

ELECTROLYTES AND $\text{Na}^+\text{-K}^+\text{-ATPase}$: POTENTIAL RISK FACTORS FOR THE DEVELOPMENT OF DIABETIC NEPHROPATHY

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ABSTRACT

Diabetic nephropathy is the leading cause of death that affects more than 40% of diabetic patients. Its metabolic derangements are frequently accompanied with electrolyte imbalances. This study was aimed to evaluate the electrolyte homeostasis during the progression of diabetic nephropathy in various stages of developing nephropathy. Patients admitted in diabetic wards of various hospitals of Karachi were selected and divided into 4 groups with 50 individuals each. Group I (healthy normotensive, non-diabetics with normal renal functions as control). Group II (diabetic patients with normal blood pressure and renal functions). Group III (diabetic hypertensive patients without renal disease). Group IV (diabetic nephropathy patients with nephropathy). Their fasting blood samples were drawn and analyzed for the estimations of intra erythrocyte and serum electrolytes and $\text{Na}^+\text{-K}^+\text{-ATPase}$ activity. Group II patients showed a significant increase in intra erythrocyte sodium, serum potassium and calcium levels where as intra erythrocyte potassium, $\text{Na}^+\text{-K}^+\text{-ATPase}$, serum sodium and magnesium were significantly decreased as compared to control. Group III showed a significant rise in intra erythrocyte sodium levels but intra erythrocyte potassium, $\text{Na}^+\text{-K}^+\text{-ATPase}$, serum sodium, calcium and magnesium were significantly lowered as compared to control. Group IV revealed a significant increase in intra erythrocyte sodium and significant decrease in intra erythrocyte potassium, $\text{Na}^+\text{-K}^+\text{-ATPase}$, serum sodium, calcium and magnesium levels as compared to control. The results suggest the progressive trends in electrolyte abnormalities in diabetes mellitus leading to end stage renal disease along with the abnormality of their chief transport mechanism. It points towards the potentiality of electrolytes disturbances as indicators for the progression of diabetic nephropathy and also beneficial in prognosis and treatment of the disease.

Keywords: Electrolytes, $\text{Na}^+\text{-K}^+\text{-ATPase}$, hypertension, diabetic nephropathy.

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder that can lead to serious cardiovascular, renal, neurologic and retinal complications (Khawaja *et al.*, 2004; Shera *et al.*, 2004). The metabolic disturbances and their consequences in diabetes mellitus are well known but still our knowledge on the diabetic disorders in electrolytes and membrane function is limited (Somogyi *et al.*, 2001). Recent studies indicated that most diabetic complications like nephropathy are vascular originated. It has been reported that there is an inverse relationship between serum sodium and potassium in diabetic coma. This association may be based on the movement of electrolytes between intra and extracellular space dependent on impaired insulin action (Saito *et al.*, 1999). The rise in plasma insulin levels may elevate blood pressure levels by a variety of mechanisms, including increased sympathetic activity and sodium retention. This association could be explained on the basis of three different pathophysiological mechanisms: 1) abnormal electrolyte transport across membrane, a defect that alters the sodium-potassium exchange, sodium-calcium exchange, increasing the concentration of intracellular calcium ions

that heightens vessels wall tension and smooth muscle contraction, 2) increased sympathetic nervous system activity and 3) altered cellular concentration that induces water logging in the peripheral arteriolar wall. These mechanisms increased peripheral resistance and arterial pressure (Reisin, 1990). Sodium retention occurs as a characteristic alteration in type 1 and 2 diabetic complications. Possible sodium retaining mechanisms include increased glomerular filtration of glucose leading to enhanced proximal tubular sodium-glucose co transport, hyperinsulinemia, an extra vascular shift of fluid with sodium and once it occurs, renal failure (Weidmann and Ferrari, 1991). This has lead to the hypothesis that an increase in the circulating concentration of $\text{Na}^+\text{-K}^+\text{-ATPase}$ inhibitor is responsible for the cause of hypertension in diabetes. Potassium depletion is the common feature of hypertension in diabetes mellitus. Treatment of hypertension at least partially restores potassium levels toward normal and fasting steady state potassium levels are closely linked to calcium and magnesium homeostasis (Resnick *et al.*, 2001). Because of the importance of magnesium and calcium in metabolic enzyme regulation and their interaction, several studies have demonstrated the

