Correlation of divalent Cat Ions (Ca^{++}, Mg^{++}) and Serum Renin in pateints of essential hypertension

Inayat-ur-Rahman Abbasi,[1] Salim-ul-Haque,[2] Muhammad Wasim Kausar,[3] Khemomal A. Karira,[4] Nudrat Anwar Zubari[5]

Department of Biochemistry, Azad Jannu & Kashmir Medical College, Muzaffarabad,[1]

Department of Biochemistry, Avicenna Medical College, Lahore,[2] Department of Biochemistry, Islamabad Medical & Dental College, Islamabad,[3]

Department of Biochemistry, Basic Medical Sciences Institute, Jinnah Postgraduate Medical Centre, Karachi.[4,5]

Abstract

Objective: To evaluate the serum status of Divalent Cat ions (Ca++ and Mg++) in hypertensive subjects along with correlation of Cat ions with serum Renin in all subjects.

Methods: Conducted at the Biochemistry Department of the Basic Medical Sciences Institute (BMSI), Jinnah Postgraduate Medical Centre, Karachi, the case control study comprised 75 subjects. Of the total, 40 were patients of essential hypertension while 35 were normal healthy subjects. The serum level of Divalent Cat ions was measured by spectrometry, Renin by RIA, and other biochemical parameters such as glucose, urea and creatinine were estimated by chemical methods.

Results: Serum calcium was significantly high ($p<0.001$) while magnesium was significantly low ($p<0.001$) in the hypertensives. Similarly, calcium had significant positive correlation (r value 0.576 and 0.593) while magnesium had significant negative correlation (r value -0.746 and -0.743) with systolic and diastolic blood pressure. The glucose, urea and creatinine were observed to be insignificant when compared to normotensive subjects. In addition, Renin has a positive correlation (r value 0.559 and 0.444) with systolic and diastolic blood pressure respectively.

Conclusion: Elevated calcium and depressed magnesium are linked with hypertension, while among the hypertensives, Renin levels need to be closely observed.

Keywords: Hypertension, Normotension, Renin Angiotension System (RAS), Calcium, Magnesium (JPMA 62: 134; 2012).

Introduction

Hypertension is the most common risk factor for the development of cardiovascular disease (CVD) and remains a major healthcare problem.[1] The objective of identifying and treating high blood pressure is to reduce the risk of cardiovascular disease and associated morbidity and mortality. Hypertension is defined as systolic blood pressure of 140 mmHg or greater, and diastolic blood pressure of 90 mmHg or greater or patients taking anti-hypertensive medicines.[2] There is still much uncertainty about the pathophysiology of hypertension. A small number of patients (between 2% to 5%) have an underlying renal or adrenal disease as the cause of their raised blood pressure called secondary hypertension. In majority (between 95% to 98%), however, no clear single identifiable cause is found of raised blood pressure and the condition is marked as primary or essential or idiopathic hypertension.[3] Essential hypertension (where cause is unknown) accounts for 95% of all cases of hypertension affecting approximately 1 billion individuals worldwide.[4]

Renin was discovered a century ago by Tiger Sted and Bergma.[5] Despite advances over all these years, there is a considerable uncertainty as to the importance of the renin angiotensin system in some disorders, most particularly in essential hypertension and its complications.[6] Renin is a proteolytic enzyme released from the renal-juxtaglomerular and tissue renin angiotensin system. It acts on angiotensinogen in plasma to generate angiotensin I which is converted into angiotensin II (Ang II) by Angiotensin Converting Enzyme (ACE) present in endothelial cells of blood vessels. Ang II is further metabolised to Ang III and Ang IV which is only poorly active. Ang II can also be formed locally in tissues such as brain, heart, aorta, arteries, adrenal glands, uterus, leukocytes, spleen and skin.[7] The Renin itself does not have a direct pressure action, but it is Angiotensin II which is produced in the body under influence of Renin and has a direct presser effect

