



THE STUDY OF ATPASE ACTIVITY IN ERYTHROCYTE MEMBRANE OF DIABETES MELLITUS-TYPE 2 SUBJECTS

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ABSTRACT

Diabetes Mellitus type-2, one of the most prevalent diseases in developing world, is a metabolic disease characterized by hyperglycemia and change in rheological properties. In type-2 Diabetes, there are many biochemical abnormalities in the plasma membrane of erythrocyte including change in ATPase activity. The study includes OPD patients of type-2 Diabetes along with control subjects. In all study subjects, blood samples were analyzed for fasting blood sugar (FBS) & ATPase activity. It was observed in the study that there is decreased Na^+ - K^+ ATPase and increased Mg^+ ATPase activity in erythrocyte membrane of diabetic patients and have a negative correlation with fasting blood sugar irrespective of the level of glycemic control in all diabetic patients. The study concluded that change in ATPase activity is one of the biochemical changes in erythrocyte cell membrane in patients of Diabetes Mellitus Type-2.

KEY WORDS: Diabetes Mellitus type-2, ATPase activity, Na^+ - K^+ ATPase, Mg^+ ATPase, erythrocyte membrane, Biochemical alteration



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INTRODUCTION

According to the World Health organization, at least 171 million people worldwide suffer from Diabetes. Its incidence is increasing rapidly, and it is estimated that by the year 2030, this number will double. The prevalence of adult diabetes in rural area is about 2.45% and 11.5% in the urban area. Diabetes mellitus occurs throughout the world but it is more common (especially type 2) in the more developed countries. The highest prevalence is however, expected to occur in Asia and Africa, where most people will likely to be found Diabetic by 2030).¹ Na/K-ATPase plays a central role in the regulation of intra- and extracellular cation homeostasis. Alteration of this transport enzyme is thought to be linked to several complications of diabetes mellitus. Aim of the present study is to determine the level of ATPase activity in the erythrocyte cell membrane of Diabetes Mellitus- type 2 patients.

REVIEW OF LITERATURE

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia resulting from defects either in insulin secretion or insulin action or both.² WHO [world health organization] recognizes three types of Diabetes Mellitus –type 1, type 2 and gestational diabetes. Type- 2 diabetes is the commonest type of Diabetes Mellitus associated with several abnormalities, such as retinopathy, neuropathy, angiopathy, nephropathy etc..The characteristics symptoms are excessive urine formation (polyuria), excessive thirst and increased fluid intake (polydipsia) and blurred vision. These symptoms are likely absent if the blood sugar is only mildly elevated. Type 1 is insulin dependent diabetes whereas type 2 is non- insulin dependent diabetes. Type 1 diabetes is due to deficiency of insulin which is due to autoimmune destruction of the pancreatic β -cells. Type 2 diabetes is due to inadequate insulin secretion with insulin resistance to target tissues. Diabetes mellitus is characterized by recurrent or persistent

hyperglycemia and is diagnosed by demonstrating any of the following:³

1. Fasting plasma glucose level at or above 126 mg /dl [7.0mmol/L]
2. Plasma glucose at or above 200mg/dl [11.1 mmol/L]—2hrs after 75 grams oral glucose load as in a glucose tolerance test.
3. Random plasma glucose at or above 200mg/dl [11.1mmol/L].

An elevated level of glucose irreversibly bound to Hemoglobin [HbA1c]. It is used as marker of fluctuation of blood glucose level over 3 months during medication of diabetes. The recommended goal for HbA1c in diabetic people is less than 7%. It is expressed into percentage which is considered as good glycemic control. Diabetic people having HbA1c less than 7 % have significantly lower incidence of diabetic complications, such as diabetic retinopathy and nephropathy.^{4,5}

TOTAL ATPase: ATPase are a class of enzymes that catalyzes decomposition of Adenosine triphosphate (ATP) into adenosine diphosphate and a free phosphate with a release of energy. These enzymes are integral membrane protein ATPase. There are F, V, E, A and P types of ATPase depending on the type of motors. The erythrocyte membrane mainly contains two types of ATPase- the Mg^{2+} ATPase and Na^+/K^+ -ATPase which account for ~60% and 40% of Total ATPase activity respectively.⁶

