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DISTURBANCES IN ELECTROLYTES, Na^+-K^+-ATPase AND TRACE ELEMENTS IN ISCHEMIC HEART DISEASE

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ABSTRACT

Ischemic heart disease still presents a major health problem. Electrolytes and to some extent trace elements play an important role in the development of cardiac diseases. This study was aimed to monitor the dysregulation and anomalies in electrolytes, their transport mechanism and role of trace elements in ischemic heart disease. Seventy-eight patients of ischemic heart disease aged between 36-60 years were selected for study. Erythrocytes were isolated from freshly drawn blood samples, washed and used for the estimation of sodium and potassium concentrations using flame photometer (Corning 410). Plasma sodium and potassium were measured by flame photometer. Erythrocyte membranes were prepared for the estimation of Na^+-K^+-ATPase activity in terms of inorganic phosphate released/mg protein/hour. Serum trace elements were investigated using atomic absorption spectrophotometry. Erythrocyte sodium was increased ($p<0.005$) and was unchanged in serum of ischemic heart disease patients as compared to control subjects. Potassium levels were found unchanged in both erythrocyte and serum of ischemic heart disease patients. A decreased level of serum calcium ($p<0.005$) and a significant increased levels of serum magnesium ($p<0.05$), copper ($p<0.005$) and zinc ($p<0.005$) were observed in patients with ischemic heart disease as compared to control subjects. An increased membrane Na^+-K^+-ATPase was observed in IHD patients as compared to control ($p<0.005$). The results suggest that the high levels of magnesium, copper and zinc are accumulated in heart tissues as infarction occurs than these trace elements comes out of the cell leaving high levels of these trace elements in serum.

INTRODUCTION

Cardiovascular diseases are associated with disturbances of electrolytes and trace elements. Patients with Ischemic heart diseases commonly exhibit acid-base and electrolyte disturbances mainly due to the activation of several neurohumoral mechanisms as well as to drugs regularly used in this population. Previous studies showed that ischemic heart disease patients exhibited electrolytes abnormalities such as hypokalemia, hypo-calcemia and hypophasphatemia (Milionis *et al.,* 2002). Intracellular calcium plays a significant role in the regulation of normal cardiac and smooth muscle physiology, biochemistry and disease induced alterations in cellular processes. It is demonstrated clinically and experimentally that calcium overload within ventricular cells is an important factor in the genesis of various serious arrhythmias (Bassett *et al.,* 1997). Magnesium is involved in all physiological interaction in myocardial tissue, coronary artery smooth muscle and sarcolemma conducting systems, including modulation of calcium and potassium channels and adenylate cyclase activity. A decrease in normal serum

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level can have serious deleterious effects (Mroczk *et al.*, 1977; Smetana *et al*., 1991; Workman, 1992). Some of these effects, especially in myocardial tissues may lead to permanent tissue damage. High serum copper is associated with increased cardiovascular mortality (Itoy and Fujita, 1996; Reunane *et al.*, 1996). In views of the above previous studies the present work was designed to investigate the changes in membrane Na^+-K^+-ATPase, serum and red cell electrolytes and serum trace elements in ischemic heart disease.

MATERIALS AND METHODS

Study Protocol:

Seventy-eight patients of either sex with ischemic heart disease admitted to the cardiovascular ward of Liaquat National Hospital, Karachi were included in the present study. All patients were aged between 36-60 years. 78 normotensive, aged 25-60 years were selected as controls. These normotensive were healthy adults and there was no known death due to hypertension in the families of any of these control subjects.

Samples Collection*:*

Blood samples of patients and control subjects were collected in lithium heparin coated tubes for analysis of erythrocytes sodium and potassium. An aliquot was taken in another tube to get serum for the estimation of serum electrolytes and trace elements. Blood samples were processed the same day for estimation of electrolytes in serum and red cells where as for estimation of trace elements serum samples were kept in the freezer until analysis.

Intra-erythrocyte sodium and potassium estimations*:*

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), centrifugation at 450 x g at 4^{o}C for 5 minutes and aspiration of the supernatant as described earlier (Fortes and Starkey, 1977). Final supernatant was retained for the estimation of intra-erythrocyte sodium and potassium concentration. Neither electrolyte was detectable in the final wash. Washed erythrocytes were then lysed and used for the estimation of intra-erythrocyte sodium and potassium.

