

**REPORT****Association between serum electrolytes and erythrocytes Na<sup>+</sup>, K<sup>+</sup> in hypertensive and normotensive male compared to female****Farzana Yasmin<sup>1,2\*</sup> and Noreen Samad<sup>3\*</sup>**<sup>1</sup>Department of Food Engineering, NED University of Engineering and Technology, Karachi, Pakistan<sup>2</sup>Department of Biomedical Engineering, NED University of Engineering and Technology, Karachi, Pakistan<sup>3</sup>Department of Biochemistry, Bahauddin Zakariya University, Multan, Pakistan

**Abstract:** Hypertension is considered as a multifactorial disorder in which a numerous of physiological mechanism take part to raise blood pressure. The present study is carried out to study the serum and red blood cell electrolytes disturbances in men and women patients of essential hypertension. The samples for analysis were obtained from two hundred four (204) age and sex matched volunteers (51 men and 51 women normotensive, 51 men and 51 women hypertensive). Erythrocytes obtained from blood samples (freshly drawn), washed and processed for the estimation of Na<sup>+</sup> and K<sup>+</sup> concentrations through flame photometer. Biochemical estimations were done by flame photometry and spectrophotometry. Data were analyzed by Two-way ANOVA followed by Newman-Keuls test. Results show the intraerythrocyte sodium levels were significantly higher in essential hypertension patients than normotensive healthy controls. Whereas serum concentrations of sodium, potassium, calcium, magnesium, phosphorus and intraerythrocyte potassium were significantly smaller in hypertensive patients with respect to normotensive control subjects. Moreover, systolic, diastolic blood pressure and intraerythrocyte sodium were higher while potassium was lower in hypertensive women compared to hypertensive men. From a clinical point of view, an inverse correlation was found between systolic blood pressure values and serum Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup> and Mg<sup>2+</sup> in the sample of essential hypertensive patients. No sex related differences were observed in serum electrolytes in normal individual and patients of essential hypertension. The results reported here suggest that serum magnesium and its interactions with monovalent cations e.g. sodium, potassium, phosphorus, RBC sodium and RBC potassium and divalent cations like calcium are the main responsible ions for the pathogenesis of hypertension. Intraerythrocyte levels of sodium perform an important role in the greater vulnerability of male sex to develop hypertension.

**Keywords:** Electrolytes, hypertension, gender difference**INTRODUCTION**

Elevation in Blood pressure is the predictive cause of mortality and morbidity associated with cardiovascular diseases, stroke and renal diseases (Selvarajah *et al.*, 2017). It is an estimation that about quarter of worldwide population is affected with heart disease that is directly linked to increased blood pressure (Nong *et al.*, 2016).

Various dietary electrolytes are associated with the progression and development of hypertension (Beyer *et al.*, 2006). Serum electrolytes also play an important role in blood pressure (BP) regulation and maintenance of water balance and hemostasis around the cells (Selvarajah *et al.*, 2017). Sodium is the most important electrolyte that is directly related to hypertension, but there is need of more ground work to establish a mechanistic role at molecular level (Strohm *et al.*, 2016).

Apart from sodium there is pronounced inactivity is noticed in the calcium (Ca<sup>2+</sup>) and magnesium (Mg<sup>2+</sup>)

metabolism in hypertensive patients (Wittman and Grobbee, 1990). Likewise, variation in the blood pressure is also associated with potassium (K). There has been found disparate studies with potassium in scientific literature related to blood pressure. Some studies claim inverse relation of potassium with BP which hence supported by decrease in systolic and diastolic pressure with intake of potassium in clinical trials (Khaw and Barrett-Conner, 1988; Beyer *et al.*, 2006; Geleijnse *et al.*, 1994). However, other studies suggested that there is no association in potassium intake and hypertension (Antzelevitch and Di Diego, 1992).

Similarly, epidemiological studies show an inverse relationship of BP and magnesium (Mg) intake (Singh *et al.*, 1997). Clinical studies in all ethnic population show decreased level of both systolic and diastolic pressures in women as compared to men. In contrast, men with middle age are more prone to hypertension than women (August, 1999).

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Researchers are contributing to find out the optimal role and involvement of serum electrolytes (Na, K) in hypertension (Makynen *et al.*, 1995; Kimura *et al.*, 2016). It has been found that the changes in metabolic and intracellular alterations are exactly the reflection of abnormalities in serum electrolytes with the development of hypertension.

