Original Article

SIMILAR NATURE OF IONIC IMBALANCES IN CARDIOVASCULAR AND RENAL DISORDERS

Shahid SM[1], Akram H[2], Jawed M[3] & Mahboob T[4]

ABSTRACT

Background: Several studies have reported improper ionic environment in cardiovascular and renal patients but how the diseases are associated on ionic basis is still not clear.

Objective: The present study was aimed to investigate sodium and potassium concentrations and their transport abnormalities in cardiovascular and renal patients.

Patients & Methods: Thirty patients of various cardiovascular and thirty patients of various renal disorders (53.33% males, 46.67% females) were selected. Erythrocytes were isolated from freshly drawn blood samples, washed and used for the estimation of sodium and potassium levels using flame photometer (Corning 410). Serum sodium and potassium were measured by flame photometer. RBC membranes were prepared for the estimation of Na^+-K^+-ATPase activity in terms of inorganic phosphate released/mg protein/hour.

Results: Intra-erythrocyte and serum sodium and potassium concentrations and Na^+-K^+-ATPase activity were different in cardiovascular and renal patients from controls. Intra-erythrocyte sodium level was increased significantly *(P<0.01)* in cardiovascular patients and non-significantly in renal patients as compared to controls. Na^+-K^+-ATPase activity and serum sodium level were decreased significantly *(P<0.01)* in both the groups as compared to controls. Serum potassium was found to be decreased significantly *(P<0.01)* in cardiovascular patients whereas it was raised significantly *(P<0.01)* in renal patients as compared to control subjects.

Conclusion: The results indicated similar nature of ionic and electrolyte imbalances in cardiovascular and renal disorders resulting from impaired Na^+-K^+-ATPase system. Further investigations in the same area, may be of help to establish an understanding of the progression of diseases, associated complications and the preventive steps that should be taken to arrest the progression of these disorders.

KEY WORDS: Cardiovascular, renal diseases, sodium, potassium, Na^+-K^+-ATPase.

Pak J Med Sci October-December 2004 Vol. 20 No. 4 331-336

1. Mr. Syed Muhammad Shahid M.Sc
Lecturer/Demonstrator
2. Ms. Hina Akram M.Sc
Research Fellow
3. Dr. Muhammad Jawed M.Phil
Assistant Professor
4. Dr. Tabassum Mahboob, PhD
Associate Professor
1 & 3:Department of Biochemistry,
Fatima Jinnah Dental College,
Bhittai Colony, Korangi Creek, Karachi-Pakistan
2 & 4: Department of Biochemistry,
University of Karachi, Karachi-Pakistan

Correspondence:
Dr. Tabassum Mahboob
Email: smshahid@super.net.pk

* Received for publication: November 3, 2003
1st Revision received: March 22, 2004
2nd Revision received: June 12, 2004
Revision accepted: July 12, 2004

INTRODUCTION

Cardiovascular and renal diseases are the major health problems and main causes of morbidity and mortality in Pakistan due to specific socio-economic and life style changes. Ischemic heart disease, hypertension and chronic cardiac failure are the most common cardiovascular problems. As indicated by various studies, dysregulation of sodium, potassium and other ions has a role in the development of various cardiovascular diseases[1]. Patients with ischemic heart disease commonly exhibit acid-base and electrolyte alterations mainly hypokalemia, hypocalcemia and hypophosphatemia and allied disturbances[2].

In 1950s and 1960s several disorders of fluid

and electrolyte metabolism were described in which the principle disturbance appeared to be a specific functional defect in the renal tubule[3]. Most important electrolytes like sodium, potassium, calcium, magnesium and bicarbonate provide inorganic chemicals for biochemical processes as well as act at the cell membrane to allow transmission of electrochemical impulse in nerve and muscle fibers[4]. The intracellular cation play a significant role in the regulation of normal physiology and biochemistry of cardiac and smooth muscles. Dysregulation of these processes is an important factor in the genesis of various serious arrythmias[5].

