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Na^+/K^+-ATPase activity as a potential biomarker for Type 2 Diabetes mellitus.

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ABSTRACT

Na^+/K^+ ATPase is one of the important protein channels integrated in cell membrane act as Na^+ / K^+ pump which influenced by uncontrolled Diabetes Mellitus type 2 (T2DM). Therefore, the purpose of this study is to evaluate the Na^+/K^+ ATPase activity as a biomarker of T2DM. This was a case control study in which Na^+/K^+ ATPase activity in patients suffering from T2DM was compared with normal subjects. Patients and controls were assessed for fasting blood glucose, post prandial glucose, glycosylated hemoglobin (HbA1c) and Na^+/K^+ ATPase activity. This study exhibited that BMI and HbA1-c were significantly higher in diabetics as compared to normal healthy individuals. Moreover, the current study revealed that erythrocytes Na^+/K^+ ATPase activity is significantly decreased in T2DM. Roc curve analysis revealed that significant area under curve for Na^+/K^+ ATPase activity. It inspects its role as a potential biomarker for T2DM.

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

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INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a disorder associated by disturbances in the metabolism of carbohydrates, Lipids and Protein. Moreover, protracted uncontrolled T2DM lead to diabetic complications like Nephropathy, Retinopathy and Neuropathy. It is a major public health problem all over the world (Zadhoush et al., 2015). Different studies in humans indicate that uncontrolled T2DM causes alterations in membrane protein structure, its organization and function which play major role in the pathogenesis of diabetic complications (Bos and Agyemang, 2013). Na^+/K^+ ATPase (EC 3.6.3.9), is a membrane enzyme (protein in nature) and is expressed in all most all eukaryotic cells. It catalyzes the counter transport of sodium and potassium across the cell membrane by using ATP as energy source (Topcu et al., 2009). Sodium pump consist of α alpha, β beta and γ gamma subunits. In diabetes mellitus along with other membrane proteins sodium Potassium pump is affected structurally as well as functionally (Vague et al., 1997).

Depletion of intracellular pool of *myo*-inositol led to increased flux through the aldose reductase pathway may play vital role in decreasing sodium pump activity (Greene et al., 1987). Extreme production of oxygen free radicals, glycated proteins and the disturbance in nerve growth factor have impact on reduction of sodium pump activity (Humayoun et al., 2016). Abnormal ratio between omega 6 and omega 3 due to abnormality in essential fatty acid metabolism also contribute in decreasing sodium pump activity (Djemli-Shipkolye et al., 2003). As Na^+/K^+ pump has role in maintaining resting membrane potential so any decrease activity in this pump, as happened in diabetes mellitus, will be harmful for the normal functions of relevant tissues (De La Tour et al., 1998). Hence it indicates that diabetes mellitus can affect the activity of Na^+/K^+ pump in different tissues. So, the aim of the present study was to evaluate the Na^+/K^+ ATPase activity as a biomarker of T2DM.

SUBJECTS AND METHODS

It was a case control study for which subjects were selected from "Internal Medicine Department", Tanta University Hospital. Forty patients with T2DM (16 male and 24 female) with age ranging from 43 to 62 years, were enrolled in the study. None of them had taken any medication known to influence Na^+/K^+ ATPase activity (thyroxin, calcium blockers and glucocorticoid). Diagnosed patients with T2DM were selected, based on the hospital record (WHO criterion) with mean duration of diabetes up to 5.5 ± 1.7 years. Patients suffering from polyneuropathy, pregnant females suffering from DM and patients taking medicine like calcium blockers, thyroxin and glucocorticoid were excluded. To exclude diabetic polyneuropathy Diabetes Control and Complications Trial (DCCT) criteria was followed (Group, 1995). Forty healthy subjects comprising 19 women and 21 men were enrolled from hospital staff and relatives of the patients with age ranging from 40 to 50 years. For estimation of fasting blood glucose level (FBG), 1.5 ml of venous blood was taken. To estimate postprandial blood glucose level (PBG), glycosylated hemoglobin (HbA1-c) and Na^+/K^+ ATPase activity, the second sample of 8 ml of venous blood was taken after two hours of the breakfast. Out of this, 1.5 ml of blood was placed in EDTA tube for the estimation of glycosylated hemoglobin and 5ml of blood was placed in sodium citrated tube for the estimation of Na^+/K^+ ATPase activity in red blood cells, while the remaining 1.5 ml of blood allowed to clot in a second tube for the estimation of blood glucose level. Clotted blood was centrifuged by 2000 rpm for three minutes and serum was separated and stored at -20 ^{0}C for biochemical analysis. FBG and PBG were estimated by glucose oxidase method using commercial kit. HbA1c was assayed by using the NycoCard READER® supplied by (Axis-Shield, Oslo, Norway).

Measurement of Erythrocyte Na^+/K^+ ATPase Activity:

Red blood cells ghost membranes prepared by procedure designed by (Dodge et al., 1963). 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 (Kitao and Hattori, 1983). The results were written as nmol Pi/mg protein/h.

Data Analysis:

All values were expressed as mean ± standard error of mean (SEM), Student T test was performed using GraphPad Prism version 7.00, GraphPad Software, Inc. A “P” value less than 0.05 was considered significant. The

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sensitivity of Na^+/K^+ ATPase activity in diagnosis of T2DM was assessed by estimating areas under curve (AUC) depending on estimating the cut-off values. Significance was adopted at $p < 0.05$ (Dawson and Trapp, 2001).

