EFFECT OF FBS AND BLOOD CHOLESTEROL ON Na\(^+\), K\(^+\)-ATPASE ACTIVITY IN TYPE-I DIABETES

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ABSTRACT:
Diabetes mellitus, a metabolic disease characterized by altered carbohydrate, lipid and protein metabolism and requiring strict glycemic control for delaying or preventing cardiovascular, nephropathic and neurological complications (WHO, 1985). Na\(^+\), K\(^+\)-ATPase is a soluble conserved trimeric pump (\(\alpha\), 133 kDa, \(\beta\), 35 kDa, \(\gamma\), 10 kDa) involved in transmembrane cation regulation via ATP - dependent eflux and influx of sodium and potassium ions in various cells (Kaplan et al., 2002). Na\(^+\), K\(^+\)-ATPase has also been reported as one of the membrane proteins affected structurally and functionally in diabetes mellitus and thought to play an important role in pathophysiology of various diseases (Naka et al., 1995). Diabetes induced persistent hyperglycemia and other metabolic aberrations result in down regulation of Na\(^+\),K\(^+\)-ATPase (Lambourne et al., 1988, Djemli et al., 2001). We have examined diabetic (n=45) and control (n=20) subjects for the association of blood sugar and lipid profile with Na\(^+\), K\(^+\)-ATPase activity. The diabetic subjects were selected having high fasting blood sugar and observed (133.511±37.3647 VS 80.255±7.3844), raised total cholesterol (239.711±15.2608 VS 158.050±11.0667), and LDL-C (183.200±19.1947 VS 104.250±8.0516) and low HDL-C Levels (37.178±4.0873 VS 42.600±3.6476) than control subjects. Variation in serum and intra-erythrocyte levels of electrolytes has also been observed between diabetic and control subjects i.e. sodium, (147.110±6.8353 VS 142.685±5.166), calcium (3.709±0.1865 VS 3.299±0.3253), potassium (4.328±0.4453 VS 4.626±0.3127) and magnesium (0.739±0.0606 VS 0.820±0.0502) has also been observed between control and diabetic subjects.

Erythrocyte membrane analysis revealed decreased amount of proteins (5.026±0.4695 VS 5.713±0.14393), while intra-erythrocyte sodium (12.300±1.61707 VS 8.206±0.5886) and potassium (139.816±7.9780 VS 119.910±5.5984) are slightly raised in diabetic subjects. Reduced total Na\(^+\),K\(^+\)-ATPase activity (0.532±0.6210 VS 0.769±0.0733) and reduced ouabain-sensitivity (0.410±0.03422 VS 0.530±0.0605) has been observed.

Keywords: Diabetes Type-I, FBS, Lipid Profile, Na\(^+\), K\(^+\)-ATPase activity.

INTRODUCTION

The basic function of the Na\(^+\), K\(^+\)-ATPase or sodium pump is to maintain the high Na\(^+\) and K\(^+\) gradient across the plasma membrane. In particular, sodium pump is the determinant of cytoplasmic sodium. As such it has an important role in regulating cell volume, cytoplasmic pH, in driving a variety of secondary transport processes such as Na\(^+\)-dependent glucose and amino acid transport, muscle contraction, nerve impulses generation and propagation (Alex et al., 2000). Though the pump is directly involved in transport of sodium and potassium, by creating an electrogenic and concentration gradient. It also affects the passage of other ions like Calcium and Magnesium. Because Na\(^+\), K\(^+\)-ATPase play a central role in exchange and transportation of the substances across cell membrane. It is regulated by multiple mechanisms according to physiological needs.
and it is accomplished by isoforms of the Na⁺, K⁺-ATPase, associated regulatory components (γ-subunit) and hormones. Because the pump affecting, transport of a variety of components and interacting with a number of molecules which, affecting its activity, this makes the Na⁺, K⁺-ATPase a target for variety of abnormalities in control and may play a pivotal role in the development of pathological conditions as sequel of disturbed transport of Na⁺ and K⁺ or other ions depending indirectly on Na⁺, K⁺-ATPase activity (Jackson et al., 2003, Kjeldsen et al., 1987 and Bamidele et al., 2007).