Erythrocyte membrane preparation*:*

The packed red cells extracted by centrifugation at 4^{o}C, 450x g for 15 minutes were resuspended and diluted in 25 volumes of 0.11 mol/L Tris-HCl buffer at pH 7.4. The hemolyzed cells were then centrifuged for 30 minutes at 12,000 rpm at 4^{o}C and the membrane pellet was resuspended in 30 ml of 0.11 mol/L Tris-HCl buffer. This centri-

Table-1

Correlation between concentration of sodium, potassium and copper with magnesium, zinc and iron in serum of Ischemic heart disease patients

	Serum Magnesium (r =)	Serum Zinc (r =)	Serum Iron (r =)
Serum Sodium	0.59*	0.17	0.28
Serum Potassium	0.24	0.16	0.30
Serum Copper	0.14	0.68**	0.12

*P<0.05, **P<0.005.

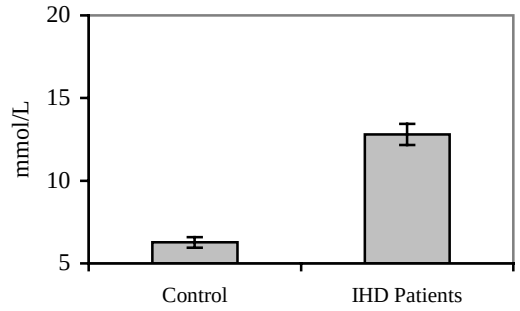

Fig. 1: Erythrocyte sodium in Ischemic heart disease.

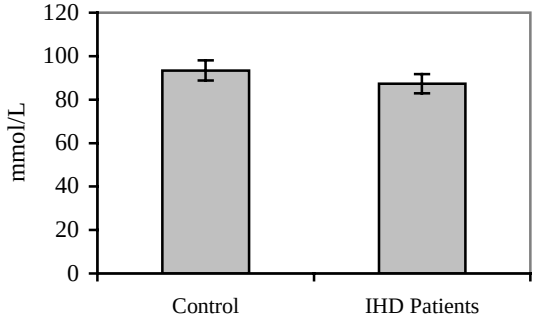

Fig. 2: Erythrocyte potassium in Ischemic heart disease.

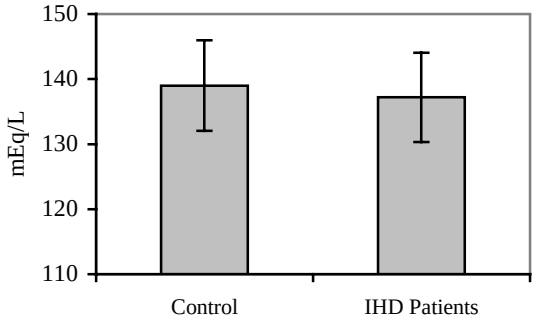

Fig. 3: Serum sodium in Ischemic heart disease.

fugation step was repeated three times. The final concentration of the membrane suspension was ~4 mg protein /ml of Tris buffer. The membrane suspension was stored at –80^{o}C until the assay was performed.

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

ATPase activity was measured in a final volume of 1 ml as follows: Membrane (400ug) were preincubated for 10 minutes at 37^{o}C in a

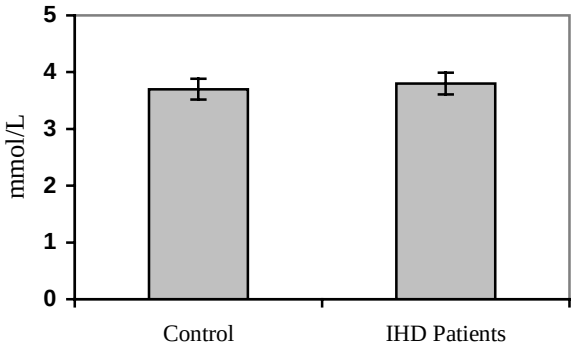

Fig. 4: Serum potassium in Ischemic heart disease

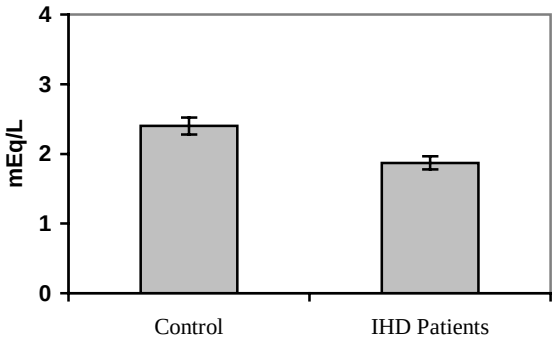

Fig. 5: Serum calcium in Ischemic heart disease.