The aim of the present study is to evaluate the accurate association of mineral elements like sodium, magnesium, phosphorous and potassium ions with hypertension in men and women.

## MATERIALS AND METHODS

**SUBJECTS:** The study was conducted at the Department of Biochemistry, University of Karachi. The samples were collected from the outpatient department in the Karachi University Clinic. The Institutional Ethics Committee approved study protocol. A total of 204 patients and volunteers (age and sex matched) were selected. Mean age of 102 hypertensive patients (51 male, 51 female) was  $46.87 \pm 7.49$  years (range 35- 61) who had been diagnosed as suffering from essential hypertension characterized by a supine systolic blood pressure (SBP) greater than 120 mmHg, and diastolic blood pressure (DBP) greater than 90 mmHg. The diagnosis of essential hypertension was considered when no cause of high blood pressure could be detected after complete clinical, biochemical, and radiological examination. Exclusion criteria were as follows: sensitivity to  $\beta$ - blocker or thiazides; pregnancy or lactation or those taking contraceptive pills; congestive heart failure; atrioventricular block or other dysrhythmia; recent history of myocardial infarction; insulin dependent diabetes; and obstructive airway disease; renal disease; alcoholism and psychiatric problem. 102 age and sex matched healthy subjects (51 male, 51 female) with age range of 34 - 59 years (mean aged  $46.62 \pm 7.42$ ) were recruited to the study. Their blood pressure never exceeded 120/85 mmHg. All subjects were healthy with no clinically significant hematological or clinical biochemical values. None of the subjects was on drug therapy. Serum creatinine was within normal range (60-120 $\mu\text{mol/l}$ ) for all participants. The complete protocol of the experiment used in this study was accepted by the institutional ethics committee.

### **Blood pressure measurement**

The subject's rested supine for 30minutes, and their systolic and diastolic blood pressures were measured. Blood pressure was measured as mean of five consecutive reading in right arm using a calibrated sphygmomanometer in term of mmHg.

### **Analytical method**

#### **Sample preparation**

Blood was withdrawn from an antecubital vein. Five milliliters of whole blood was collected into a tube in

order to collect serum. For this purpose blood was allowed to clot for 15 minutes at 22°C, than centrifuged and serum was transferred into polystyrene tubes. Remaining blood was taken into heparinized tube for intraerythrocyte electrolyte concentration.

### **Erythrocytes preparation**

Heparinized blood was centrifuged, plasma was separated, buffy coat was aspirated and discarded. Red cells were washed three times at room temperature by suspension in magnesium chloride solution (112mmol/L) and centrifugation at 450 X g at 4°C for 5 minutes. Washed red cells were then used for the estimation of intraerythrocytes sodium, potassium by method of Fortes Meyer and Starkey (Fortes-Mayer and Starkey, 1977). Intraerythrocyte magnesium did not monitored in erythrocytes. Serum separated by centrifugation was analyzed for sodium, potassium and calcium by flame photometer (Coring 410) as described earlier (Shahid *et al.*, 2005). Magnesium was determined by Titan method (Hallry and Skyeck, 1964). Phosphorus was determined by Gomorri method.

## STATISTICAL ANALYSIS

Results are presented as means  $\pm$  S.D. Statistical analysis was performed by 2-Way ANOVA. Post hoc comparisons were done by Newmann-Keuls statistics.  $P < 0.05$  was considered significant. All the data were analyzed by SPSS version 20.

## RESULTS

Fig. 1 shows the levels of DBP, SBP, intraerythrocyte sodium and potassium in male and female patients of hypertension. 2-Way ANOVA showed significant disease effects on DBP, SBP, intraerythrocyte sodium and RBC potassium. Sex effects were significant for DBP, SBP, RBC sodium and potassium.

The post-hoc analysis by Newmann-Keuls test showed that DBP, SBP and intraerythrocyte sodium were significantly ( $p < 0.01$ ) higher in men and women patients of hypertension than their respective controls. Values of DBP, SBP and intraerythrocyte sodium in normal healthy men and normal healthy women were comparable. DBP, SBP and intraerythrocyte sodium were higher in hypertensive male patients than hypertensive female patients. Intraerythrocyte potassium levels were significantly ( $p < 0.01$ ) smaller in both male and female patients in their respective normal healthy controls. Male and female controls exhibited comparable values. Male and female patients also exhibited comparable values.