The possible role of Divalent Cat ions in the pathogenesis of essential hypertension has recently received increasing attention. Certain studies have suggested a positive correlation of serum total calcium level with the height of

blood pressure. However, other reports have raised the opposite possibility that an actual deficiency of calcium may somehow be the cause.[8] Epidemiological data suggest that high calcium intakes are associated with low body weight and high blood pressure.[9] Increased Ca^{+2} enhances vascular reactivity to a variety of vasoactive agonists and increased levels have been reported in hypertensives when compared with normotensives. Ca^{+2} and Mg^{+2} are inversely related to each other in their effect on blood pressure. Ca^{+2} has been documented to be positively correlated with increments in blood pressure whereas low Mg^{+2} has been suggested to increase vascular reactivity in vitro studies.[10] Dietary intake of calcium is also considered to be an important factor in etiology of essential hypertension. A fall in blood pressure has been observed in about one-third of patients with essential hypertension following administration of calcium carbonate.[11] Most of the clinical studies to date have shown minor effect of role of calcium supplementation in lowering blood pressure.[12] Magnesium has been implicated in the regulation of blood pressure on the basis of its role in cellular Cat ion metabolism. Defect in Mg^{+2} metabolism have been demonstrated in animal and human hypertension.[13] An inverse association between Mg^{+2} intake and blood pressure has been reported in population studies.[14] But it is not clear, however, whether Mg^{+2}supplementation lowers blood pressure in hypertensive subjects.[15] Lower Mg^{+2} levels in vitro cause vasoconstriction while increased Mg^{+2} level dilates blood vessels and blocks the vasoconstrictor effect of substance such as nor epinephrine. Similarly, parenteral Mg^{+2} sulphate administrations reduces blood pressure due to vasodilation or other mechanism in eclampsia, pre-eclampsia and in malignant hypertension.[16] Though adequate dietary intake of Mg^{+2} was recommended in the VIth report of Joint National Committee (JNC-VI), increasing Mg^{+2} intake is not accepted as a general application in the treatment of hypertension.[17]

Patients and Methods

This case-control study was carried out in the Department of Biochemistry, Basic Medical Sciences Institute (BMSI), Jinnah Postgraduate Medical Centre (JPMC), Karachi. A total of 75 subjects were included in the study, out of which 40 were suffering from essential hypertension selected from medical OPD, JPMC, Karachi. These hypertensive patients were either taking no medicine or, if they were taking medicines, their medications were stopped at least three days before obtaining the blood sample. Patients of either sex were selected, whereas children and pregnant women were not included in the study. In addition, 35 normal healthy subjects of both sexes of similar ages with normal blood pressure were also included in the study.

Six to eight ml of venous blood was collected from each subject and control. The blood was immediately transferred into a clean test tube and allowed to clot at room temperature for 20 to 30 minutes. The blood was then centrifuged for 10-15 minutes at 3000 rpm (revolution per minute). Serum was separated and kept in serum cups with labels, stored at -50 to -60°C until biochemical parameters were performed. All the selected subjects were divided into two groups.

Group A: Normal control healthy subjects.

Group B: Diagnosed patients of essential hypertension.

The inclusion criteria comprised two factors: age between 20-50 years (male and female), and the patients to fulfill the WHO criteria of Hypertension, having Diastolic Blood Pressure (DBP) $\geq$ 90 mmHg, or having Systolic Blood Pressure (SBP) $\geq$ 140 mmHg, or taking anti-hypertensive medications.

The exclusion criteria had several factors, including children, pregnant women, anyone with age > 55 years, recent major systemic illness, suffering from diseases of liver, and kidney, diabetes mellitus, Post Myocardial Infarction (MI) and CCF (Congestive Cardiac Failure). Also excluded were patients suffering from endocrine diseases like hyperaldosteronism, Cushing disease or Pheochromocytoma, and patients using diuretic, calcium channels blocker or ACE-inhibitors.

Both subjects and controls were assessed to meet inclusion and exclusion criteria listed above. The data collected of both groups were standardised through the use of similar methodology, protocol and procedures. The questionnaire provided information about type of work, smoking habit, family history of hypertension, past history of any major illness or diabetes, etc. Blood pressure of subjects was measured in supine position after 5 minutes of rest and mean of three readings was recorded. Hypertension status of patients was defined as Systolic Blood Pressure $\geq$ 140 mm Hg or Diastolic Blood Pressure $\geq$ 90 mm Hg or current use of anti-hypertensive medications.

Serum calcium and magnesium both were determined by colorimetric method using Kit Cat no CA-590 and MG-573 of Randox Company. The plasma Renin was determined by Renin IRMA (radioimmunoassay) RIA kit ref no. RIA-4541 manufactured by DRG diagnostics, Germany; while serum urea, creatinine and glucose were measured by chemical methods. The statistics was applied by using SPSS version 10.0 and the Pearson correlation was applied to find out the correlation among different parameters.

