inducing diabetes by Streptozotocin- and alloxa which decreased sodium pump activity¹⁸. The decrease activity of sodium pump in sciatic nerve was first proposed by Das and colleagues in 1976, but an increased activity in sodium pump in mucosa of small

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intestine of diabetic rats was also found in another study. Hence it indicates that diabetes mellitus can affect the activity of sodium potassium pump in different tissues.

The aim and objective of the current study is to evaluate the Na+/K+ATPase activity in patients suffering from Diabetes Mellitus type 2.

MATERIALS AND METHODS

It was a case control study for which subjects were selected from diabetes center of Jinnah Hospital Lahore after getting written consent. Forty patients with Type 2 diabetes mellitus (22 male and 18 female) with age ranging from 38 to 55 year, were enrolled in the study. None of them had taken any medication known to influence Na+/K+ATPase activity (calciumblockers, thyrroxin, glucocorticoid or digitalislikedrugs). Diagnosed patients of Type 2 diabetes mellitus were selected on the basis of hospital record (WHO criterion) with mean duration of diabetes up to 8.5 ± 0.9 years. The patients, having diabetes mellitus type2 according to WHO criteria were included in the study. Patients suffering from polyneuropathy, pregnant females suffering from Diabetes Mellitus and patients taking medicine like calcium blockers, thyrroxin, glucocorticoid or digitalis were excluded.

To exclude diabetic polyneuropathy Diabetes Control and Complications Trial (DCCT) criteria was followed. Forty healthy subjects comprising 17 women and 25 men were enrolled from hospital staff and relatives of the patients with age ranging from 40 to 50 year.

For estimation of fasting blood glucose level, 1.5 ml of venous blood was taken in 5cc disposable syringe. To estimate postprandial blood glucose level, glycosylated hemoglobin (HbA 1-c) and Na+/K+ATPase activity, the second sample of 10cc of venous blood was taken after two hours of the breakfast. Out of this, eight ml of blood was placed in EDTA tube for the estimation of glycosylated hemoglobin and Na+/K+ATPase activity in red blood cells, while the remaining two ml of blood was allowed to clot in a second test tube for the estimation of blood glucose level. Clotted blood was centrifuged by 2000 Rev/min for three minutes and serum was separated and stored at -20 °C for biochemical analysis. Plasma glucose level was estimated by glucose oxidase method using commercial kit.HbA1c was analyzed using high performance liquid chromatography (HPLC).

Measurement of Erythrocyte Na+/K+ ATPase Activity: Red blood cells ghost membranes prepared by procedure designed by DeLuise and Flier. Na+/K+ATPase activity was estimated on these ghost membranes. Na+/K+ATPase activity was measured with and without ouabain (specific inhibitor of sodium pump) and expressed as the difference between inorganic phosphate released from ATP during separate assays. Results were given in nmol Pi x mg protein x 1 x h ± 1.

Data Analysis: All values were expressed as mean ± standard derivation (SD), and then compared by statistical package for social sciences software (SPSS), version 20. A “P” value less than 0.05 was considered significant. Student T test was applied to calculate P value.

RESULTS

BMI and HbA1-c were higher in diabetics as compared to normal healthy individuals but the difference is not significant in case of BMI, as P-value (0.07) is more than 0.05, but this difference was significant in case of HbA1c as P-value (0.02) is less than 0.05 as shown in table 1. Moreover, erythrocytes Na+/K+ATPase activity is lower in patients with diabetes type 2 than in normal healthy control. But difference between these two groups is not significant as P value (0.08) is greater than 0.05 (Table-1).

Table 1: Comparison of BMI, HbA1c and NaK ATPase activity in Controls and Diabetics

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (Mean)</th>
<th>Diabetics type 2 (Mean)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI</td>
<td>22±1.5</td>
<td>25.7±1.5</td>
<td>0.07</td>
</tr>
<tr>
<td>HbA1c</td>
<td>4.6±0.7</td>
<td>6.5±1.1</td>
<td>0.02</td>
</tr>
<tr>
<td>Na+/K+ATPase Activity nmol Pi/mg protein/h</td>
<td>398±5</td>
<td>374±9</td>
<td>0.08</td>
</tr>
</tbody>
</table>

Table 2: Comparison of fasting & Postprandial Blood Glucose levels in controls and Diabetics

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (Mean)</th>
<th>Diabetics type2(Mean)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting Blood glucose level</td>
<td>91±07</td>
<td>124±08</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Postprandial Blood glucose level</td>
<td>119±09</td>
<td>189±21</td>
<td>&lt; 0.05</td>
</tr>
</tbody>
</table>

DISCUSSION

This case control study was conducted to compare sodium potassium ATPase activity in cell membrane of RBCs in patients having type 2 diabetes mellitus and the normal healthy controls. Blood glucose level, HbA1c and BMI were higher in patients suffering from diabetes type 2 but the increase is significant only in case of blood glucose level. There was a decrease in the activity of Na+/K+ATPase, which was not significant. These results are similar with the results of Bozo et al (1990) who reported that there was a decrease in lymphocytes Na+/K+ATPase activity in
patients with Diabetes type 2 but the decrease was not significant\(^{(25)}\). The study of Mimura et al; \((1994)\) revealed slight reduction in RBCs Na\(^+\)/K\(^+\)ATPase activity in type 2 diabetes with micro albuminuria. In our study we also concluded the same but we did not include the parameter of micro albuminuria\(^{(26)}\). Das and colleagues \((1976)\) first described a decrease of Na\(^+\)/K\(^+\) ATPase enzyme activity in sciatic nerve of diabetic rats where as an increase in enzyme activity was found in mucosa of small intestine of diabetic rat\(^{(21)}\). This shows effects of diabetes on Na\(^+\)/K\(^+\)ATPase in different tissues. Rauh et al. narrated that Na\(^+\)/K\(^+\)ATPase activity significantly decreases in RBCs of diabetics type 2, this contradict our findings\(^{(27)}\). According to Finotti and verbaro,\(\) and Rahmani Jourdheil et al., Na/K ATPase activity of RBC reduces up to 30% in type1 diabetes mellitus\(^{(28,29)}\). Raccach et al also concluded no correlation of decrease Na\(^+\)/K\(^+\)ATPase activity with BMI, blood glucose level and HbA1-c. In our study Na\(^+\)/K\(^+\)ATPase activity was not related to BMI but decrease in enzymatic activity was associated with high blood glucose and HbA1-c levels and this contradicted the finding of Raccach et al\(^{(30)}\).

**CONCLUSIONS**

Na\(^+\)/K\(^+\)ATPase activity was lower in patients suffering from type 2 diabetes mellitus as compared to normal healthy controls but that was not significant. Poor glycemic control decreases Na\(^+\)/K\(^+\)ATPase activity in cell membrane of erythrocytes. Proper functioning of this pump is crucial for the energy balance and prevention of the disease mechanisms which can happen if it is not functioning correctly.

**REFERENCES**