Kidneys being the vital organs of the body perform reabsorption of these important electrolytes by means of active transport and electrochemical gradient[6]. Changes in renal medulla, resulting in activation of the renin-angiotensin system may also contribute to sodium retention and hypertension[7]. Treatment of hypertension at least partially restores potassium levels towards normal and fasting steady state potassium level is closely linked to calcium and magnesium homeostasis[8]. Abnormal sodium metabolism has a critical role in etiology of cardiovascular and renal disorders that can increase the circulating concentration of Na^+-K^+-ATPase inhibitors Dopamine and Catecholamine for example, which is responsible for the cause of essential hypertension. The Na^+-K^+-ATPase pump has an important role in maintenance of cellular ionic composition and volume by promoting the entry of nutrients into the cell by cotransport with sodium and is involved in the generation of membrane potential and is also essential for the movements of solutes across the cells in renal tubules[9]. This mechanism can be associated with urinary loss of sodium and bicarbonate, leading to volume depletion and metabolic acidosis[10]. Other channels also have their roles in regulation of cations in the compartments of blood including Ca^{++}-dependent K^+-channels, Na^+-carriers, K^+-carriers, Na^+-Li^+ exchangers, Na^+-K^+ exchangers[11]. Some of these effects especially in myocardial tissues may lead to permanent tissue damage directly associated with cardiovascular mortality[12].

The present study was carried out to find out sodium and potassium transport abnormalities in serum and red blood cells of various cardiovascular and renal patients, and to compare these with the transport of these cations in normal healthy subjects.

PATIENTS & METHODS

Study Population:

Thirty cases of cardiovascular diseases (16 males and 14 females) admitted to coronary care unit of Liaquat National Hospital and thirty cases of renal disorders (16 males and 14 females) admitted to Urology units of Liaquat National Hospital, Civil Hospital and Jinnah Postgraduate Medical Center were selected randomly for the present study after informed consent was obtained. The mean ± SD ages of cardiovascular and renal patients were 58.23 ± 8.86 and 51.18 ± 10.49 years, respectively. The cardiovascular patients were suffering from ischemic heart disease and hypertension; where as renal patients were suffering from nephritis, renal hypertension and diabetic nephropathy. These diagnoses were taken from the medical records of these patients on the basis of laboratory investigations. Same number (N=30) of age and sex matched healthy subjects with no known history of cardiovascular and renal diseases were selected as controls.

Sample Collection:

The blood samples of patients and control subjects were collected in lithium-heparin coated tubes after an informed consent was obtained for analysis of erythrocyte sodium and potassium. Blood samples were processed the same day for estimations.

Erythrocyte electrolyte estimation:

Heparinized blood was centrifuged and plasma was separated. Buffy coat was also removed leaving behind erythrocytes. Erythrocytes were washed three times at room tem-

perature by suspension in the magnesium chloride solution (112 mmol/L) and using centrifugation at 450x g at 4°C for 5 minutes and aspiration of the supernatant as described earlier [13]. Final supernatant was retained for the estimation of erythrocyte sodium and potassium concentrations. Neither electrolyte was detectable in the final wash. Washed erythrocytes were then lysed with the help of 20% Saponin solution and the lysate was used for the estimation of erythrocyte sodium and potassium concentrations.

Plasma electrolyte estimation:

Plasma sodium and potassium were estimated by flame photometer (Corning 410) by previously described method[14].

Erythrocyte membrane preparation:

The red cell pack extracted by centrifugation at 4°C was resuspended and diluted in 25 volumes of 0.11mol/L Tris buffer, pH 7.4. The hemolyzed cells were then centrifuged at 12,000 rpm at 4°C and the membrane pellet was again suspended in 30 ml of 0.11 mol/L Tris-HCl buffer, pH 7.4. This centrifugation step was repeated three times. The final concentration of the membrane suspension was ~4 mg protein/ml of Tris buffer. The concentration of protein was estimated by Biuret method[15]. The membrane suspension was stored at –80°C until the assay was performed.

Erythrocyte Na^+-K^+-ATPase activity measurement[16]:

ATPase activity was measured in a final volume of 1 ml briefly: membrane (400mg) were preincubated for 10 minutes at 37°C in a mixture containing 92 mmol/L Tris-HCl (pH 7.4), 100 mmol/L NaCl, 20 mmol/L KCl, 5 mmol/L $MgSO_4$ and 1 mmol/L EDTA. Assays were performed with and without 1mmol/L Ouabain, a specific inhibitor of Na^+-K^+-ATPase. After incubation with 4 mmol/L ATP (Vanadate free, Sigma) at 37°C for 10 minutes, the reaction was stopped by adding ice-cold trichloroacetic acid to a final concentration of 5%. After centrifugation at 5500g at 4°C for 10 minutes the amount of inorganic phosphate in the supernatant was determined[17]. Na^+-K^+-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 /milligram protein/ hour.