Na^+/K^+ -ATPase: Also known as the Na^+/K^+ pump. It is a transmembrane ATPase enzyme (EC 3.6.3.9) Located in the plasma membrane. It is found in the human cell and is common to all cellular life. Na^+-K^+ ATPase is a soluble conserved trimetric pump (α , 133 kDa, β , 35 kDa, γ , 10kDa) involved in trans membrane cation regulation via ATP – dependent efflux and influx of sodium and potassium ions in various cells. The pump with bound ATP binds 3 intracellular Na^+ ions. ATP is hydrolyzed, leading to phosphorylation of the pump at a highly conserved aspartate residue and subsequent release of ADP. A conformational change in the pump exposes the Na^+ ions to the outside. The

phosphorylated form of the pump has a low affinity for Na^+ ions, so they are released. The pump binds 2 extracellular K^+ ions. This cause the dephosphorylation of the pump, reverting it to its previous conformational state and transporting the K^+ ions into the cell. The unphosphorylated form of the pump has a higher affinity for Na^+ ions than K^+ ions, so the two bound K^+ ions are released. ATP binds, and the process starts again.⁷ Na^+-K^+ ATPase has been reported as one of the membrane proteins affected structurally and functionally in diabetes mellitus. In Diabetes there is a conformational change in the structure of the enzyme due to glycation and oxidative stress hence reducing its activity.^{1, 8}

Mg²⁺-ATPase: It is an enzyme (E.C.3.6.3.2) located in the plasma membrane. It is a P-type ATPase which undergoes covalent phosphorylation during the transport cycle. It pumps Mg^{2+} inside from outside.⁹ Alteration of lipid composition can affect the physiochemical properties of the erythrocyte membrane including the plasma membrane Na^+-K^+ ATPase and Mg^{2+} ATPase pump activities.¹ Insulin within a physiological range of concentration normalizes lipid composition and order in erythrocytes in diabetic patients. It stimulates Na^+-K^+ ATPase activity and translocation to plasma membrane phosphorylation of α - subunit by protein kinase. The insulin is low so there is a no change in the total ATPase activity, but the Mg^{2+} -ATPase activity is increased and activity of Na^+-K^+ ATPase is reduced.^{1,8}

ABNORMALITIES IN ERYTHROCYTE: In erythrocytes, the hypoglycemia leads to a reduced life span of RBC, increased viscosity, excessive aggregation and increased tendency to adhere to the endothelial lining cells. Change in the level of cholesterol, phospholipids and their ratio, unsaturated fatty acids and altered membrane phospholipids asymmetry have been reported in erythrocyte membrane of Type-2 diabetic subjects.¹ Numerous experimental studies conducted on streptozotocin- induced diabetic rats showed delayed motor nerve conduction velocity, decreased nerve blood flow, degeneration of peripheral nerves, persistent hyperglycemia