The Na⁺, K⁺-ATPase has been thought to play an important role in pathophysiology of obesity, hypertension and renal diseases (Kjeldsen et al., 1987 and Adewoye et al., 2001). As disturbed or decreased activity of Na⁺, K⁺-ATPase has been observed in these and various other pathological conditions. In the present study, we have studied the relationship of blood sugar & cholesterol and total Na⁺, K⁺-ATPase with aubain-sensitive Na⁺, K⁺-ATPase activity in diabetic patients.

Diabetic syndrome is a cascade of abnormalities affect Na⁺, K⁺-ATPase in different ways. Persistently high blood sugar results glycosylation of various proteins including the Na⁺, K⁺-ATPase and may directly or indirectly affect the Na⁺, K⁺-ATPase activity (Nandhini et al., 2003 and Gonzalez et al., 1990). Not only that, Persistent hyperglycemia also results in impairment of Na⁺, K⁺-ATPase pump activity (Sandeep et al., 2002). The main energy source, the glucose deprivation of the cell also results in low level of ATP which is the main driving force for the Na⁺, K⁺-ATPase. Cholesterol serves as precursor for the synthesis of a number of molecules, some of these like CS (cholesterol sulphate) can serve as inhibitor for Na⁺, K⁺-ATPase and affect its activity negatively. Low levels of insulin are also a down regulator of Na⁺, K⁺-ATPase, results in reduced number of Na⁺, K⁺-ATPase in the cell membrane. The reduced concentration and activity of Na⁺, K⁺-ATPase results in an abnormal ionic distribution between extra and intracellular environment and may lead to accumulation and loss of some of these electrolytes. Abnormal sodium metabolism has critical role in etiology of cardiovascular and renal disorders. Reduced pump activity results in increased intracellular Na⁺ which is one of the most consistently reported abnormalities in essential hypertension, although a link between intracellular cation metabolism and salt-induced blood pressure has not been established (Jackson, 2003). A higher intracellular sodium secondary to decreased sodium pump activity will activate Na⁺/Ca²⁺ exchange and calcium calmodulin signaling pathways in smooth muscle cells, thereby increasing vascular smooth muscle reactivity and tone by enhancing vascular responsiveness to vasoactive agonists (Douglas et al., 1996). K⁺ is known as great alkalizer as it is a primary electrolyte, important in pH and water balance. Increased tissue and blood alkalinity depends on potassium level and we know that potassium is also the natural diuretic, helping in excreting the water and sodium through renal kallikrein-kinin system (Lanjin et al., 1999), attract oxygen towards tissue and lack of it reduces tissue oxygenation. Potassium has vasodilation effects. Not only that, Mg²⁺ deficiency is also responsible for K⁺ deficiency. Mg²⁺ deficiency further weakens the Na⁺, K⁺-ATPase and this also change the intracellular Ca²⁺-accumulation, that results in decreased membrane potential, increased energy consumption and disturbed perfusion. This way directly leads to cellular ischemia. Mg²⁺ deficiency also results in accumulation of free radicals, tissue injury (Ernesto et al., 2005), accumulation of oxidative products in heart, liver, kidney, skeletal muscle tissue and RBCs.

**MATERIAL AND METHOD**

Forty five (45) type-I diabetic subjects have been selected and having persistent hyperglycemia based on the criteria proposed by American Diabetes Association. Subjects having other inflammatory or infectious
complications have been excluded from the study. Similarly, twenty (20) subjects have been selected as controls, are normoglycemic with repeated check of serum glucose and are free of any observable inflammatory or infectious complication. Samples have been collected with the consensus of the subjects, in both lithium heparin tubes and plain tubes.