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disturbed concentrations of erythrocyte sodium, potassium, magnesium and calcium in diabetic patients with hypertension (Romano *et al.*, 2002). Due to similar nature of ionic imbalances in diabetes, hypertension and nephropathy the study was aimed to evaluate the potentiality of electrolytes imbalance and alterations in their transport mechanisms as a marker for the progression of diabetic complications like hypertension and nephropathy.

METHODS AND MATERIALS

Study Population

Previously registered patients with type 1 and type 2 diabetes mellitus of either sex admitted in diabetic wards or visiting out patient departments of Civil Hospital Karachi, Jinnah Postgraduate Medical Center Karachi and M.S. General Hospital, Karachi were selected. The aim and procedures were explained to patients and/or attendant and informed consent was obtained. The age of patients was 48.48 ± 11.52 (mean $\pm$ SD) years. Their diabetes age was more than five years. The diagnosis of diabetes was made according to the World Health Organization's (WHO) criteria (WHO, 1985). The study protocol was approved by the regulations of institutional ethical committee for the use of human subjects in research. The patients were divided into four groups (50 each) as follows:

- Group I:** Non-diabetic, normotensive control subjects.
- Group II:** Diabetic, normotensive patients.
- Group III:** Diabetic, hypertensive patients.
- Group IV:** Diabetic, Hypertensive patients with nephropathy.

Sample Collection

The blood samples of patients and control subjects were collected in lithium heparin coated tubes after the patients have been taken no drugs for the last 12 hours or more. An aliquot was taken separately in order to get serum. Blood samples were processed the same day for estimations, in accordance with the ethical guidance and regulation of institution and with generally accepted guidelines governing such work.

Intra erythrocyte electrolyte estimation

Heparinized blood was centrifuged and plasma was separated. Buffy coat was aspirated and discarded. Erythrocytes were washed three times at room temperature by suspension in the magnesium chloride solution (112 mmol L^{-1}), centrifugation at $450 \times g$ at 4°C for 5 minutes and aspiration of the supernatant as described earlier (Fortes and Starkey, 1977). Neither electrolyte was detectable in the final wash. Washed erythrocytes were then lysed and used for the estimation of intra-erythrocyte sodium and potassium.

Serum electrolyte estimation

Serum sodium, potassium and calcium were estimated by flame photometer (Corning 410). Serum magnesium was estimated by the method described earlier (Hallry and Skypeck, 1964).

Erythrocyte membrane preparation

The red cell pack extracted by centrifugation at 4°C were resuspended and diluted in 25 volumes of Tris-HCl buffer at pH 7.4. The hemolyzed cells were then centrifuged at 12,000 rpm at 4°C and the membrane pellet was suspended in 30 ml of 0.11 mol L^{-1} Tris-HCl buffer. This centrifugation step was repeated three times. The final concentration of the membrane suspension was $\sim 4 \text{ mg protein ml}^{-1}$ of Tris buffer. The membrane suspension was stored at -80°C until the assay was performed.

Erythrocyte $\text{Na}^+ - \text{K}^+ - \text{ATPase}$ activity measurement (Raccah *et al.*, 1996)

ATPase activity was measured in a final volume of 1 ml as follows: Membrane (400 ug) were preincubated for 10 minutes at 37°C in a mixture containing 92 mmol L^{-1} Tris-HCl (pH=7.4), 100 mmol L^{-1} NaCl, 20 mmol L^{-1} KCl, 5 mmol L^{-1} $\text{MgSO}_4 \cdot \text{H}_2\text{O}$ and 1 mmol L^{-1} EDTA. Assays were performed with and without 1 mmol L^{-1} Ouabain, a specific inhibitor of Na-K-ATPase. After incubation with 4 mmol L^{-1} ATP (Vanadate free, Sigma) at 37°C for 10 minutes, the reaction was stopped by adding ice-cold trichloroacetic acid (5%). After centrifugation at 4°C , $5500 g$ for 10 minutes. The amount of inorganic phosphate in the supernatant was determined (Dryer and Tammes, 1957). $\text{Na}^+ - \text{K}^+ - \text{ATPase}$ activity was calculated as the difference between inorganic phosphate released during the 10-minute incubation with and without Ouabain. Activity was corrected to a nanomolar concentration of inorganic phosphate released $\text{milligram}^{-1} \text{ protein hour}^{-1}$. The concentration of protein was estimated by Biuret method.