and other metabolic abnormalities involving a common denominator of down regulated Na^+-K^+ ATPase.^{7,9,10} Reduced activity of Na^+-K^+ ATPase has also been implicated in streptozotocin induced diabetic rats with nephropathy.^{8, 11} De Leo et al and Kowluru also reported impairment in the level of this enzyme in diabetic rats and mice with retinopathy.^{12,13} However in most of the studies, the observed Na^+-K^+ ATPase reduction was found with other metabolic derangements which include myo-inositol depression, aldolase reduction, sorbitol accumulation and protein kinase-C activity.^{7,14} Subsequent association of Na^+-K^+ ATPase with these parameter have been unfolded.^{10,14} Several studies in humans also consistently reported a decline Na^+-K^+ ATPase activity from the membranes of nerve cells and RBC-cells of Type-1 and Type-2 diabetic patients coupled with alterations in membrane proteins composition.^{15,16} Diabetes mellitus induces a reduction in erythrocyte membrane Na^+-K^+ ATPase activity which results in hemodynamic dysfunction due to altered micro-vascular blood flow. Rheological abnormalities precipitated by decreased erythrocyte deformability and raised fluidity and complications such as nephropathy, neuropathy, cardio vascular disorder and microangiopathy.^{8, 17, 18} Research is still needed to enhance present understanding about factors responsible for the decreased Na^+-K^+ ATPase observed in diabetes mellitus.¹⁹ Furthermore, this group of diabetics is also susceptible to cardiovascular complication of diabetes mellitus.^{7,19} In type-2 diabetic patients, reduced V_{max} connotes a reduction in the enzyme ability to hydrolyzed ATP in-vitro, which undoubtedly has physiological implications in the regulation of trans-membrane cation transport in the erythrocytes. While diabetes may be a factor for this event, numerous secondary factors have been reported. They include decreased number of pump units on the erythrocyte membrane, altered lipid protein interaction, depleted membran charge and enzyme glycation and peroxidation.^{14, 15, 20, 21}

MATERIALS AND METHODS

The study was done in Out Patient Department patients at the Major S.D.Singh Medical College and hospital, Farrukhabad, (U.P.), India. 20 male and 20 female diabetic patients were enrolled for study, whereas 30 patients (both male and female) were enrolled as control subjects in study. Blood samples from the enrolled subjects were analyzed for fasting plasma glucose, isolation of RBC membrane and assay of ATPase activity. ATPase activity includes total ATPase assay, Mg²⁺ ATPase assay and Na⁺-K⁺ATPase

assay. Plasma glucose estimation was done by the semi auto-analyzer in Biochemistry lab of the Hospital. Plasma glucose were estimated by enzymatic method.²² RBC membrane was isolated by a simplified procedure of Deluise and flier.²³ Estimation of ATPase activity was done by Pearson's method.²⁴ Inorganic phosphate was measured by Fisk and Subharao method.²⁵ATPase activity is expressed in $\mu\text{moles}/\pi/\mu$ erythrocyte membrane protein/60 minute. The study was done under the approval of research and ethical review committee of the institute.

RESULTS

Parameters	Male		Female	
	Control	Type 2 Diabetic subjects	Control	Type 2 Diabetic subjects
Age (in years)	55 ± 8	58 ± 6	45 ± 7	48 ± 4
Blood Glucose (mg/dl)	87.8 ± 10.1	197 ± 42	91 ± 3	178 ± 28
Total ATPase (μmoles/π/mg erythrocyte membrane protein/60 minute)	1.2 ± 0.05	1.4 ± 0.05	1.1 ± 0.02	1.4 ± 0.02
Mg ²⁺ ATPase (μmoles/π/mg erythrocyte membrane protein/60 minute)	0.72 ± 0.08	1.0 ± 0.03	0.7 ± 0.02	0.98 ± 0.02
Na ⁺ -K ⁺ -ATPase (μmoles/π/mg erythrocyte membrane protein/60 minute)	0.48 ± 0.02	0.4 ± 0.03	0.42 ± 0.02	0.40 ± 0.03

TABLE-1
Comparison of Biochemical Parameters of serum sample and erythrocyte membrane of with type-2 Diabetes Mellitus with control.