Samples have been collected after overnight fast. Other information has been collected using a questionnaire. Samples have been processed immediately for the estimation of blood glucose, TC, LDL, HDL and the serum and intracellular electrolytes. Lithium heparinized tubes have been centrifuged at 7000 rpm for 10 min, plasma and buffy coat has been discarded. Glucose was estimated by GOD-PAP method, and total cholesterol, LDL cholesterol and HDL cholesterol were measured using CHOD-PAP method. For the estimation of intracellular electrolytes, the sedimented erythrocytes have been washed five times with magnesium chloride solution (112 mmol/L), centrifugation at 10000 rpm at 4°C and discard of supernatant has been performed with every wash. The cells are, then lysed with ice-cold double distilled water and centrifuged at 14000 rpm at 4°C for 30 min. Supernatant was removed for the estimation of electrolytes. Electrolytes, sodium and potassium were measured using the flame photometer, calcium using O-CPC method and magnesium using xylidyl blue method. For erythrocyte membrane preparation, simplified procedure (DeLuise et al., 1982) was used with some modifications. The Plasma and buffy coat removed and cells were centrifuged at 14000 rpm at 4°C and the residual supernatant has been removed. The sedimented cells are then lysed in 10 volumes of ice-cold 5mM Tris in 0.1 mM Na₂EDTA, pH 7.6. Tubes were then centrifuged at 14000 rpm at 4°C for 30 min. The supernatant was removed and the membrane are then washed three times with 5mM Tris-HCl in, pH 7.6,0.017 M NaCl and three times with 10 mM Tris-HCl, pH 7.5. The membranes are then aliquoted and are stored at -40°C for Na⁺, K⁺-ATPase activity measurements (Bamidele et al., 2007). Aliquot from each sample has been removed and the membranes are solublized in the presence of 0.2% SDS for protein estimation. This was determined using method by Lowry et al. For Na⁺, K⁺-ATPase activity determination, the membranes are resuspended in a solution containing 92 mmol/L HCl (pH=7.4), 100 mmol/L NaCl, 20 mmol/L KCl, 5 mmol/L MgSO₄·H₂O and 1 mmol/L EDTA, to obtain final concentration of 400μg/ml of proteins. Assays were performed with or without 1 mmol/L Ouabain. After incubation with 4 mmol/L ATP (Vanadate free) at 37°C for 10 minutes, the reaction was stopped by adding of ice-cold trichloroacetic acid to a final concentration of 5%. After centrifugation at 14000 rpm at 4°C for 5 min, the amount of inorganic phosphate in the supernatant was determined.

Na⁺, K⁺-ATPase activity was calculated as the difference between inorganic phosphate released during 10 min incubation with and without ouabain. Activity was corrected to a nanomolar concentration of inorganic phosphate released/milligram protein/hour.

Table 1
Age, Duration of Diabetes and Hemoglobin Concentration

<table>
<thead>
<tr>
<th></th>
<th>Age</th>
<th>Duration of Diabetes</th>
<th>Hb (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetic</td>
<td>36.178±8.074</td>
<td>4.22±2.04</td>
<td>11.920±2.5202</td>
</tr>
<tr>
<td>Control</td>
<td>35.500±8.876</td>
<td>N.A.</td>
<td>12.390±2.1901</td>
</tr>
</tbody>
</table>

No significant difference has seen in mean age of diabetic and control subjects (36.178±8.074 VS 35.500±8.876). Duration of diabetes ranged 1 to 9 years, (4.22±2.04) similarly no significant difference has been found in the Hb concentration (11.920±2.5202 VS 12.390±2.1901) of diabetic and control subjects
RESULTS

Table 2
FBS and Lipid Profile levels in Diabetic and Control Subjects

<table>
<thead>
<tr>
<th></th>
<th>FBS (mg/dl)</th>
<th>TC (mg/dl)</th>
<th>HDL-C (mg/dl)</th>
<th>LDL-C (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetic</td>
<td>133.511±37.3647</td>
<td>239.711±15.2608</td>
<td>37.178±4.0873</td>
<td>183.200±19.1947</td>
</tr>
<tr>
<td>Control</td>
<td>80.255±7.3844</td>
<td>158.050±11.0667</td>
<td>42.600±3.6476</td>
<td>104.250±8.0516</td>
</tr>
</tbody>
</table>

Comparison of diabetic with control subjects, shows significant difference in the level of Fasting Blood Sugar (FBS) (133.511±37.3647 VS 80.255±7.3844), total cholesterol (239.711±15.2608 VS 158.050±11.0667), HDL cholesterol (37.178±4.0873 VS 42.600±3.6476) and LDL cholesterol (183.200±19.1947 VS 104.250±8.0516) levels.