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 ± SD. Statistical significance and differences between groups were determined by Student's t-test.

RESULTS

Results presented in Tables 1 and 2 show differences among cardiovascular patients, renal patients and control subjects with respect to sodium and potassium homeostasis. Intra-erythrocyte sodium and potassium levels were

Table-I: Intra-erythrocyte and serum sodium and potassium concentrations and Na^+-K^+-ATPase activity in control subjects and cardiovascular patients

	Control	*Cardiovascular Patients*
RBC Sodium (mmol/L)	8.92 ± 0.89	10.65±2.11*
RBC Potassium (mmol/L)	121.73 ± 11.98	133 ± 18.83*
Na^+-K^+-ATPase (nm/mg/hr)	443.37 ± 282.03	182.71±30.75*
Serum Sodium (mmol/L)	143.3 ± 0.68	122.27 ± 13.3*
Serum Potassium (mmol/L)	4.9 ± 0.39	4.2 ± 1.26*

Values are mean ± SD.
Significant difference was determined by Student's t-test
* P<0.01 controls vs. cardiovascular patients (n=30)

significantly increased (P<0.01) where as Na^+-K^+-ATPase activity, serum sodium and potassium levels were significantly decreased (P<0.01) in cardiovascular patients as compared to control subjects (Table-I).

Table-II represents the comparison of renal patients with control subjects. Intra-erythrocyte and serum potassium levels were significantly raised (P<0.01) while serum sodium and Na^+-K^+-ATPase activity were significantly decreased (P<0.01) in renal patients as compared to control subjects (Table-II). No significant difference was observed in intra-erythrocyte sodium concentrations of renal patients and control subjects.

Table-II: Intra-erythrocyte and serum sodium and potassium concentrations and Na^+-K^+-ATPase activity in control subjects and renal patients

	Control	*Renal Patients*
RBC Sodium (mmol/L)	8.92 ± 0.89	9.82 ± 5.04
RBC Potassium (mmol/L)	121.73 ± 11.98	133.7 ± 13.9*
Na^+-K^+-ATPase (nm/mg/hr)	443.37 ± 282.03	28.85 ± 12.22*
Serum Sodium (mmol/L)	143.3 ± 0.68	130.9 ± 14.92*
Serum Potassium (mmol/L)	4.9 ± 0.39	5.99 ± 0.21*

Values are mean ± SD.
Significant difference was determined by Student's t-test
* P<0.01, controls vs. cardiovascular patients (n=30)

DISCUSSION

The dysregulation of chief electrolytes especially sodium, potassium and calcium has a characteristic role in the cardiovascular and renal diseases. The results from the presented study expose the similar nature of ionic imbalances as intra-erythrocyte sodium and potassium levels were significantly increased and Na^+-K^+-ATPase activity and serum sodium level was significantly decreased in cardiovascular as well as renal patients as compared to control subjects (Table -I & II).

This study has shown altered sodium and potassium concentrations in cardiovascular and renal patients primarily due to impaired Na^+-K^+-ATPase activity. The results also show that alterations occur in erythrocyte and plasma ionic environment in cardiovascular and renal patients. Under physiological conditions, the Na^+-K^+-ATPase pump is the principal transporter, accounting for 1.4-2.0 mmol/RBC/Hr. The Na^+-K^+-cotransport and sodium leak pathway are each responsible for approximately 0.2 mmol/RBC/Hr. Most of the previous studies indicate that elevation of intracellular sodium and potassium was associated with a reduced activity of erythrocyte Na^+-K^+-ATPase pump[18], that also observed in this study (Table I-II). Inhibition of Na^+-K^+-ATPase activity is the main factor as in most of the cardiovascular diseases, inhibited or reduced ATPase activity has been observed. Various studies have reported inhibition of Na^+-K^+-ATPase activity during cardiovascular problems[19-23]. The plasma sodium and potassium concentrations were found to be decreased in cardiovascular patients (Table-I). This has been indicated by several previous investigators suggesting that increased Na^+-H^+-exchanger activity might be involved in this functional impairment[24]. In spontaneously hypertensive rats, increased functional sodium reabsorption and blunted pressure natriuresis have been reported[25].