Fig 2 shows serum concentrations of sodium, potassium, calcium, magnesium and phosphorus in male and female patients of hypertension. Two-Way ANOVA showed

significant disease effects ( $p < 0.01$ ) on serum sodium, potassium, calcium, magnesium and phosphorus. Sex effect was found to be significant ( $p < 0.01$ ) for serum potassium. Effect of interactions between sex and disease were not significant for all these measurements.

Post-hoc analysis by Newman-Keuls test showed that in men and women patients of hypertension serum levels of sodium, potassium, calcium, magnesium and phosphorus were smaller ( $p < 0.01$ ) in both male and female patients of essential hypertension. Serum values of these electrolytes in men and women controls were comparable. Levels of these electrolytes in men and women diseased patients were also comparable.

## DISCUSSION

Hypertension is a multifactorial phenomenon that may have some final common pathway that is yet to be determined. Many reasons are found to explain population differences with blood pressure, associated with psychosocial stresses, diet, sodium intake, cultural variability, ecological factors and underlying predisposition based on ethnicity and biology, no clear explanatory model for blood pressure differentials have been emerged so far. Several epidemiological, experimental and clinical studies have shown that electrolyte (e.g. sodium, potassium, calcium and magnesium) changes occur in hypertension in both human and experimental animals (Beyer *et al.*, 2006; Witteman and Grobbee, 199; Yasmin, 2015). However, it has been difficult to evaluate precisely the role of the electrolytes in the hypertension. Since it is not at present clear whether changes in the concentration of electrolytes are involved in the development of hypertension or conversely hypertension produces disturbances of tissue electrolytes.

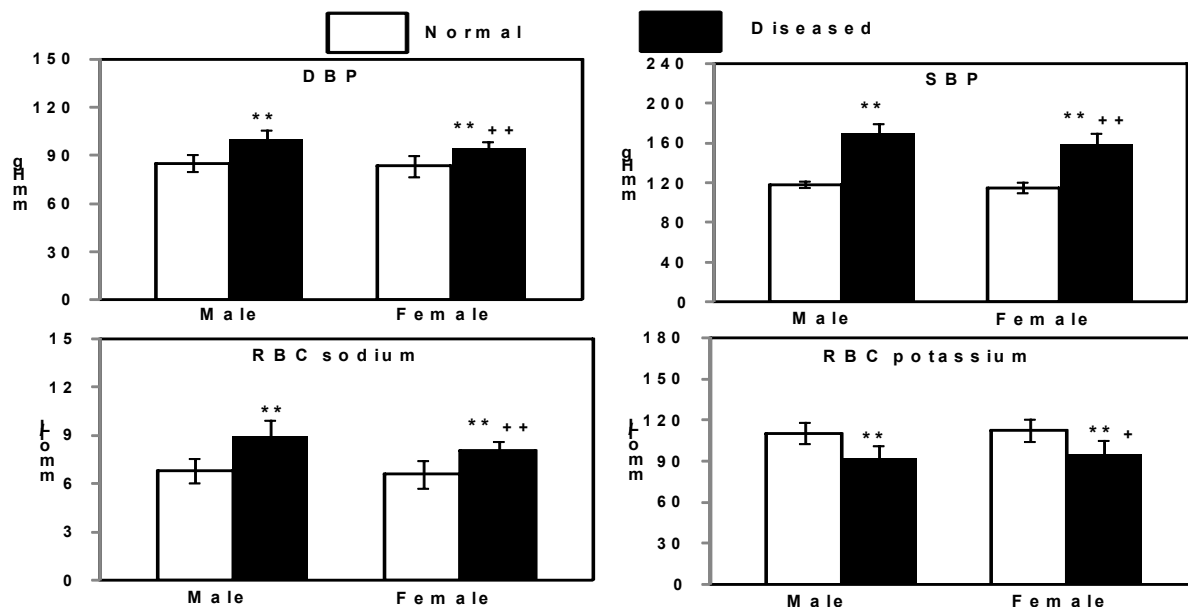
The present study is focused on investigating role of electrolytes in male and female patients of essential hypertension. Stamler (1976) studied that normotensive women at all ages has low mean diastolic blood pressure than normotensive men. Whereas mean systolic blood pressure was higher in normotensive men and women in comparison to women until age 50 years for blacks and 65 years for white and was higher in women thereafter (Stamler *et al.*, 1976). However in the present study where samples were collected from city areas of Karachi, Pakistan diastolic (1.12%) and systolic (2.69%) blood pressure were slightly greater in men than women but the differences were not significant. It has been suggested that lower blood pressure is associated with the presence of estrogen in young women (Regensteiner *et al.*, 1991). A study of hemodynamics changes related with the menstrual cycle is reported with lower blood pressure during the luteal phase rather than the follicular phase (Chapman *et al.*, 1997). This variability may explain the