All assays were performed in duplicate, and blanks for substrate, membrane and incubation time were included to compensate for endogenous phosphate and non-enzyme related breakdown of ATP. Under these experimental conditions, the coefficient of variation was 7.5%.

STATISTICAL ANALYSIS

Results are presented as mean $\pm$ SD. Statistical significance and difference from control and test values evaluated by Student's t-test. Correlation coefficient and regression analysis were used to describe the effects of one variable on the other by Pearson's Correlation test. All statistical analyses were done by using MINITAB version 11.0 for Windows 2000/XP.

RESULTS

Electrolyte Homeostasis in Diabetic, Normotensive patients

The intra erythrocyte sodium level was significantly increased ($p < 0.01$) and intra erythrocyte potassium level was decreased significantly ($p < 0.05$) in diabetic patients as compared to control subjects (table 1). The $\text{Na}^+ - \text{K}^+ - \text{ATPase}$ activity, serum sodium and magnesium concentrations were found to be significantly decreased ($p < 0.01$) in diabetic subjects as compared to controls (table 1). Serum potassium and calcium levels were found to be increased significantly ($p < 0.01$) in diabetic subjects as compared to controls (table 1).

Electrolyte Homeostasis in Diabetic, Hypertensive patients

The intra erythrocyte sodium level was significantly increased and intra erythrocyte potassium level was decreased significantly in diabetic hypertensive patients as compared to control subjects ($p < 0.01$) (table 2). The $\text{Na}^+ - \text{K}^+ - \text{ATPase}$ activity, serum sodium, calcium and magnesium levels were found to be decreased significantly in diabetic hypertensive patients as compared to control subjects ($p < 0.01$) (table 2). No significant difference was observed in case of serum potassium levels in diabetic hypertensive subjects as compared to controls (table 2).

Electrolyte Homeostasis in Diabetic, Hypertensive patients with Nephropathy

The intra erythrocyte sodium was increased and intra erythrocyte potassium was decreased significantly in diabetic hypertensive patients with nephropathy as compared to control subjects ($p < 0.01$) (table 3). The $\text{Na}^+ - \text{K}^+ - \text{ATPase}$ activity, serum sodium, calcium and magnesium levels were found to be decreased significantly in diabetic hypertensive subjects with nephropathy as compared to control subjects ($p < 0.01$) (table 3). Serum potassium level was not significantly changed in diabetic hypertensive subjects with nephropathy as compared to controls (table 3).

DISCUSSION

Several studies on the mechanisms and progression of diabetic complications with hypertension have been done in recent years on human and animal subjects, but these studies some times are contradictory, although the role of sodium, potassium, calcium and magnesium in the blood pressure regulation particularly during diabetes mellitus is well established (Zochary and Bloomgarden, 2001). In present study a progressive percentage increase in intra erythrocyte sodium was observed in diabetic, diabetic hypertensive and diabetic nephropathy patients (fig. 1) and a progressive percentage decrease was observed in intra erythrocyte potassium levels in various diseased groups of patients (fig. 2). Similarly serum sodium levels

Table 1: Electrolyte Homeostasis in Control and Diabetic subjects

Parameters	Control subjects	Diabetic subjects
Intra erythrocyte Sodium (mmol/L)	11.84 ± 5.14	$14.49 \pm 3.63^*$
Intra erythrocyte Potassium (mmol/L)	107.46 ± 22.61	$99.91 \pm 13.39^{**}$
$\text{Na}^+ - \text{K}^+ - \text{ATPase}$ Activity (nm/mg/hour)	433.35 ± 257.28	$100.52 \pm 41.55^*$
Serum Sodium (mmol/L)	138.96 ± 14.42	$107.1 \pm 15.12^*$
Serum Potassium (mmol/L)	4.99 ± 1.52	$7.42 \pm 1.82^*$
Serum Calcium (mmol/L)	2.09 ± 0.38	$2.46 \pm 0.69^*$
Serum Magnesium (mmol/L)	1.14 ± 0.59	$0.83 \pm 0.32^*$

n = 50; Values are mean $\pm$ SD.