The decreased serum sodium and increased serum potassium concentrations in renal patients (Table-II) have also been reported previously[6], indicating that hyperkalemic state might have developed from a shift of potassium from intracellular to extracellular compartment or it could have been secondary to decreased renal potassium excretion. This increased potassium could also result from decreased renin production, which affects the aldosterone synthesis due to adrenal defect, which then would produce renal tubular secretory defect leading to abnormal distribution of potassium between intra and extracellular compartments[26].

Cardiovascular diseases especially ischemic heart disease account for the majority of mor-

bidity. It involves the atherosclerotic vascular changes in the coronary circulation leading to angina pectoris and myocardial infarction. It has previously been suggested that higher sodium and potassium concentrations in hypertensive patients are related to an elevated blood pressure and an inappropriately high secretion of aldosterone, which is a potent salt retaining hormone[27].

Kidneys are primary target of diabetes mellitus. In fact renal, failure is second only to myocardial infarction as a cause of death from this disease. The biochemical and structural alterations responsible for renal vascular complications of diabetes mellitus, such as, diabetic nephropathy are likely to be the result of common processes[28]. Diabetes raises the blood glucose level by increasing the amount of glucose in glomerular filtrate. Reabsorption of glucose takes place by Na^+-glucose cotransport mechanism, increasing both sodium and glucose concentrations. This raised sodium level returns to normal by pressure natriuresis because in response to an acute increase in arterial pressure there is a rapid natriuresis and diuresis that occurs in the absence of a change in renal blood flow or glomerular filtration rate[29]. The results also suggest that there is a loss or damage of major part of nephron due to which the adrenal release of aldosterone is decreased, which in turn reduces potassium secretion. Therefore, decreasing plasma sodium and increasing potassium concentrations were observed. The decreased plasma sodium also caused by an increased activity of adrenaline stimulates the excretion of prostaglandins, which causes increased excretion of sodium in urine, and a further decrease in plasma sodium level takes place. The principal function attributed to renal prostaglandins is modulation of renal blood flow and electrolyte excretion, activation of renin secretion and antagonism to the antidiuretic action of vasopressin[30].

The altered similar status of sodium and potassium in patients of cardiovascular and renal disorders suggested that abnormal plasma and erythrocyte sodium and potassium levels are possibly produced due to abnormal physiochemical and structural condition of heart muscles as well as kidneys. Further elucidation of exact mechanism by which these ionic disturbances occur for example the various cation transport mechanisms, their kinetics at the cellular level, the membrane abnormalities resulting in receptors modulation and the secondary changes at the hormonal level in normal and kidney patients may provide new insights regarding the pathogenesis and treatment of various kinds of cardiovascular and renal disorders.

