

Evaluation of Sodium Pumps Activity in Patients of Lahore City Suffering from Diabetes Mellitus Type 1

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ABSTRACT

Background: Sodium pump, also called as Na⁺/K⁺ ATPase, considered as membrane protein that can be affected structurally and functionally by the prolonged hyperglycemia in diabetes mellitus. This effected sodium pump may play a vital role in the pathogenesis of polyneuropathy in diabetes mellitus.

Aims: To evaluate the sodium pump activity in type 1 diabetic patients of Lahore city in Pakistan

Methods: It was a case control study conducted on type 1 diabetics patients of Lahore, Pakistan and compared with normal healthy individuals of the same city. Diabetic patients were selected from diabetes center of Jinnah Hospital Lahore. We enrolled 40 patients with Type I diabetes mellitus. The control group was 40 healthy subjects enrolled from hospital staff. Plasma glucose level was estimated by using glucose oxidase method. HbA1c was analyzed using high performance liquid chromatography (HPLC) method.

Results: A simplified procedure of DeLuise and Flier was used for erythrocyte ghost membrane preparation and this was used for estimation for sodium pump activity. We find no significant correlation between erythrocytes sodium pump activity and body mass index of the two groups. Erythrocytes sodium pump activity was significantly lowered in patients suffering from diabetes type 1 than in normal healthy control as P value was less than 0.05. To investigate what had influenced erythrocytes sodium pump activity in type 1 diabetes mellitus we analyzed all the parameters that could affect the enzymatic activity.

Conclusions: sodium pump activity was significantly lowered in patients suffering from diabetes mellitus type 1. The blood glucose level was the positive factor that correlate with decrease erythrocytes sodium pump activity.

Keywords: Diabetes mellitus type 1, sodium pump, BMI, HbA1c

INTRODUCTION

Diabetes mellitus is a syndrome in which there is disturbance in normal metabolism of dietary macromolecules like carbohydrate, lipid and protein. To prevent or to delay polyneuropathy, patients should have good control on their blood glucose level. It is still a public health problem all over the world. At present it is an alarming issue and its prevalence rises by the past twenty years¹. Pakistan have high prevalence of diabetes mellitus, and at present there are 6.9 million people suffering from this disease and it is expected to 11.5 million people by 2025. It is a serious problem and a challenge for Health department and health policy makers in Pakistan². Most previous studies had confirmed that uncontrolled diabetes mellitus leads to disturbance in structure and function of membrane proteins of cells

that can leads to diabetic complications^{3,4,5}. The sodium potassium ATPase maintains sodium and potassium concentration across the cell membrane and thus maintains membrane potential in almost all eukaryotic cells⁶. Sodium pump activity can be decrease in diabetes mellitus as there is rise in oxidative stress, formation of advance glycated products and abnormal ration of $\omega 6/\omega 3$ in red blood cells membrane^{7,8}. In diabetes mellitus along with other membrane proteins, sodium pump also affected structurally and functionally^{9,10}. This may play a critical role in the pathogenesis of diabetic complications, as normal sodium potassium ATPase is vital to maintain membrane potential^{11,12,13,14,15}.

The aims and objects of the current study were to evaluate the sodium pump activity in type 1 diabetic patients of Lahore city in Pakistan.

MATERIALS AND METHODS

This was a case control study conducted on patients suffering from diabetes mellitus type 1 and compared with normal healthy individuals. 40 consented Patients (25 males and 15 females, age 27 to 45 years) suffering from diabetes mellitus type 1 were

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selected from outpatient department (Diabetic center) of Jinnah Hospital Lahore. The control group was 40 healthy subjects (13 women and 17 men, 22 to 45 years of age) enrolled from hospital staff. All enrolled persons were requested to come in the morning with fasting of at least 8-12 hours for the assessment of fasting blood glucose level. We took 1.5ml of blood to estimate fasting blood glucose level and second sample of 10ml blood, two hours after breakfast and divide it into two parts, eight ml was placed in EDTA test tube for the estimation of HbA_{1c} and Na⁺/K⁺ATPase activity, and two ml of blood was placed in a second test tube and allowed to clot for determination of glucose level. Clotted samples were centrifuged at 2000 Rev/min for three minutes and serum was separated and stored at -20 °C till shifted to laboratory for biochemical analysis. Plasma glucose level was estimated by using glucose oxidase method by using commercial kit. HbA_{1c} was analyzed using high performance liquid chromatography (HPLC) method.