The Mean and SD were found out and further statistical analysis was performed. The mean values of both groups were compared by student's t-test, and when p value was < 0.001 it was considered as significant and shown in

tables as *. In this study, patients and controls of both sexes were included. Of the total 75 subjects, 35 were in Group A (Normotensive controls) and 40 were in Group B (Hypertensive patients).

Results

After the two groups were formed, their basic data was compared, showing that mean value of age, height, weight and BMI had non-significant difference (Table-1). The mean value of Systolic and Diastolic Blood Pressure had statistically significant difference in hypertensive group when compared to the normotensive group with $p < 0.001$.

The mean values of Divalent Cat ions (Ca^{++} and Mg^{++}) and other biochemical parameters in both groups were also worked out (Table-2). The serum calcium concentrations in normotensive and hypertensive groups were 8.84 ± 0.92 and 11.26 ± 2.05 respectively with $p < 0.001$; while serum magnesium concentration in the two groups was 2.21 ± 0.04 and 1.19 ± 0.34 respectively with $p < 0.001$. Similarly, Renin had significant difference ($p < 0.001$), whereas urea, creatinine and glucose showed non-significant difference when compared to the controls. It was observed that calcium and Renin were high whereas magnesium was low in hypertensive subjects.

The mean Renin value in hypertensives showed significant difference when compared to normal controls. Moreover, when hypertensive subjects were sub-grouped on the basis of Renin value (3.00 to 33.00 pg/ml) it was observed that the value did not rise in all hypertensive subjects. In a total of 40 hypertensives, only 16 (40%) showed high Renin, while 24 (60%) showed normal or low value. On this basis, two sub groups were formed; one with high Renin value with range of 40.84 to 121.12 pg/ml and having mean value of 70.82 ± 6.85, while the other with normal renin value with range of 10.01 to 32.91 pg/ml and having mean of $20.93 \pm .1.70$.

Table-1: Comparison of biophysical variables in Normotensive and Hypertensive subjects (Values expressed as mean ± SD).

S. No.	Biophysical Parameters	Normotensive Group	Hypertensive Group
1	Number of Subjects (n)	35	40
2	Age (Years)	44.17 ± 4.19	44.32 ± 5.61^{NS}
3	Height (Meters)	1.71 ± 0.09	1.75 ± 1.74^{NS}
4	Weight (Kg)	66.54 ± 7.389	69.70 ± 6.26^{NS}
5	BMI (Kg/m2)	22.90 ± 2.428	22.72 ± 1.68^{NS}
6	Systolic B.P.(mm Hg)	115.14 ± 3.950	148.75 ± 10.84*
7	Diastolic B.P.(mm Hg)	74.63 ± 3.590	95.00 ± 5.77*

NS: Non-Significant; *P<0.001: significant. BMI: Body Mass Index.

Table-2: Comparasion Of Biochemical Parameters In Normotensive and Hypertensive Subjects (Values are expressed as mean ± SD).

S.No.	Biochemical Parameters	Normotensive Group (n=35)	Hypertensive Group (n=40)
1	Calcium (mEq/L)	8.84 ± 0.92	11.26 ± 2.05*
2	Magnesium (mEq/L)	2.21 ± 0.04	1.19 ± 0.34*
3	Serum Renin(Pg/ml)	20.91 ± 7.09	41.86 ± 29.95*
4	Serum Urea(mg/dl)	33.88 ± 5.85	32.17 ± 9.35^{NS}
5	Serum Creatinine (mg/dl)	0.95 ± 0.100	0.91 ± 0.18^{NS}
6	Blood Glucose (mg/dl)	88.74 ± 6.77	90.87 ± 7.17^{NS}

NS=Non-Significant; *p < 0.001: significant: mEq/L= milli Equilant per Liter, pg/ml= pico gram per milli liter.

Table-3: Coefficient correlation among Cat Ions with Systolic, Diastolic blood pressure and in high and normal Renin hypertensive subjects.

	Calcium (r value)	Magnesium (r value)	SBP (r value)	DBP (r value)	High Renin HT (r value)	Normal Renin HT (r value)
Calcium (r value)	1	-0.255	0.576*	0.593*	-0.273	0.026
Magnesium (r value)	-0.255*	1	-0.746**	-0.743**	0.039	0.111
SBP (r value)	0.576*	-0.746**	1	-0.686**	0.559*	0.016
DBP (r value)	0.593*	-0.743**	-0.686**	1	0.444*	0.116
High Renin (r value)	-0.273	0.039	0.559*	0.444*	1	-
Normal Renin (r value)	0.026	0.111	0.016	0.116	-	1

*Correlation is significant at the 0.05 level (2-tailed). ** Correlation is significant at the 0.01 level (2-tailed). SBP=Systolic Blood Pressure, DBP=Diastolic Blood Pressure, HT= hypertensives.