REFERENCES

1. Blaustein MP. Sodium ion, blood pressure regulation and hypertension: a reassessment and a hypothesis. Am J Physiol 1977; 232: 165-73.
2. Milionis HJ, Alexandrides GE, Liberopoulos EN, Bairaktari ET, Goudevenos J, Elisaf MS. Hypomagnesemia and concurrent acid-base and electrolyte abnormalities in patients with congestive heart failure. Eur J Heart Fail 2002; 4(2): 167-73.
3. Scheinman SJ, Guay-Woodford LM, Thakker RV, Warnock DG. Genetic disorders of renal electrolyte transport. N Engl J Med 1999; 340: 1177-87.
4. Kokko and Tannen (eds.) Fluid and electrolyte abnormalities of disease, "Fluids and Electrolytes" (second edition), W.B. Saunders Company, Philadelphia, PA 19106, USA, pp 647-80, 1990.
5. Bassett AL, Chakko S, Epstein M. Are calcium antagonists proarrhythmic? J Hypertens 1997; 15: 915-23.
6. Bear RA, Neil GA. A clinical approach to common electrolyte problems. Can Med Assoc J 1983, 129: 28-32.
7. Hall JE. Renal and cardiovascular mechanisms of obesity. Hypertension 1994, 23: 381-394.
8. Resnick LM, Barbagallo M, Dominguez LJ, Veniero JM, Nicholson JP & Gupta RK. Relation of cellular potassium to other mineral ions in hypertension and diabetes. Hypertension 2001; 38 (3 pt 2): 709-12.
9. Jorgenson PL. Structure, function and regulation of sodium-potassium ATPase in kidney. Kidney Int 1986;29: 10.
10. Hricik DE, Chareandee C, Knauss TC. Hypertension after pancreas-kidney transplantation: role of bladder versus enteric pancreatic drainage. Transplantation 2000;70:494-6.
11. Roberto Paterno, Donald D. Functional activity of calcium-dependent potassium channels in increased in basilar artery during chronic hypertension. Am J Physiol 1997; H1287-H1290.
12. Reunane A, Knekt P, Maruiemi J, Maki J, Aromaa A. Serum calcium, magnesium, copper and zinc and risk of cardiovascular death. Eur J Clin Nutr 1996; 50(7): 431-37.
13. Tabassum M, Mumtaz M, Haleem MA. Electrolyte content of serum, erythrocyte, kidney and heart tissue in salt induced hypertensive rats. Life Sci 1996; 59:731-7.
14. Fortes KD, Starkey BJ. Sompler flame photometric determination of erythrocyte sodium and potassium. The reference range for apparently healthy adults. Clin Chem 1977;23:257-8.

15. Savory J, Pu PH, Sunderman FW. A Biuret method for determination of protein. Clin Chem 1968; 14:1168-71.
16. Raccah D, Cloudie A, Azulay J P, Philipe V. Erythrocyte Na^+-K^+-ATPase activity, metabolic control and neuropathy in IDDM patients. Diabetes Care 1996; 19: 564-8.
17. Dryer RL, Tammes AR. Method for estimation of phosphorus in biological sample. J Biol Chem 1957;255:177.
18. Garay R, Adrangna N, Canessa M, Tostenson D. Outward sodium and potassium cotransport in human red blood cells. J Membr Biol 1981; 62: 169-74.
19. Lees GH. Inhibition of sodium potassium ATPase; a potentially ubiquitous mechanism contributing to central nervous system neuropathology. Brain Res Rev 1991;16(3):283-300.
20. Walter V, Muller S. Evidence for sodium potassium ATPase inhibitor in erythrocytes in patients with essential hypertension. Eur J Clin Invest 1985;15(4):209-14.
21. Hamlyn JM, Ringel R. A circulating inhibitor of sodium potassium ATPase associated with essential hypertension. Nature 1982; 300: 650-2.
22. Gault MH, Vasdev SC, Longerrich LL, et al. Plasma digitalis-like factor(s) increase with salt loading. N Engl J Med 1983;309:1459-61.
23. Lijnen P. Erythrocyte concentrations and transmembrane fluxes of sodium and potassium in essential hypertension. Cardiovasc Drugs Ther 1990; 4 suppl (2):321-33.
24. Daugher G, Sauterey. H pump and Na^+-H^+-exchange in isolated single proximal tubules of spontaneously hypertensive rats. J Hypertns 1992; 10:969-78.
25. Hayashi M, Yoshida T, Monhawa T, et al. Na^+-H^+-exchanger activity and its gene in the spontaneously hypertensive rat kidney. J Hypertens 1997; 15: 43-8.
26. Zeidek W, Losse H, Schmidt W, Vetter H. Potassium load in spontaneously hypertensive rats. Effects on blood pressure, renin-angiotensin, aldosterone and intracellular electrolytes. Res Exp Med 1983;183:147.
27. Kisters K, Tepel M, Spieker, et al. Decreased cellular magnesium concentration in a subgroup of hypertensives Cell models for the pathogenesis of primary hypertension. J Hum Hypertens 1997;11(6):367-72.
28. Jensen T, Deukert T. Generalized vascular damage in insulin dependent diabetic patients. Horm Metab Res Suppl 1992;26:68-70.
29. Guyton AC. Kidney and fluids in pressure regulation. Hypertension Dallas 1992; 19 (suppl 1): I-2-I-8.
30. Dunn MJ, Hood VL. Prostaglandins and the kidney. Am J Physiol 1977; 233: F169-F184.