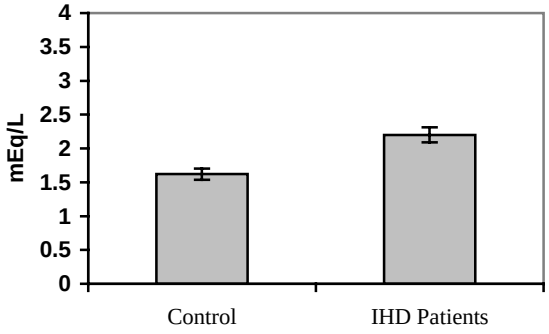

Fig. 6: Serum magnesium in Ischemic heart disease.

mixture containing 92 mmol/L Tris-HCl (pH=7.4), 100 mmol/L NaCl, 20 mmol/L KCl, 5 mmol/L $MgSO_4$ .H_2O and 1 mmol/L EDTA. Assays were performed with or without 1mmol/L Ouabain, a specific inhibitor of Na^+-K^+-ATPase. After incubation with 4 mmol/L ATP (Vanadate free, Sigma) at 37^{o}C for 10 minutes, the reaction was stopped by adding of

ice-cold trichloroacetic acid to a final concentration of 5%. After centrifugation at 4°C, 5500g for 10 minutes. The amount of inorganic phosphate in the supernatant was determined (Dryer and Tammes, 1957). 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%.

Serum sodium and potassium and calcium were estimated by flame photometer (Corning 410). Serum magnesium was estimated by the method of Hallry and Sky Peck (Hallry and Sky Peck, 1964). Serum Iron was estimated by Iron the method described earlier (Artiss *et al*., 1981; Hennessy, 1984). Serum copper and zinc were estimated by atomic absorption spectrophotometer.

Statistical Analysis:

Results are presented as mean ±SD. Statistical significance of the difference from control and test values were evaluated by student's t-test. Correlation coefficient and regression analysis was used to describe the

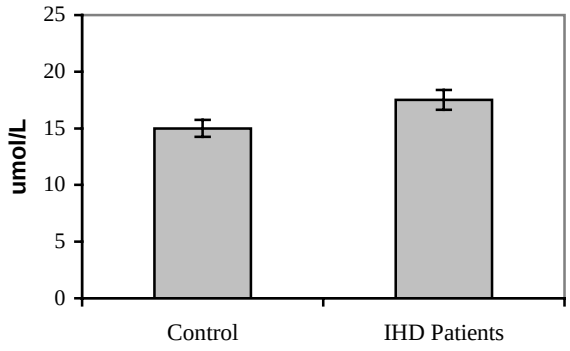

Fig. 7: Serum iron in Ischemic heart disease.

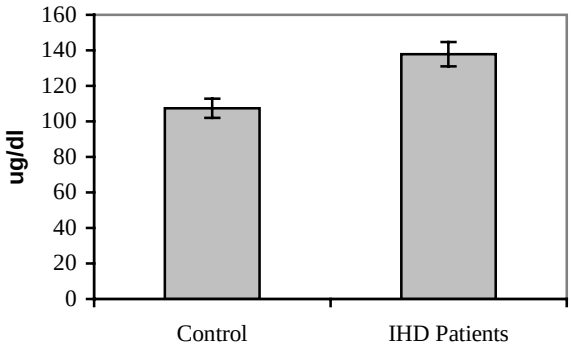

Fig. 8: Serum copper in Ischemic heart disease.

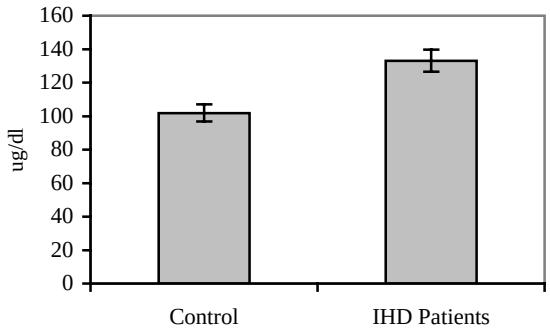

Fig. 9: Serum zinc in Ischemic heart disease.

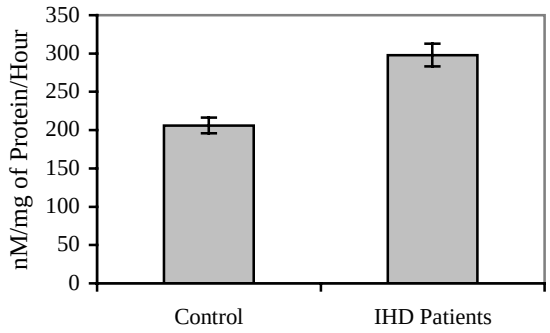

Fig. 10: Erythrocyte Na-K-ATPase activity in Ischemic heart disease.

effect of ischemic heart disease on various electrolytes and trace elements.