* $p < 0.01$ as compared to control subjects. ** $p < 0.05$ as compared to control subjects.

Table 2: Electrolyte Homeostasis in Control and Diabetic hypertensive subjects

Parameters	Control subjects	Diabetic hypertensive subjects
Intra erythrocyte Sodium (mmol/L)	11.84 ± 5.14	$17.64 \pm 3.13^*$
Intra erythrocyte Potassium (mmol/L)	107.46 ± 22.61	$97.37 \pm 9.90^*$
$\text{Na}^+ - \text{K}^+ - \text{ATPase}$ Activity (nm/mg/hour)	433.35 ± 257.28	$57.24 \pm 25.38^*$
Serum Sodium (mmol/L)	138.96 ± 14.42	$93.24 \pm 12.2^*$
Serum Potassium (mmol/L)	4.99 ± 1.52	4.74 ± 1.72
Serum Calcium (mmol/L)	2.09 ± 0.38	$1.57 \pm 0.45^*$
Serum Magnesium (mmol/L)	1.14 ± 0.59	$0.72 \pm 0.25^*$

n = 50; Values are mean $\pm$ SD; * $p < 0.01$ as compared to control subjects.

Table 3: Electrolyte Homeostasis in Control and Diabetic hypertensive subjects with nephropathy

Parameters	Control subjects	Diabetic hypertensive subjects with nephropathy
Intra erythrocyte Sodium (mmol/L)	11.84 ± 5.14	21.67 ± 4.53*
Intra erythrocyte Potassium (mmol/L)	107.46 ± 22.61	91.88 ± 13.30*
Na ⁺ -K ⁺ -ATPase Activity (nm/mg/hour)	433.35 ± 257.28	24.69 ± 14.07*
Serum Sodium (mmol/L)	138.96 ± 14.42	85.92 ± 15.15*
Serum Potassium (mmol/L)	4.99 ± 1.52	5.36 ± 1.42
Serum Calcium (mmol/L)	2.09 ± 0.38	1.43 ± 0.49*
Serum Magnesium (mmol/L)	1.14 ± 0.59	0.52 ± 0.26*

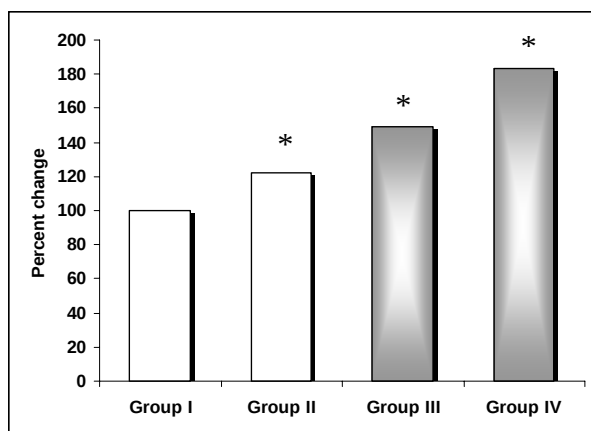
n = 50; Values are mean ± SD; * p<0.01 as compared to control subjects.