difference observed in blood pressure between men and women. In the present study systolic blood pressure of hypertensive men and women were 43.12 % and 38.42 % respectively higher than normotensive men and women (fig. 1). The diastolic blood pressure of hypertensive men and women were 16.81 % and 10.59% respectively higher than normotensive men and women (fig. 1).

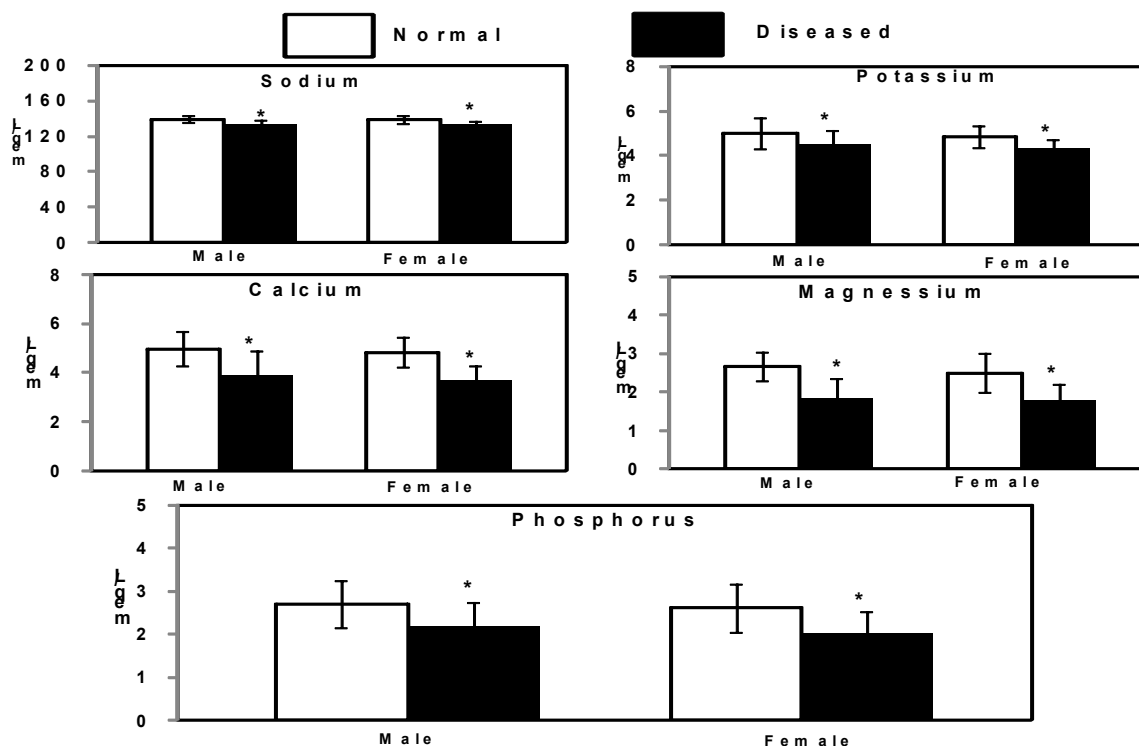
The present study (fig. 1) shows that intraerythrocyte sodium levels were smaller in hypertensive women than men. The present results therefore tend to suggest that sex difference in the levels of systolic blood pressure and diastolic blood pressure may be due to the differences in the concentration of intraerythrocyte sodium in the two sexes. The elevated intraerythrocyte sodium concentration in hypertensive men than women could result because of lower activity of  $\text{Na}^+$ ,  $\text{K}^+$  - ATPase. A number of authors have reported that men have a higher erythrocyte sodium concentration than women (Lasker *et al.*, 1985; Norman *et al.*, 1985, Nakajima *et al.*, 2016). Norman *et al.* (1985) reported that red blood cell  $\text{Na}^+$ ,  $\text{K}^+$  - ATPase activity is smaller in males than females and that lower activity of the enzymes is associated with higher intracellular sodium concentration. The present results suggest that observed decrease in serum sodium; increase intracellular sodium and decrease intracellular potassium may be due to smaller  $\text{Na}^+$ ,  $\text{K}^+$  - ATPase activity in hypertensive men than normotensive subjects (Canestrari *et al.*, 1994). Another cause of observed increase in the intraerythrocyte sodium concentration could be an altered activity of  $\text{Na}^+$  -  $\text{K}^+$  - co-transport (Garay *et al.*, 1983).