RESULTS

BMI, FBG, PBG and HbA1c were higher in diabetic group as compared to normal healthy individuals with significant difference as *P* value (< 0.0001). Moreover, erythrocytes Na^+/K^+ ATPase activity is significantly lower in patients with T2DM than in normal healthy control *P* value (< 0.0001) (Table 1, fig.1).

Table 1: Biochemical parameter in studied groups.

Parameter	Control (n=40)	Diabetics type 2 (n=40)	p-value
BMI (kg/m^2) Mean ± SEM	23.5 ± 0.24	32.8 ± 0.3	< 0.0001
FBG (mg/dl) Mean ± SD	88.6 ± 0.68	163 ± 0.98	< 0.0001
PBG (mg/dl) Mean ± SD	108.8 ± 1.01	249 ± 1.25	< 0.0001
HbA1c (%) Mean ± SEM	5.8 ± 0.08116	7.0 ± 0.08	< 0.0001
Na^+/K^+ ATPase activity (nmol Pi/mg protein/h) Mean ± SEM	427.4 ± 6.09	318.5 ± 3.076	< 0.0001

BMI: body mass index; FBG: fasting blood glucose; PBG: postprandial blood glucose; Hb_{A1c}: glycosylated hemoglobin.

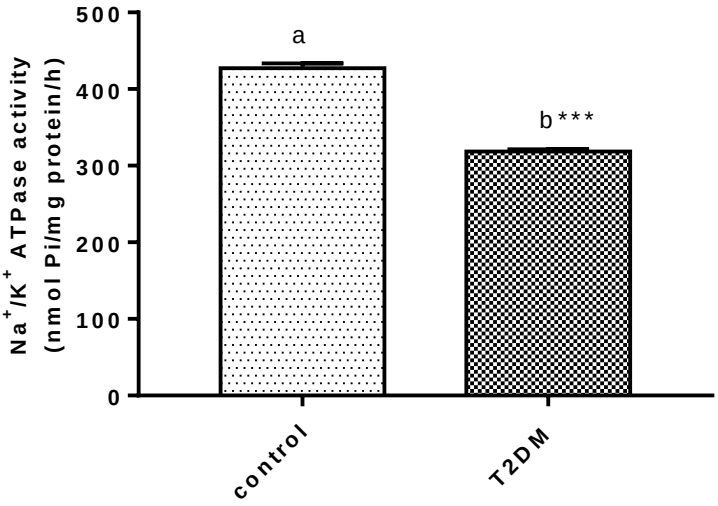

Figure 1: Na^+/K^+ ATPase activity in studied groups.

The area under the ROC curve of Na^+/K^+ ATPase activity was equal (0.997), *p* value <0.001. The best cutoff value of Na^+/K^+ ATPase activity was (337.6 nmol Pi/mg protein/h). Applying this cutoff value, the maximum sensitivity and specificity of Na^+/K^+ ATPase activity was 93.3% and 100% respectively (Fig.2).

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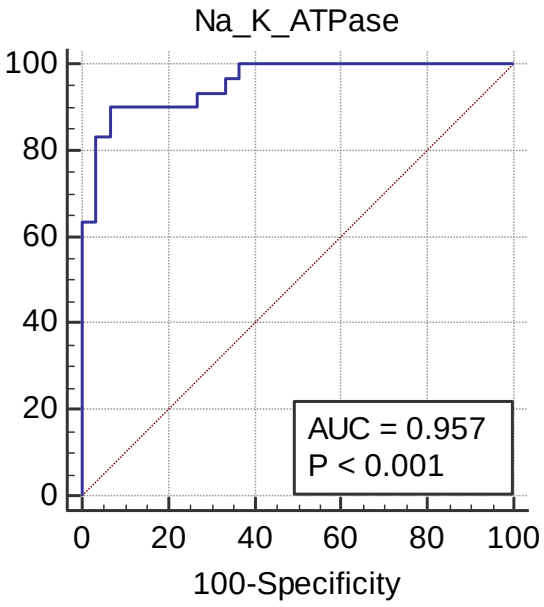

Figure 2: ROC curve analysis of Na^+/K^+ ATPase activity in T2DM group versus Control group. Area under the curve (AUC) is 0.997.

DISCUSSION

This case control study was conducted to evaluate the Na^+/K^+ ATPase activity as a biomarker of T2DM. Our results revealed significant increase in FBG, PBG, HbA1c and BMI in T2DM patients. However, the activity of Na^+/K^+ ATPase showed extremely significant decline in diabetic group. These results are similar with the results of (Koc et al., 2003) who reported that there was a decrease in Na^+/K^+ ATPase activity in patients with T2DM. The study of (Mimura et al., 1994) revealed slight reduction in RBCs Na^+/K^+ ATPase activity in type 2 diabetics with micro albuminuria. (Das et al., 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. This shows effects of diabetes on Na^+/K^+ ATPase in different tissues. (Raccah et al., 1996) reported that Na^+/K^+ ATPase activity significantly decreases in RBCs of T2DM patients, this compatible with our findings. Our data proved that Na^+/K^+ ATPase activity to be an excellent predictive biomarker by ROC-AUC, which showed that Na^+/K^+ ATPase activity as a sensitive parameter for T2DM (AUC=0.997).

CONCLUSIONS

Na^+/K^+ ATPase activity was significantly decreased in patients suffering from type 2 diabetes mellitus as compared to controls. Regarding the ROC curve Na^+/K^+ ATPase activity is a potential biomarker for T2DM.

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