Table 4: Electrolyte Homeostasis in Control and non diabetic hypertensive subjects

Parameters	Control subjects	Non diabetic hypertensive subjects
Intra erythrocyte Sodium (mmol/L)	11.84 ± 5.14	13.09 ± 4.13
Intra erythrocyte Potassium (mmol/L)	107.46 ± 22.61	102.69 ± 16.84
Na ⁺ -K ⁺ -ATPase Activity (nm/mg/hour)	433.35 ± 257.28	108.56 ± 38.06*
Serum Sodium (mmol/L)	138.96 ± 14.42	115.3 ± 19.68*
Serum Potassium (mmol/L)	4.99 ± 1.52	5.54 ± 1.89
Serum Calcium (mmol/L)	2.09 ± 0.38	2.21 ± 0.51
Serum Magnesium (mmol/L)	1.14 ± 0.59	1.05 ± 0.66

n = 50; Values are mean ± SD; * p<0.01 as compared to control subjects.

were observed symmetrically decreased in diabetic, diabetic hypertensive and diabetic nephropathy patients (fig. 4). This drastic change is supposed to be due as the activity of Na⁺-K⁺-ATPase was also observed significantly decreased in all the groups of patients in a similar trend (fig. 3).

**Fig. 1:** Percentage difference in Intra erythrocyte sodium levels in various disease groups.

*p<0.01 as compared to control subjects

Na⁺-K⁺-ATPase pump is a ubiquitous enzyme that ensures that the transmembrane gradients of sodium and

potassium concentrations are maintained. Alterations of this transport system are thought to be linked to several complications of diabetes mellitus, hypertension and nephropathy for example (Totan and Greaby, 2002). This enzyme is found on the surface of all eukaryotic cells and actively extrudes sodium from cells in exchange for potassium at a ratio of 3: 2 respectively (Sweaney *et al.*, 1998). In humans, this enzyme's activity is mainly studied in the erythrocyte membranes because these cells are easily accessible. A significant decrease has also been reported in uncontrolled diabetic patients (Issautier *et al.*, 1994). Na⁺-K⁺-ATPase pump simultaneously transport sodium ions out of the cell and potassium ions into the cell and pump rate is enhanced by a rise in intracellular sodium concentrations. This hypothesis is confirmed as we observed a significant positive correlation between serum sodium and Na⁺-K⁺-ATPase activity in diabetic, diabetic hypertensive and diabetic nephropathy patients (tables 5, 6 and 7).

It is recently suggested that higher sodium concentration in hypertensive patients is related to an elevated blood pressures and an inappropriately high secretion of aldosterone because aldosterone is a potent salt retaining hormone (Tabassum *et al.*, 2004). This enzyme's dysfunction is probably connected with the relative insulinopenia of hyperglycemic diabetic type 1 patients. Intensive insulin therapy from an artificial pancreas for 24

hours restores erythrocyte $\text{Na}^+\text{-K}^+\text{-ATPase}$ activity in diabetic patients. In another connection disturbances of the membrane lipid organization can also explain the decrease in $\text{Na}^+\text{-K}^+\text{-ATPase}$ activity (Racah *et al.*, 1996). Since involvement of glycemic status by means of hyperglycemia and insulin resistance is considerably involved in the electrolyte imbalances in diabetes and its complications as investigated by previous workers (Fein *et al.*, 1980).

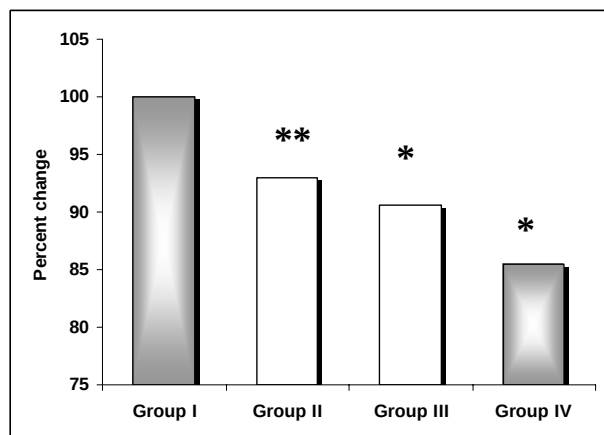


Fig. 2: Percentage difference in Intra erythrocyte potassium levels in various disease groups.