Previously studies have shown that serum magnesium levels were higher in hypertension (Frohlich, 1964). However, some studies shown inverse correlation between blood pressure and magnesium (Resnick *et al.*, 1983). The present results show significant decrease in serum  $\text{Mg}^{2+}$  concentration. A significant negative correlation was observed between SBP and serum magnesium ( $r = -0.677$ ,  $p < 0.01$ ) in patients with essential hypertension in the present study (fig.1).

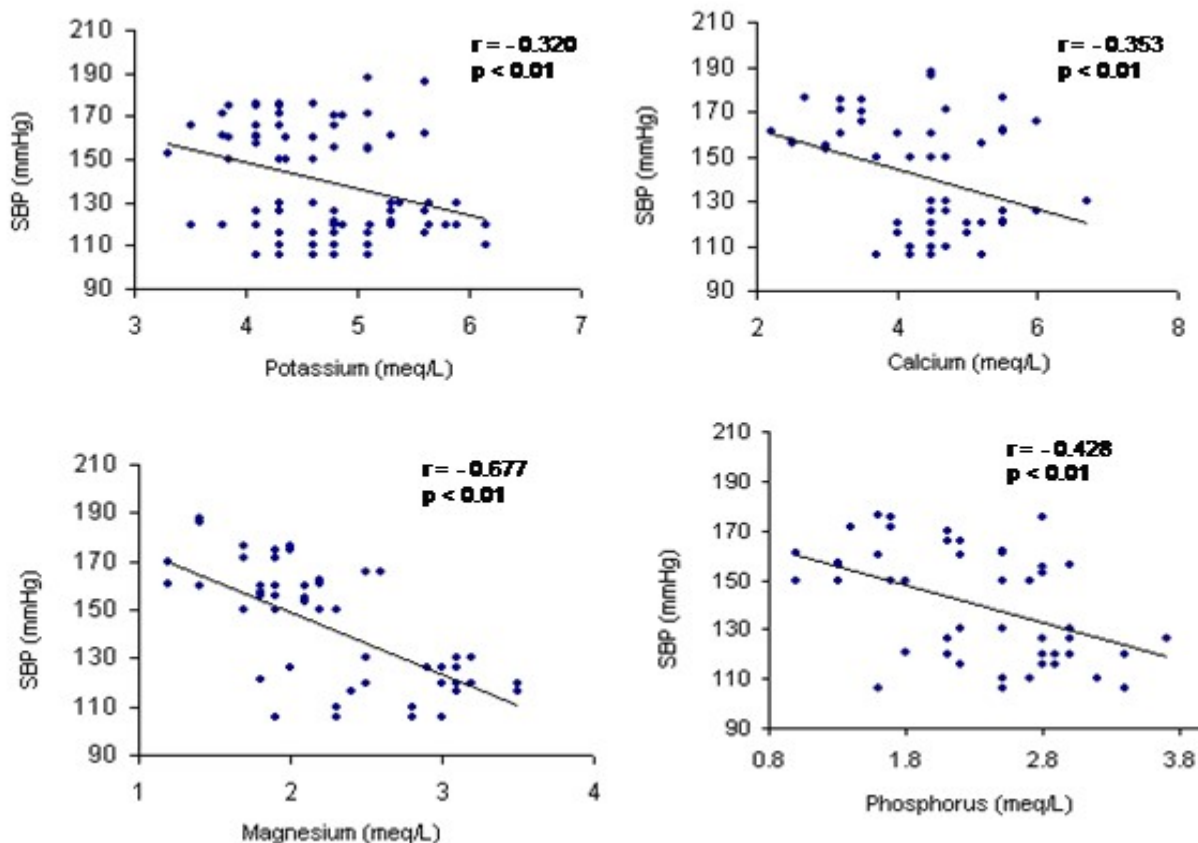
$\text{Mg}^{2+}$  is among the fourth major ion in extra cellular, while the second most abundant intracellular ion in human body. Its deficiency is associated with different cardiovascular disorders (Hordyjewska and Pasternak, 2004). It serves as a cofactor of many enzymes and involved in the regulation of different physiological processes. It also involved in the regulation of concentration of various ions such as  $\text{Na}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{K}^+$  through channels in cell thus; prevents the cell from excessive  $\text{Ca}^{2+}$  ions, suppresses lack of  $\text{K}^+$  ions, maintains the correct level of acidosis, equalizes pH of cell, inhibits sodium influx into the cell, accelerates bioelectrical potential and supplies energy for calcium and sodium pump (Yunfeng *et al.*, 1998).