Measurement of Erythrocyte sodium pump Activity: Erythrocyte ghost membranes prepared by following procedure designed by DeLuise and Flier(16). sodium pump activity was estimated on these ghost membranes with and without ouabain (specific inhibitor of sodium pump) and expressed as the difference between inorganic phosphate released from ATP during separate assays^{13,14}. Results are given in nmol Pi x mg protein/hour.

Inclusion Criteria: Diagnosed type 1 diabetic patients.

Exclusion criteria: Patients suffering from polyneuropathy. Pregnancy in case of female patient. Patients taking medicine like calcium blockers, thyroxin, glucocorticoid or digitalis like drugs (17).

Data analysis: All means values were expressed as mean±standard deviation (SD), and then these were compared by statistical package for social sciences (SPSS), software version 10 for windows. Student T test was applied to calculate the P - Value. A "P" value less than 0.05 was considered significant.

RESULTS

It was a simple case control study in which we had compared sodium pump activity of RBCs membrane from the type 1 diabetic patients and that of normal healthy individuals. BMI was not significantly higher but HbA_{1c}, fasting and postprandial blood glucose levels were significantly higher in patients as compare to normal healthy individuals as p value is less than 0.05 as shown in tables. So it the raised blood glucose level which decreases the activity of sodium potassium pump in membranes of red blood

cells and this raised blood glucose level also raised the HbA_{1c} to a significant level.

Table 1: Comparison of BMI, HbA_{1c} and Na⁺/K⁺ATPase Activity between two groups.

Parameter Patients=40, Control=40	Control (mean)	Diabetics type 1 (mean)	P value
BMI	21.9±1.5	25.6±1.8	□ 0.07
HbA _{1c}	4.6±0.7	6.8±1.9	< 0.03
Na ⁺ /K ⁺ ATPase Activity nmole Pi./ mgprotein/h	398±9.1	341±5.6	< 0.02

P-value less than 0.05 is significant

Table 2: Comparison of fasting blood sugar level and 2HAB between two groups.

Parameter Patients=40, Control=40	Control (Mean)	Diabetics type 1 (Mean)	P value
Fasting Blood sugar level	91.5±7.1	129.2±9.2	< 0.03
Blood sugar level 2HAB	119.2±8.9	201.9±24.7	< 0.02

P-value less than 0.05 is significant

DISCUSSION

It was a simple case control study in which sodium pump activity in type 1 diabetic patients was significantly lowered as compared to normal healthy controls as P-value is less than 0.05. Raised blood glucose level was positively correlated with the decrease sodium potassium ATPase activity in red blood cell membrane. There was significant decrease in Na⁺/K⁺ATPase activity of RBCs in type 1 diabetics when we compare with normal healthy individuals. This type of observation also made by Mimura et al; (1994) who studied slight reduction in sodium pump activity in type 2 diabetic patients but those patients also having microalbuminuria¹⁸. Das and colleagues (1976) had different type of observation in sciatic nerve of diabetic rats and mucosal cells of small intestine, in sciatic nerve cells there was decrease activity and in mucosal cells there was increase in sodium pump activity. This shows different tissues cells can have different enzyme activity. We observed significant decrease sodium pump activity in red blood cells membrane¹⁹. Raccach et al. also found decrease sodium pump activity in red blood cells membranes of diabetics which support our conclusion of significant decrease in sodium pump activity in same type of cells¹⁴. Finotti and verbaro, R. 1987, and Rahmani Jourdehil et al 1987, concluded up to 30% decrease in sodium pump activity in same type of

cells of diabetics type 1, but in our study the reduction was significant^{20,21}.

CONCLUSIONS

Sodium pump activity was significantly decreased in type 1 diabetic patients as compared to normal healthy controls. Raised blood glucose level was positively correlated with the decrease sodium pump activity in red blood cell membrane.

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