HYPERLIPIDEMIA, INCREASED LIPID PEROXIDATION AND CHANGES IN ANTIOXIDANT ENZYMES, Na⁺-K⁺-ATPASE IN ERYTHROCYTES OF TYPE 2 DIABETIC PATIENTS IN ANDHRA PRADESH

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ABSTRACT

Plasma levels of lipids, lipoproteins and lipid peroxides and erythrocyte Na⁺-K⁺ ATPase, Mg²⁺ATPase and antioxidant enzymes were measured in type-2 diabetic patients. A significant decrease in Na⁺-K⁺-ATPase activity was observed in diabetic patients which was negatively correlated with blood glucose and lipid peroxides, while the Mg²⁺-ATPase activity was increased. In the diabetic subjects the plasma concentrations of Na⁺ and K⁺ were increased where as erythrocyte levels of Na⁺ were increased and K⁺ were decreased. Hyperlipidaemia and increased levels of lipid peroxides were observed in the diabetic subjects. There was a significant increase in erythrocyte catalase activity in diabetics which positively correlated with their lipid peroxides. There was no change in GPx activities between controls and diabetics.

KEY WORDS : Na⁺-K⁺ ATPase, Hyperlipidaemia, antioxidant enzymes, NIDDM, Lipid peroxides.

INTRODUCTION

Na⁺-K⁺-ATPase is an enzyme present in the membranes of all eukaryotic cells, which actively extrudes Na⁺ from cells in exchange for K⁺ at a ratio of 3:2 respectively. Its activity also provides the driving force for Na⁺-coupled transport of nutrients such as amino acids and vitamins in all cells and glucose into intestinal and renal epithelial cells (1).

In addition to the regulation of carbohydrate metabolism, insulin plays a pivotal role in Na⁺-K⁺-homeostasis, (2,3). Diabetes mellitus is a metabolic disorder resulting in due to

insufficient secretion or action of insulin. Decreased Na⁺-K⁺-ATPase activity has been reported in the vasculature of diabetic patients and experimental diabetic animals (4,5). Lipid metabolism is also affected in association with impairment in carbohydrate metabolism in diabetes mellitus. Abnormalities in serum lipids and lipoproteins are highly prevalent in both types of diabetes (6). It has also been shown to be a state of increased free radical activity (7). Increased lipid peroxides, a consequence of free radical activity have been reported in diabetic subjects (8). Reports about the state of antioxidants and scavengers as defense mechanisms in diabetic patients are very contradictory (9).

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The diabetic population in India is heterogenous and shows considerable differences in the clinical and biochemical patterns in comparison with the Western diabetic population (10). South Indian diabetics, especially from the states of Andhra Pradesh and Tamil Nadu have considerable differences in their socio-economic conditions and dietary patterns compared to the diabetic patients from the northern part of the country. The staple food of the people of Andhra Pradesh is rice.

Hence, this study was taken up to assess the abnormalities in the $\text{Na}^+\text{-K}^+\text{-ATPase}$ and $\text{Mg}^{2+}\text{-ATPase}$ activities of erythrocytes, serum lipids, lipoproteins, lipid peroxides and the status of erythrocyte antioxidant enzymes in the type-2 diabetic patients.

MATERIALS AND METHODS

The study group consisted of 40 type-2 normotensive diabetic patients and 26 age and body weight matched normotensive healthy control subjects. All the diabetic patients selected for this study were attending to the Diabetes Research Centre, Tirupati, for treatment. Before visiting the centre, all of them were on irregular therapy with oral hypoglycemic agents and predominant rice eaters without observing the dietary restrictions for diabetes. Blood samples were collected from the study subjects on the first day of their visit to the hospital, in fasting state in heparinized tubes. The plasma was separated immediately by centrifugation and used for measurement of glycosylated haemoglobin (HbA_1) (11), glucose (12), total cholesterol (13), high density lipoprotein cholesterol (HDL) (14), triglycerides (15), and lipid peroxides (thiobarbituric

acid reactive substances (TBARS) (16). Low density lipoprotein cholesterol (LDL-C) and very low density lipoprotein cholesterol (VLDL-C) were calculated by using Friedwald formula (17). Red blood cells were used for enzyme assays. Erythrocyte plasma membranes were prepared from fresh blood according to Hanahan (18). Erythrocyte catalase (CAT) activity was assessed according to Aebi (19). Glutathione peroxidase (GPx) activity in erythrocytes was measured according to the method of Wendel (20). Sodium (Na^+) and potassium (K^+) concentrations in plasma and RBC hemolysates were measured using an electrolyte analyzer and expressed as millimoles / litre.