**Fig. 1:** Diastolic, systolic blood pressure, intraerythrocyte sodium and potassium levels in male and female patients of hypertension. Values are means  $\pm$  SD (n = 204). Significant difference by Newman- Kuels test; from respective controls \*p<0.01; from respective males +p< 0.05; \*\*p<0.01; following two- way ANOVA.



**Fig. 2:** Level of serum sodium, potassium, calcium, magnesium and phosphorus in male & female patients of hypertension values are means  $\pm$  SD (n = 204). Significant differences by Newman -Keuls test; from respective controls \*p< 0.05; following two-way ANOVA.



**Fig. 3:** Correlation between systolic blood pressure and serum potassium, calcium, magnesium and phosphorus in male and female hypertensive.

$Mg^{2+}$  is a cofactor for the  $Na^+ - K^+$  ATPase, which catalyses the active transport of sodium from the cell and the entrance of  $K^+$  into the cell (Yunfeng *et al.*, 1998). Smaller serum  $Mg^{2+}$  levels in hypertensive patients of both sexes in the present study (fig. 2) may be explained in terms of a decrease in  $Na^+ - K^+$  ATPase activity, thereby decreasing electrolyte transport and increasing vascular tone and blood pressure (Hordyjewska and Pasternak, 2004; Cunha *et al.*, 2013).

Present study is based on observing a significant decrease in serum sodium levels in hypertensives men and women than their normotensive counterpart (fig. 2). The decrease may be due to greater influx of sodium into cell as intracellular  $Na^+$  was found to be significantly high in the hypertensive groups of both sexes (fig. 1). This is consistent with findings of other studies (Jhetam *et al.*, 1982; Cohen *et al.*, 1982). Potassium also plays an important role in regulating arterial blood pressure, either directly or indirectly (Haddy *et al.*, 2006). Some investigator have reported there was no significant difference between mean serum level for  $Na^+$  and  $K^+$  of the hypertensive than normotensive group whereas others have reported increases in  $K^+$  (Frohlich, 1964; Tostes *et*

*al.*, 1997). In the present study smaller serum  $K^+$  concentration in patients with essential hypertension could be due to a decrease in outflow of sodium ions and inflow of potassium ions in cell cause a rise in intracellular sodium concentration due to altered  $Na^+ - K^+$  ATPase activity.

In the pathogenesis of hypertension, calcium has been implicated as an important factor. Some investigators reported a highly significant correlation between serum calcium and hypertension (Tostes *et al.*, 1997). Whereas other studies could not establish any significant differences in the serum  $Ca^+$  level of hypertensive patients (Frohlich, 1964). But from our study we found a significant decrease in serum calcium in both male and female hypertensive patients. It is possible that intracellular calcium level may be more important in systemic hypertension which was not measured in our study.