RESULTS

Changes in erythrocytes sodium and potassium in ischemic heart disease are shown in figure 1 & 2. Erythrocyte sodium was significantly ($p<0.005$) increased in ischemic heart disease whereas no significant difference in erythrocyte potassium was observed as compared to control subjects. Serum sodium and potassium remains normal in Ischemic heart disease (Fig 3 & 4). A significant decrease in serum calcium ($p<0.005$) was observed (Fig. 5) whereas serum magnesium was significantly increased ($p<0.005$) in ischemic heart disease patients as compared to control subjects (Fig. 6). Serum iron remains normal in patients with Ischemic heart disease as compared to control subjects (Fig. 7). A significant increase in serum copper and zinc was observed in Ischemic heart disease (Fig. 8 and 9). An increased membrane Na^+-K^+-ATPase was observed in IHD patients as compared to control ($p<0.005$) (Fig. 10).

DISCUSSION

Cardiovascular diseases especially ischemic heart disease accounts for the majority of morbidity. It involves the atherosclerotic vascular changes in the coronary circulation leading to angina pectoris and myocardial infarction. It is previously suggested that higher sodium concentration in essential hypertensive patients is related to an elevated blood pressure and an inappropriately high secretion of aldosterone because aldosterone is a potent salt retaining hormone.

Magnesium as a cofactor of Na^+-K^+-ATPase may play an important role (Kisters *et al*., 1997). Results showed that erythrocyte sodium was significantly ($p<0.005$) increased in IHD (Fig. 1) whereas erythrocyte potassium, serum sodium and potassium remain normal. Erythrocyte Na^+-K^+-ATPase activity was increased ($p<0.005$) in IHD patients as compared to control subjects. Several lines of evidence suggested that increased Na/H exchanger activity might be involved in this functional impairment. In SHR strain increased functional sodium reabsorption and blunted pressure natriuresis have been reported (Daugher and Sauterey, 1992; Hayashi *et al*., 1997). Sodium potassium exchange 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 concentration as shown in the present study (Fig. 1). Results showed that serum magnesium was significantly ($p<0.005$) increased in ischemic heart disease. It my be raised due to two major factor: a) Cytolysis due to tissue necrosis leads to general magnesium leakage from cardiac cells and b) Anoxia and hypoxia because myocardial aerobic metabolism is magnesium and potassium dependant (Nagas, 1996). Increased magnesium also accelerates the activity of Na^+-K^+-ATPase (Laurant *et al*., 1997; Speich *et al*., 1980; Hollifield, 1984). A significant inverse correlation between serum sodium and serum magnesium in ischemic heart disease was observed during the present study (Table-1).

Results showed significantly ($p<0.005$) high serum copper and zinc concentration in patients with ischemic heart disease. High serum copper is associated with increased cardiovascular mortality (Reunane *et al*., 1996). It is previously suggested that zinc levels increases in heart tissue in patients with cardiovascular disease. High serum zinc levels in patients with IHD may be due to the tissue necrosis after chronic ischemic heart disease. Results showed a positive correlation between serum copper and zinc (Table 1). Results showed a complete picture of electrolyte disturbances. The alterations of electrolytes are of similar type in IHD associated with tissue necrosis. Intra-erythrocyte sodium level found high but serum sodium concentration remains normal (Fig. 1 & 3). Serum potassium and intra-erythrocyte potassium remain normal (Fig. 2 & 4). High serum magnesium and low serum calcium was found in IHD (Fig. 5 & 6).

Normal ionic balance in cardiac muscle cell is maintained by various co transport and antiport systems. These systems maintain action potential in cardiac cells. The slow calcium current give rise the plateau of cardiac action potential. A small rise in cytosolic calcium ion can trigger a large release of calcium ions from binding sites within the cell, mainly within the sarcoplasmic reticulum (Sarineau and Marthan, 1997). Sodium calcium exchanger across the plasma membrane is a bi-directional exchange system. Sodium is a controlling ion. Increased intracellular sodium leads to enhance calcium uptake by slowing calcium efflux (Ducceschi *et al*., 1996). An ATP dependent calcium ion pump is present in the plasma membrane. Its activity is catalyzed by Calcium magnesium ATPase. It efflux calcium out of the cardiac cell.

Present study showed that calcium, magnesium, copper and zinc are the most affected ions in IHD that is associated with tissue necrosis. Distribution of intracellular sodium, potassium and serum sodium potassium and calcium also altered. The high levels of magnesium, copper and zinc are accumulated in heart tissues and as infarction occurs these trace elements come out of the cell leaving high serum levels of these trace elements.

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