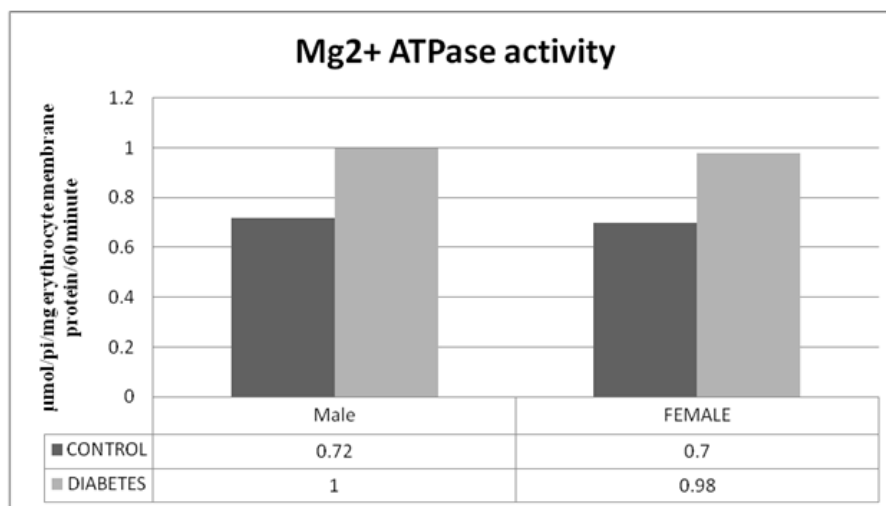


CHART 1
Mg²⁺ ATPase activity in male and female Type-2 diabetic subjects in comparison to control groups.

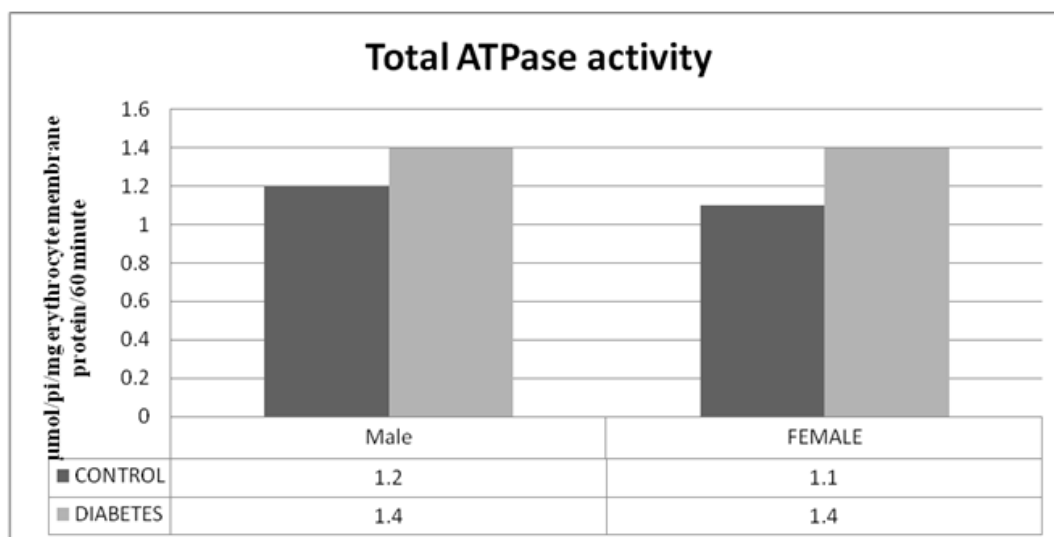


CHART 2
Total ATPase activity in male and female Type-2 diabetic subjects in comparison to control groups.

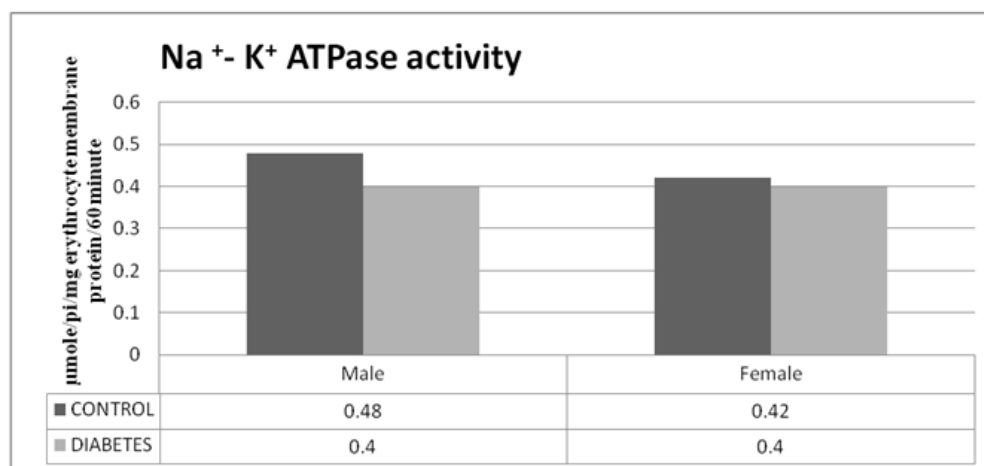


CHART 3
Na⁺ - K⁺ ATPase activity in male and female Type-2 diabetic Subjects in comparison to control groups.