* $p < 0.01$, ** $p < 0.05$ as compared to control subjects

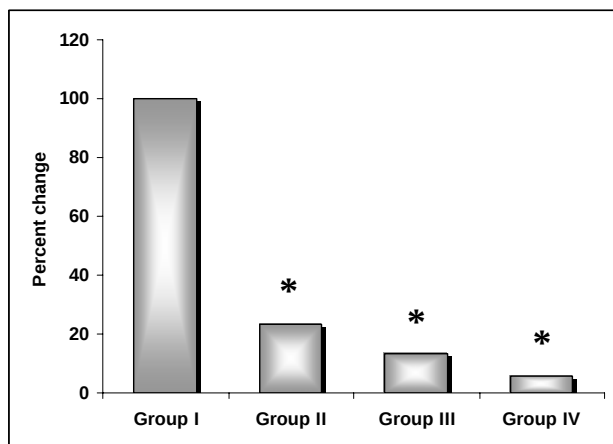


Fig. 3: Percent difference in $\text{Na}^+\text{-K}^+\text{-ATPase}$ levels in various disease groups.

* $p < 0.01$ as compared to control subjects

The other factors involved in increased intra erythrocyte sodium and decreased intra erythrocyte potassium concentrations during hypertensive diabetic disease are impaired $\text{Na}^+\text{-Ca}^{2+}$ -exchange system as the significant increase in serum calcium is also observed in diabetic patients during presented study (fig. 6) and enhanced activity of $\text{Na}^+\text{-Li}^+$ -counter transport system. The $\text{Na}^+\text{-Li}^+$ -counter transport activity is significantly higher in

patients with positive family history of hypertension renal disease. These facts strongly reflect a predisposition of hypertension in diabetes mellitus (Fujita *et al.*, 1994). These counter transport mechanisms are implicated in our study as we observed a significant positive correlation between serum sodium and calcium in diabetic hypertensive and diabetic nephropathy patients (table 6 and 7).

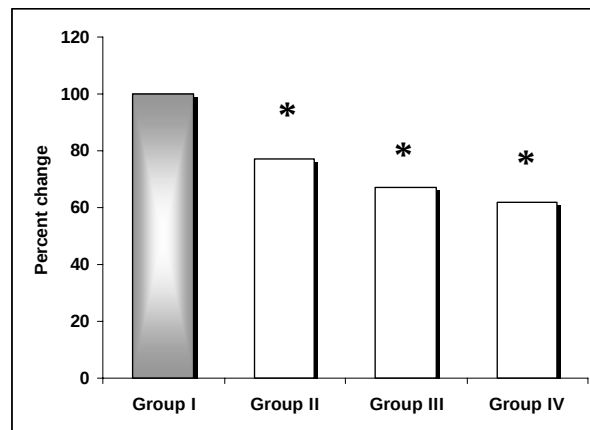


Fig. 4: Percent difference in serum sodium levels in various disease groups.

* $p < 0.01$ as compared to control subjects.

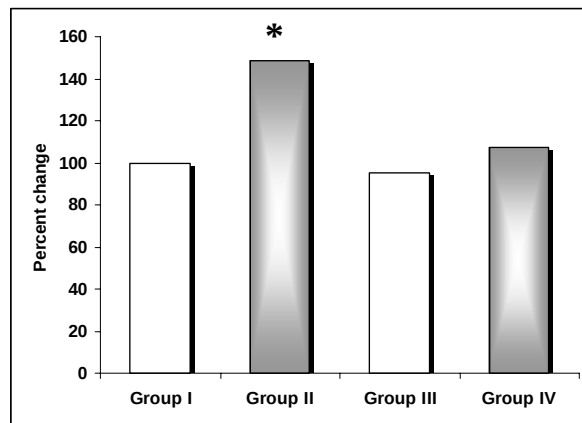


Fig. 5: Percent difference in serum potassium levels in various disease groups.