Table 3
Serum Electrolytes level in Diabetic and Control Subjects

<table>
<thead>
<tr>
<th></th>
<th>Sodium (mmol/l)</th>
<th>Potassium (mmol/l)</th>
<th>Calcium (mmol/l)</th>
<th>Magnesium (mmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetic</td>
<td>147.11±6.8353</td>
<td>4.328±0.4453</td>
<td>3.709±0.1865</td>
<td>0.739±0.0606</td>
</tr>
<tr>
<td>Control</td>
<td>142.685±5.166</td>
<td>4.626±0.3127</td>
<td>3.299±0.3253</td>
<td>0.820±0.0502</td>
</tr>
</tbody>
</table>

Serum sodium (147.11±6.8353 VS 142.685±5.166) and calcium (3.709±0.1865 VS 3.299±0.3253) are found significantly increased, while it is inverse in the case of serum potassium (4.328±0.4453 VS 4.626±0.3127) and magnesium (0.739±0.0606 VS 0.820±0.0502) which are significantly decreased in diabetic subjects in comparison to control subjects.

Table 4
Erythrocyte Membrane Protein, intra-erythrocyte Electrolytes and ATPase (Total and Ouabain-sensitive) Activity

<table>
<thead>
<tr>
<th></th>
<th>Ery. Mem.-Protein</th>
<th>Ery. Sodium</th>
<th>Ery. Potassium</th>
<th>Total ATPase Activity (µmol Pi/me./h)</th>
<th>Ouabain Sensitive ATPase Activity (µmol Pi/ me./h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetic</td>
<td>5.026±0.4695</td>
<td>12.300±1.61707</td>
<td>139.816±7.9780</td>
<td>0.532±0.6210</td>
<td>0.410±0.03422</td>
</tr>
<tr>
<td>Control</td>
<td>5.713±0.14393</td>
<td>8.206±0.5886</td>
<td>119.910±5.5984</td>
<td>0.769±0.0733</td>
<td>0.530±0.0605</td>
</tr>
</tbody>
</table>

Reduced membrane protein concentration have been observed in erythrocyte membrane preparations in diabetic subjects in comparison to control subjects (5.026±0.4695 VS 5.713±0.14393). Likewise serum sodium, intracellular sodium level (12.300±1.61707 VS 8.206±0.5886) is also raised significantly. The potassium level (139.816±7.9780 VS 119.910±5.5984) is raised intracellularly in contrast to serum potassium level. A reduced total (0.532±0.6210 VS 0.769±0.0733) and ouabain-sensitive ATPase (0.410±0.03422 VS 0.530±0.0605) has been observed in diabetic over control subjects.
Fig. 1: Age and Hemoglobin.

Fig. 2: FBS and Cholesterol Parameters

Fig. 3: Ions
**DISCUSSION**

In order to assess the biochemical parameters in normal/control and diabetic subjects, this piece of quantitative work was restricted to few cellular measurements, very related to major causes of diabetes mellitus. At present, this metabolic disease has been, on sharp rise and reported to be very common in our country (Baig, 2008). In order to observe the changes in Hgb concentration, first, (age-wise and time duration) in diabetes mellitus. Thus, an attempt has been made, to see:

a) The changes in blood sugar level especially, FBS,
b) Total blood cholesterol, HDL-C, LDL-C,
c) Electrolytes i.e. sodium, potassium, calcium and magnesium (serum and intra erythrocyte)
d) Erythrocyte membrane proteins, total and Oubain-sensitive ATPase activity.