Measurement of ATPase activities

Ouabain sensitive $\text{Na}^+\text{-K}^+\text{-ATPase}$ and $\text{Mg}^{2+}\text{-ATPase}$ activities were measured in the erythrocyte membranes according to the method of Luly et. al. (21). Assays were carried out in a final volume of 1 ml containing $200 \pm 50 \mu\text{g}$ proteins as the enzyme source, 30 mM Tris-HCl buffer, 3mM MgCl_2 , 40 mM NaCl, 10 mM KCl and 3 mM ATP as the substrate at a final pH value of 7.5. Erythrocyte membranes were pre-incubated in the reaction mixture for 5 min at 37°C before the addition of the substrate. The reaction was stopped by the addition of 1 ml ice cold TCA (10%). All assays were run in triplicate. $\text{Mg}^{2+}\text{-ATPase}$ activity is expressed as the amount of phosphorus (Pi) released in the presence of ouabain (22). $\text{Na}^+\text{-K}^+\text{-ATPase}$ activity was calculated by subtracting the activity assayed with ouabain from the activity assayed without ouabain. The protein content was determined by the method of Lowry et. al. (23).

Statistical evaluation was done by Student's t-test. Correlation was calculated using Pearson test.

RESULTS

The clinical details and biochemical parameters of the study subjects are given in Tables 1 and 2 respectively. All the diabetic patients had higher levels of blood glucose and glycosylated haemoglobin (HbA_{1c}) than those in controls.

The diabetic patients had hyper-lipidaemia as indicated by high concentrations of plasma triglycerides, total cholesterol, LDL-cholesterol and low levels of HDL-cholesterol compared to those in controls. Plasma levels of lipid peroxides (TBARS) were significantly increased in diabetics compared to those in controls. There was no correlation between TBARS and HbA_{1c} in diabetic patients.

The erythrocyte Na⁺-K⁺ATPase activities were significantly lower and Mg²⁺-ATPase

Table 1. Clinical details of controls and diabetic subjects.

Particulars	Controls	Diabetic patients
Number	26	40
Age (yrs)	45 ± 12.0	53 ± 11.0
Body Mass Index (BMI) (Kg/m ²)	21.2 ± 2.2	23.9 ± 3.8
Duration of diabetes (yrs)	—	6.0 ± 5.6
Plasma glucose (mg%)	86.7 ± 14.5	154.8 ± 46.1*
HbA _{1c} (%)	7.1 ± 0.6	10.7 ± 2.3*

* P < 0.001 compared with controls

Table 2. Plasma lipids, lipoproteins and TBARS in controls and diabetics

	Controls	Diabetic patients
Total cholesterol (mg %)	168.4 ± 35.5	256.8 ± 5.2*
HDL-cholesterol (mg %)	42.2 ± 7.3	32.6 ± 9.2*
LDL-cholesterol (mg %)	111.0 ± 39.6	183.0 ± 46.2*
VLDL-cholesterol (mg %)	22.1 ± 11.1	34.6 ± 16.9**
Triglycerides (mg %)	114.3 ± 53.5	165.3 ± 102.8+
TBARS (nm MDA/ml)	5.3 ± 0.3	6.0 ± 0.6*

* P < 0.001 compared to controls, ** P < 0.01, + P < 0.05

activities were higher in diabetic patients than those in controls (Table 3). The blood glucose levels correlated negatively with the erythrocyte Na⁺-K⁺-ATPase activity ($r=-0.406$, $P<0.01$) and positively with Mg²⁺-ATPase activity ($r=0.36$, $P<0.05$). Plasma levels of Na⁺ and K⁺ were significantly increased in diabetics than those in controls. Erythrocyte sodium levels in diabetic

patients were higher compared to those in controls, while it was reverse in the case of K⁺ levels.

Among the antioxidant enzymes, the erythrocyte catalase activity was significantly higher in diabetics than that in controls, while there was no significant difference between the

Table 3. Erythrocyte ATPase activities and Na⁺, K⁺ levels in controls and diabetics.