* $p < 0.01$ as compared to control subjects

Sodium retention occurs as a characteristic alteration in type 1 as well as type 2 diabetes mellitus; exchangeable body sodium is increased by 10% on average. This abnormality develops in the uncomplicated stage of diabetes and differentiates diabetic from non-diabetic essential hypertensive subjects. Possible sodium retaining mechanisms include increased glomerular filtration of glucose leading to enhanced proximal tubular sodium – glucose cotransport, hyperinsulinemia, which activates several tubular sodium transporters, an extravascular shift

Table 5: Correlations among various electrolytes in diabetic patients (r=)

	RBC Na ⁺	RBC K ⁺	Na ⁺ -K ⁺ -ATPase	Serum Na ⁺	Serum K ⁺	Serum Ca ⁺⁺
RBC K ⁺	-0.345					
Na ⁺ -K ⁺ -ATPase	-0.132	0.153				
Serum Na ⁺	-0.256	0.276	0.493*			
Serum K ⁺	0.414	-0.279	-0.365	-0.519*		
Serum Ca ⁺⁺	0.052	-0.030	-0.267	-0.271	0.162	
Serum Mg ⁺⁺	-0.116	0.276	0.350	0.458	-0.181	-0.128

* p<0.01

Table 6: Correlations among various electrolytes in diabetic hypertensive patients (r=)

	RBC Na ⁺	RBC K ⁺	Na ⁺ -K ⁺ -ATPase	Serum Na ⁺	Serum K ⁺	Serum Ca ⁺⁺
RBC K ⁺	-0.344					
Na ⁺ -K ⁺ -ATPase	-0.362	0.233				
Serum Na ⁺	-0.505*	0.249	0.617*			
Serum K ⁺	-0.042	-0.198	0.055	0.215		
Serum Ca ⁺⁺	-0.311	0.232	0.306	0.494*	-0.121	
Serum Mg ⁺⁺	-0.190	0.292	0.438	0.463	0.152	0.191

* p<0.01

Table 7: Correlations among various electrolytes in diabetic hypertensive patients with nephropathy (r=)

	RBC Na ⁺	RBC K ⁺	Na ⁺ -K ⁺ -ATPase	Serum Na ⁺	Serum K ⁺	Serum Ca ⁺⁺
RBC K⁺	-0.490*					
Na⁺-K⁺-ATPase	-0.505*	0.313				
Serum Na⁺	-0.600*	0.351	0.654*			
Serum K⁺	0.182	-0.179	-0.079	-0.133		
Serum Ca⁺⁺	-0.436	0.323	0.394	0.525*	-0.155	
Serum Mg⁺⁺	-0.350	0.321	0.537	0.567*	0.096	0.317

* p<0.01

of fluid with sodium, and once it occurs, renal failure. This pathogenetic role of sodium in diabetes-associated hypertension is supported by positive correlation between systolic or mean blood pressure and exchangeable sodium of body (Shahid and Tabassum, 2003). The progressive decrease in Na⁺-K⁺-ATPase activity, observed in all patients groups as compared to control subjects (fig. 3) in present study is also implicated in the development of vascular diseases in diabetic patients. Previous studies showed that renin-angiotensin involvement especially changes in angiotensin converting enzyme (ACE) activity level are considered to be a key factor in the progression of diabetic nephropathy. Since ACE converts angiotensin I to angiotensin II which is a potential vasoconstrictor and plays a vital role in the regulation of blood pressure. Previous studies have indicated that hyperreactivity of adrenal renin-angiotensin system under hyperreninemia is associated with severe diabetes mellitus (Unsung et al., 2000; Nakayama et al., 1998; Biwititi et al., 2000).

Intracellular potassium depletion is a common feature of diabetic complications. That was consistently found in various stages of progression of diabetic nephropathy (fig. 2). On the other hand, diabetics differ markedly in their erythrocyte reactions regarding potassium permeability. Therefore we did not find significant changes in case of serum potassium levels in various disease groups except diabetic patients (fig. 5). Where as patients with renal insufficiency show an efflux of potassium during investigation as there is a decrease of potassium concentration in serum of diabetic patients (Kraat et al., 1997).