All above mentioned parameters have an extreme degree of importance to perform physiological functions of the various systems in the body and it is well known, that, pancreatic dysfunction leads to changes in metabolism of carbohydrates, proteins and fats. Now in the light of our present results and observations, it has been clearly shown that, all observations are, very similar to previous reports (Hina et al., 1993; Thomas, 2008). Not only the causing factors of diabetes mellitus have been determined, is enough, but these changes in normal concentrations of said parameters, also do however, allow some specificity of therapy also (Dowling et al., 1970). So differences in cell or body behavior must depend on these mentioned measurements, that results in disturbances in control systems of cells also (Dendy et al., 1983).

The present study recruited almost 45 diabetic and 20 normal subjects. And to evaluate the parameters, are very related to causation and development of the diabetes mellitus. The results are summarized in the following tables and figures e.g. Tables 1, 2, 3 and 4 as well as the Figs. 1, 2, 3 and 4 respectively.

As apparent from the Table-I that, no significant difference has been observed in mean age and duration of disease ranged 1-9 years. Similarly, no significant reduction has been found in the concentration of Hgb of diabetics when compared to control subjects. Same data have been expressed in Fig. 1, for a clear picture. This comparative study of normal and diabetic subjects through many means has been strengthen, by previously reported results and suggestions in relation to fall and rise in concentrations of biochemical parameters in normal and diabetic subjects.
The data have been shown in Table 2 as well as in Fig. 2, explain the changes in concentrations of FBS in diabetics in comparison to control subjects. In the same one, lipid profile is affected and shows a significant rise in total blood cholesterol, LDL-C and fall in HDL-C levels in diabetics in comparison to control samples and these encouraging results strengthen by previous reports that lipid profile rise is one of the risk of metabolic syndromes i.e. cardiac and other disorders including diabetes mellitus.

These alterations in concentrations of above mentioned (Table 1 and 2, Fig. 1 and 2) Parameters, were reported previously. The Table 3 and Fig. 3, data shows the difference in concentrations of serum electrolytes (Na⁺, K⁺, Ca++ and Mg++) and has been compared on the basis of normal and diabetic subjects. Significantly increased concentrations of serum sodium, calcium cations in diseased subjects, as well as in erythrocytes sodium and potassium when compared to control subjects, respectively.

The data shows in Table 4 and illustrated in Fig. 4, a significant fall in RBCs-membrane proteins and total and Ouabain-sensitive ATPase activity compared in diabetic and control subjects. Same one shows a raised concentration of most important electrolytes e.g. sodium and potassium (cations) in erythrocytes, likewise serum sodium in diabetics than control subjects. The previous reports also show the supporting and promoting observations to our results.

So overall results, showing the significant differences in all observed parameters, when diabetics compared to control subjects. While, all the measured parameters when compared by the fraction, was found to have a good linear correlation with total and Ouabain-sensitive ATPase activity. This agrees with the observations of many researchers, reported earlier (Hina, 2004 and Thomas, 2008). Most of them observed, are very dependent to one another, directly or indirectly. All biochemicals responsible for normal/routine regulations, if disturbed and then caused development of diabetes mellitus (Bamidele et al., 2007). Not only that, these all have been regulated in a very narrow limits through the neuro-endocrine control (Panet, 1979, Alton et al., 1980 and Thomas, 2008) and it could be a change, produced in membrane permeability, that has been proposed to be a primary cause of diabetes mellitus and lastly, resulting in the metabolic disturbances (Saba et al., 1993) and it might be that, the diabetes mellitus, produced and developed because of the changes in (Carbohydrates, Proteins and Fat) metabolism (Bamidele et al., 2007), and that obviously dependent on cell-permeability and the transport mechanisms. The disturbances in monovalent (cation) transports are manifested by osmotic alterations, because the elevated concentrations of intracellular Na⁺ and K⁺, is also associated with lower activity of red cell-Na⁺, K⁺-ATPase activity. Abnormal membrane function plays a relevant role in the alterations of membrane cations transport, as observed in RBCs of diabetic subjects. These results summarize that, abnormal cation-homeostasis may contribute to the development of diabetes mellitus (Hina et al., 2004) but there is still need of many more studies to classify their mechanisms of generation and pathological significance (Kjeldse et al., 1987 and Jackson et al., 2003) that depends on synthesis that require energy for all anabolic activities/concentration, a part of carbohydrates, proteins and fats.