	Controls	Diabetic patients
Total ATPase (μ mols Pi / mg protein / hr)	1.07 ± 0.08	1.08 ± 0.09
Na ⁺ -K ⁺ -ATPase (μ mols Pi / mg protein / hr)	0.43 ± 0.05	0.27 ± 0.04*
Mg ²⁺ -ATPase (μ mols Pi / mg protein / hr)	0.65 ± 0.07	0.80 ± 0.1*
Plasma		
Na ⁺ (mmols/L)	133.40 ± 5.8	142.70 ± 4.2*
K ⁺ (mmols/L)	4.90 ± 0.4	5.80 ± 0.6*
Erythrocyte (RBC)		
Na ⁺ (mmols/L)	7.20 ± 0.4	9.80 ± 3.6*
K ⁺ (mmols/L)	88.80 ± 2.8	79.80 ± 10.4*

* P < 0.001 compared to controls.

glutathione peroxidase (GPx) activities of controls and diabetics (Table 4). A positive correlation was obtained between the lipid

peroxides and erythrocytes catalase activity in the diabetic patients ($r=0.441$, $P<0.01$).

Table 4. Erythrocyte GPx and catalase activities in controls and diabetics.

	Controls	Diabetic patients
Glutathione peroxidase	35.6±10.5	33.8±10.0
(Units /g Hb)		
Catalase (K units / g Hb)	98.7±24.9	130.7±26.3

* P < 0.001 compared to controls

DISCUSSION

Na⁺-K⁺-ATPase activity contributes to the control of cellular pH, osmotic balance and therefore cell volume, as well as to the Na⁺ coupled transport of nutrients such as amino acids and vitamins in all cells. In addition, it provides the driving force for the uptake of glucose into intestinal and renal epithelial cells (1). In our study the erythrocyte Na⁺-K⁺-ATPase activity accounted for ~39% of total ATPase activity in control subjects while in the diabetic patients it was 25% of the total ATPase. Mg²⁺-ATPase activity which was 59% of the total ATPase activity in controls was 74% of the total ATPase in type 2 diabetic patients.

Weidmann et. al. have reported a decrease in Na⁺-K⁺-ATPase activity and consequently Na⁺ retention in diabetics (24). Decreased Na⁺-K⁺-ATPase activity has also been claimed to be associated with the development of diabetic neuropathy and nephropathy (25, 26). The poor control of diabetes, as indicated by higher levels of HbA_{1c} and the negative correlation between the blood glucose and erythrocyte Na⁺-K⁺-ATPase activities, in the diabetic patients in our study indicate that hyperglycaemia could be the causative factor for the decrease in Na⁺-K⁺-ATPase activity. This is in agreement with the earlier reports which have shown decrease in Na⁺-K⁺-ATPase activity by elevated glucose concentration in diabetics (5).

Recent information from the National Health and Nutrition Examination Survey (NHANES II) indicates that LDL levels above 160 mg/dl occur more frequently among individuals with NIDDM than among non-diabetic individuals (29).

In our study all the diabetic patients had hyperlipidemia with increased LDL and decreased HDL-cholesterol levels in comparison with control subjects.

Lipid peroxides are thought to be formed by free radicals and may play an important role in the development of atheromatous vascular disease. Several authors have reported increased levels of lipid peroxides in diabetic patients (8, 16, 27) while a few could not find any significant increase in lipid peroxidation in the diabetics (28). In the present study serum levels of lipid peroxides were significantly increased in type-2 diabetic patients and they negatively correlated with the Na⁺-K⁺-ATPase activities of erythrocytes.

The role of antioxidative defense mechanisms in diabetic patients is controversial (9, 10). Selvam and Anuradha (30) have shown an increased activity of membrane bound catalase in the diabetic erythrocytes while others (31, 32) could not detect any change in erythrocyte catalase activity in diabetics as compared to controls. In the present study there was a significant increase in the erythrocyte catalase activity in the diabetic patients, and their lipid peroxides correlated positively with the catalase activity.

In one study GPx activity in RBC of type 2 diabetics was increased (9) but was decreased in other studies (33). In our study there was no change in erythrocyte GPx activities in diabetics as compared to controls.

In conclusion, in diabetic patients of Andhra Pradesh area also there is decrease in

Na⁺-K⁺-ATPase activity which could be due to the elevated levels of blood glucose and there are abnormalities in plasma lipids, lipoproteins, lipid peroxides and antioxidant enzymes in type-2 diabetic patients as observed in others..

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