The coefficient correlation among Divalent Cat ions with systolic (SBP), diastolic blood pressure (DBP) in high and normal Renin hypertensive subjects was calculated (Table-3). A significant positive correlation was observed in calcium with r-value of +0.576 with SBP, +0.593 with DPB, -0.273 with high Renin, and 0.026 with normal renin hypertensives. A significant negative correlation was observed in magnesium with r-value of - 0.746, -0.743 with SBP and DBP respectively. Similarly SBP and DBP are inversely correlated to each other with r value of -0.686.

Discussion

Hypertension is one of the most common diseases afflicting humans worldwide. Because of the associated morbidity, mortality and the cost to society, hypertension is an important public healthcare challenge. In our study, we measured serum levels of Renin and Divalent Cat ions in hypertensive patients of local population and then matched them to our control group. The results show that the hypertensives had slightly high calcium level and slightly low magnesium levels. These results are in agreement with most of the researches,[14] which found an inverse association between magnesium intake and blood pressure level in population studies. Similarly, another study[18] found that magnesium administered intramuscularly lowers diastolic blood pressure in hypertensive subjects with high plasma Renin activity. Ried[9] suggested that hypertension is associated with elevated calcium, depressed magnesium and reduced pH. Our results of low Mg in hypertensive subjects are in disagreement with Witteman[15] who had not clearly found weather Mg^{+2} supplementation could lower blood pressure in hypertensive subjects. Similarly Durlach[19] in his study did not establish a major role of Mg^{+2} as an anti-hypertensive factor.

Our results of high calcium in hypertensive patients are also in agreement with the study of Wright[10] who found increased level of serum calcium in hypertensive subjects when compared to the normotensives. Similarly, Resnick[18] and Weidmann[20] both suggested that sudden elevation and depression of serum calcium level are followed by rising and falling of blood pressure respectively. Our results of high calcium in hypertensive patients are in disagreement with the study of Ackley[21] who suggested that high calcium content of milk derivatives seems to exert a protective effect on the rise of blood pressure in communities.

We found significant positive correlation between calcium with systolic and diastolic blood pressure; in addition to significant negative correlation between magnesium with systolic and diastolic blood pressure. These findings are in agreement with Staessen[22] who found that Ca^{+2} and Mg^{+2} are inversely correlated to each other in their effects on blood pressure. Calcium has been documented to be positively correlated with increments in blood pressure; while Magnesium has been found to be negatively correlated with blood pressure. Similarly, Resnick[14] also reported negative correlation between free magnesium with both systolic and diastolic blood pressure.

Although serum Renin was found significantly raised in hypertensives, but when we analysed the Renin value, we noticed that not all hypertensive subjects had raised Renin levels. We found that only 16 (40%) had raised and 24 (60%) had normal Renin. So the hypertensives can be divided into 'high' and 'normal' Renin sub-groups. These results matched and justified with most of the researchers who also formed sub-groups of hypertensives into' high' 'normal' or 'low' Renin. Resnick[8] also divided essential hypertensive subjects into 'high' 'normal' or 'low' Renin groups. Thurston[23] claimed that the measurement of Renin levels in patients with essential hypertension enables identifiable subgroups of patients to be demarcated and that these subgroups exhibit important differences in prognosis and response to therapy. Patients were divided into 'high', 'normal' and 'low' Renin subgroups.

Allikmets[24] suggested that the importance of the Renin-angiotensin system (RAS) in blood pressure regulation is well established. High RAS activity has also been implicated in connection with elevated cardiovascular risk in patients with essential hypertension. Data from epidemiological studies have related high plasma Renin levels in essential hypertensive patients leading to more cardiovascular complications. Similarly, Hans and Gavros[25] stated that evidence suggest that hypertensive subjects with low concentration of Renin were less likely to have heart attacks, strokes or renal complications than those with high Renin activity.

Conclusion

Essential hypertension has an established association with elevated calcium and depressed magnesium levels. Besides, the measurement of Renin levels in hypertensives patients is recommended to keep the blood pressure in check and thereby reduce chances of mortality or morbidity.

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