DISCUSSION

The study showed slight differences in total ATPase in male and female diabetic subjects but there was a marked difference in Na⁺-K⁺ATPase activity and Mg⁺² ATPase activity in control and Type-2 diabetic subjects. In both male and female diabetic subjects, the Na⁺-K⁺ ATPase activity is markedly decreased when compared to age and sex matched controls. The total ATPase is more in diabetic subjects (1.4± 0.05) than in controls (1.2 ±

0.05) as shown below in Table 1 and Chart2. The Na⁺- K⁺ ATPase activity was decreased (0.4 ± 0.03) and Mg⁺²ATPase was higher (1.0± 0.03) in diabetes type-2 subjects when compared with the controls (0.48± 0.02 for Na⁺- K⁺ ATPase and 0.72 ± 0.08for Mg⁺²ATPase). There was also marked difference in the Na⁺- K⁺ ATPase activity and Mg⁺²ATPase activity in control subjects and Type-2 diabetic subjects (both male and female)as shown in Chart 1, 3 and Table 1. Studies conducted in human and animals

have consistently showed that diabetes mellitus induced a reduction in erythrocyte membrane Na^+ - K^+ ATPase activity leading to hemodynamic dysfunction due to altered micro-vascular blood flow, decreased erythrocyte deformability, raised plasma fluidity and diabetic complication such as nephropathy, neuropathy, vascular disorder and microangiopathy. The greater reduction in Na^+ - K^+ ATPase activity observed among Type-2 diabetic subjects with poor glycemic control may be due to greater susceptibility of the membrane protein to oxidative stress. In the present study there is a decrease in erythrocyte membrane Na^+ - K^+ ATPase activity and have a significant negative correlation with fasting blood sugar (FBS) irrespective of the level of glycemic control in all diabetic subjects. Observation with respect to fasting blood glucose is supportive of previous findings in which glucose level in diabetic condition exhibited direct toxicity on erythrocyte membrane protein including Na^+ - K^+ ATPase through non-enzymatic glycosylation.^{26, 27} The Na^+ - K^+ ATPase which account for ~ 40% of total ATPase activity is reduced to ~ 30% of total ATPase activity and Mg^{+2} ATPase activity which accounts for about ~ 60% of total ATPase activity is increased to about ~ 70% of total ATPase activity as shown in table 1. This association among diabetes, erythrocyte membrane proteins and Na^+ - K^+ ATPase activity is in accordance with previous findings.^{1, 8} On the basis of above results study concludes that total ATPase activity is slightly higher in diabetic subjects (1.4 ± 0.02) than in controls

(1.2 ± 0.02). The Na^+ - K^+ ATPase activity is decreased (0.4 ± 0.030) and Mg^{2+} ATPase is increased (1.0 ± 0.03) In diabetes type 2 subjects when compared with the controls (48 ± 0.02 for Na^+ - K^+ ATPase and 0.72 ± 0.08 for Mg^{+2} ATPase). There is also a marked difference in the Na^+ - K^+ ATPase activity and Mg^{2+} ATPase activity in male and female control and diabetic subjects. So this study is also consistent with the previous studies done on Na^+ - K^+ ATPase, Mg^{2+} ATPase and total ATPase in RBCs membrane of diabetic subjects.

CONCLUSION

The study suggested that change in ATPase activity is one of the biochemical changes in erythrocyte cell membrane which may be associated with pathophysiology of diabetes mellitus Type-2.

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