Extracellular Mg ions exert important actions upon divalent cation metabolism, transport and release of  $[Ca^{2+}]_i$  and  $[Mg^{2+}]_i$  in both vascular smooth muscle and endothelial cells. Because  $Mg^{2+}$  is known to be an

important cofactor for  $\text{Ca}^{2+}$ -ATPase, which catalyses the active transport of  $\text{Ca}^{2+}$  ions from the cytoplasm to the endoplasmic reticulum (Hordyjewska and Pasternak, 2004; Yunfeng *et al.*, 1998). Because  $\text{Na}^+$  and  $\text{Ca}^{2+}$  transport systems across the plasma membrane as well as contractile processes of vascular smooth muscle are regulated by magnesium (Patel *et al.*, 2015). It is possible that a decrease in blood  $\text{Mg}^{2+}$  levels causes inhibition of  $\text{Na}^+$ ,  $\text{K}^+$ -ATPase. Indeed, it has been reported that low concentrations of ouabain increases free calcium concentration in the cytosol of vascular smooth muscle cell (Zhu *et al.*, 1996). Therefore elevation of intracellular free  $\text{Ca}^{2+}$  concentration causes vasoconstriction resulting in an increase in peripheral resistance to increase blood pressure (Kesteloot and Geboers, 1982). A decrease in serum  $\text{Ca}^{2+}$  concentration observed in essential hypertension in the present study is indicative of an increase in intracellular  $\text{Ca}^{2+}$  concentration. Conversely, high intracellular  $\text{Ca}^{2+}$  levels have been also reported in hypertensive patients (Orlov and Li, 1995; Shamsi and Katz, 1987). Many researchers report a positive correlation between serum  $\text{Ca}^{2+}$  and blood pressure (Kesteloot and Geboers, 1982). In a large population study of normotensive individuals, both SBP and DBP were directly correlated with the serum  $\text{Ca}^{2+}$  levels (Kesteloot and Geboers, 1982). These apparently contradictory results can be explained on the basis that certain hypertensives have a low rennin profile, whereas serum  $\text{Ca}^{2+}$  is decreased (Orlov and Li, 1995) and others have a high rennin profile, where, serum  $\text{Ca}^{2+}$  is raised. Plasma rennin profile is therefore known to contribute to changes in  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  transmembrane fluxes (Resnick *et al.*, 1983; Cooper *et al.*, 1991). Results show a significant decrease in serum calcium in patients of hypertension that may be due to greater influx of calcium into the cells leaving decrease serum calcium level. There is negative correlation ( $r = -0.433$ ,  $p < 0.01$ ) between SBP and serum calcium in hypertensive patients (Fig. 1). The present study shows no sex difference in the levels of serum calcium in these patients.

Ljunghall *et al.* (1977) have reported a decrease in serum phosphorus in hypertensive patients of both sexes (Ljunghall and Hedstrand, 1977). Similarly in the present study serum phosphorus inversely correlated to SBP ( $r = -0.433$ ,  $p < 0.01$ ) in hypertensive patients (fig. 1). Phosphorus deficiency often secondary to smaller intake of phosphorus could exacerbate hypertension by impairing function of ion transport system that regulate cellular electrolyte homeostasis (Levy *et al.*, 1994) may induce alteration in cellular calcium metabolism to increase vascular resistance and precipitate hypertension (Blaustein, 1977). Energy in the form of ATP is required for many ion transport systems. Phosphorus depletion is often associated with increased urinary excretion of  $\text{Ca}^{2+}$  and this may lead to  $\text{Ca}^{2+}$  deficiency (Ulmann *et al.*, 1985). Decreased circulating levels of inorganic

phosphorus have been reported to induce increases in circulating level of 1, 25-dihydroxy vitamin D (Vukicevic *et al.*, 1986) that may lead to an increase in blood pressure.

## CONCLUSION

In conclusion, the present study shows that hypertension is associated with altered electrolyte and water homeostasis. Gender differences in the dysregulations of serum electrolyte balance were not observed in the present study but intraerythrocyte  $\text{Na}^+$  levels were greater and  $\text{K}^+$  levels smaller in hypertensive women than men.

These finding play an important role not only for prevention and treatment of hypertension but also for development of population-based strategies helpful in decreasing cardiovascular disease risk by lowering the overall blood pressure distribution. The present study was conducted on a small sample distributed on an age span of 27 years. Increasing sample size may provide more conclusive effects. It will also be interesting to monitor hormonal and electrolytes changes associated with different age groups and the related sex difference.

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