Ouabain-binding (an indirect measure of Na⁺, K⁺-ATPase activity), ATPase is lower in severely obese patients (genetically determined) than in normal control. In contrast, no difference was found in red cell-Na⁺, K⁺-ATPase activity between severely obese and normal persons (Jackson et al., 2003 and Beutler et al., 1983). This enzyme must be able to adapt, changing the cellular and physiological stimuli. Many mechanisms in place to regulate, sodium pump activity in a tissue-specific manner (Alex et al., 2000). As far as diabetes is concerned, decreased Na⁺, K⁺ pump activity/concentration in skeletal and other muscles and nerves of rat, especially
contractility and excitability of nerves has been observed. In certain instances, alteration in Na⁺, K⁺-ATPase activity and kinetic behavior, results from specific interaction with other membrane components and such interactions are mostly, tissue-specific. The nature and mechanism of regulation by these components, nowadays, are current, topical and exciting area of investigation (Alex et al., 2000 and Jackson et al., 2003). Our data suggests that higher the Na⁺ level of serum and Intracellular RBCs, more specifically might have been secondary to decreased activity of Na⁺, K⁺-ATPase. RBCs have low sodium pump activity when compared with other cell types (Jackson et al., 2003). This enzyme must be able to adapt to changing the cellular and physiological stimuli (Alex, 2000). Our data indicate a significant reduction in Na⁺, K⁺-ATPase activity in the type-I diabetic patients when compared to the control subjects. The elevated LDL-C was found and also reported earlier (Bamidele et al., 2007). The observed decrease in Na⁺, K⁺-ATPase pump concentration may be important for the pathophysiology of diabetes (Kjeldsen, 1987). However, findings are still needed to enhance our present understanding about factors, responsible for decreased, Na⁺, K⁺-ATPase, observed in type-I diabetes mellitus, which occurs early in life and requires life long insulin therapy.

The lipid metabolites are more-concerned with CVS-risk and complications than plasma lysophosphate choline (LPC) in diabetic mellitic patients (Bamidele et al., 2007). It is very clear to us through too much work on biochemicals involved in diabetes mellitus type-I and our results also indicate significant (p = 0.005) reduction in total Na⁺, K⁺-ATPase activity. So, no doubt, the membrane is a dynamic barrier, affecting the movements of substances across the lipid bilayer by virtue of its property of selective permeability, considerable progress towards this goal, has been made with human erythrocytes. The resulting altered levels of Na⁺ and K⁺ in diabetic subjects, could affect the process of protein synthesis, etc. (Panet, 1979 and Alton et al., 1980).

This change might be due to some functional (neuro-endocrine) changes, taking place in order to alter membrane potential or electro-chemical gradient. Especially, Na⁺, K⁺-ATPase pump maintains the concentration gradients for Na⁺ and K⁺ and without these cations, have a tendency to leak through channels. As far as, patho-physiology of diabetes mellitus is concerned, we emphasize that, the quantification of Na⁺, K⁺ ATPase responsible for so many other biochemical parameters transport (Kjeldsen et al., 1987). Sodium, potassium have maintained a central role in investigation of patho-physiology of diabetes mellitus type-I. A second equally important aspect of these studies was the magnitude of ATPase activity i.e. elevation or fall especially, reduced activity of Na⁺, K⁺-ATPase (Soroku et al., 2001; Zachary et al., 2004).

This presentation can not allow us to arrive at any definite conclusion, regarding the exact change in the transport, enzyme-activity, but they conclusively show that diabetes mellitus type-I causes some inactivation of total-ATPase and Ouabain-sensitive ATPase activity. This might be one aspect through which diabetic or pancreatic changes interferes, the cellular functions by inducing alterations in membrane-activity to restrict the entry of certain substances into the cell.

REFERENCES

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