Fasting steady state levels are closely linked to calcium and magnesium homeostasis (Resnick et al., 2001). This link is observed as the significant and progressive percentage decrease of serum magnesium levels in all patients groups in presented study (Figure 7). The present study revealed decreased levels of serum calcium and magnesium. This might have an association with

increased blood pressure. These were also reported in hypertensive patients as compared to normotensive patients. Magnesium appears to be a special kind of calcium antagonist in vascular smooth muscles. At vascular membrane it can lower peripheral and cerebral vascular resistance. Decreased level of magnesium also results in the inhibition of $\text{Na}^+\text{-K}^+\text{-ATPase}$ enzyme because magnesium is a co factor for this enzyme system (Kristers *et al.*, 1997). This mechanism has been shown in our study as both magnesium concentrations and $\text{Na}^+\text{-K}^+\text{-ATPase}$ activity are markedly decreased in various disease groups. It also works as our observation in which we found a strong positive correlation between serum magnesium and $\text{Na}^+\text{-K}^+\text{-ATPase}$ activity in diabetic nephropathy patients (table 7). Magnesium is the second most abundant intracellular cation in a number of important biochemical reactions, including all ATP-transfer reactions. Possibly because of its relevance to all protein kinases, magnesium appears to mediate hormonal as well as other aspects of cellular glucose utilization. There was significant decreased level of magnesium and calcium found in diabetic patients with complications such as hypertension and nephropathy as described by various previous workers. Magnesium depletion is also clinically associated with the poor glycemic control. The magnesium deficiency has been demonstrated in insulin resistance such as hypertension and type 2 diabetes mellitus may thus contribute to suppress glucose metabolism and insulin action (Djurhuus *et al.*, 2000; Resnick *et al.*, 1999; Paolisso and Barbagallo, 1997).

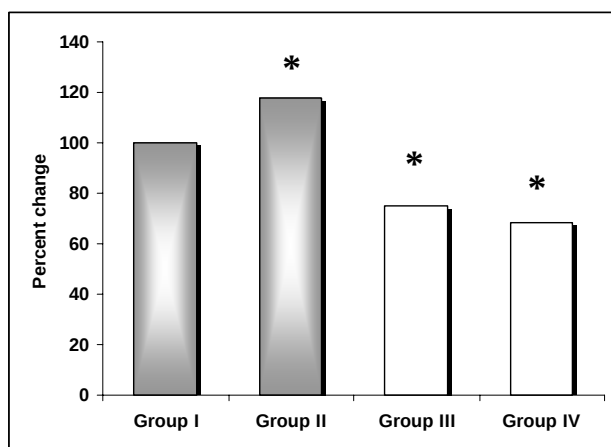


Fig. 6: Percent difference in serum calcium levels in various disease groups.

* $p < 0.01$ as compared to control subjects

In conclusion, the results reported in this study suggest that the electrolyte aberration and $\text{Na}^+\text{-K}^+\text{-ATPase}$ dysfunction is significantly associated in the progression of diabetic renal disease. The observations are also focused on the possibility that the basic cellular abnormality in diabetic nephropathy is an inability to

maintain normal transmembrane electrolyte gradient. In long standing diabetes with hypertension, the measurement and interpretation of these cellular markers may be of help to identify the patients who are at risk of developing nephropathy and it should be considered while treating the diabetic patients to get rid of renal insufficiencies.

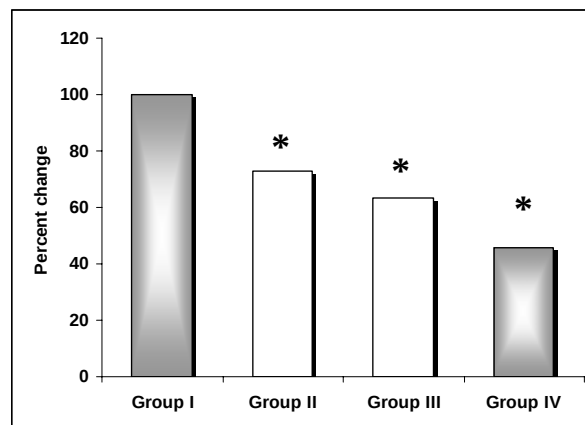


Fig. 7: Percent difference in serum magnesium levels in various disease groups.

* $p < 0.01$ as compared to control subjects

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