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STUDY OF SERUM CALCIUM LEVELS IN HYPERTENSIVE PATIENTS

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ABSTRACT

Introduction: Hypertension is an major health related risk factor in India with major contribution to burden of disease and mortality .It contributes to 1.6 million deaths annually in India due to ischemic heart disease and stroke. 57% of deaths related to stroke and 24% of death are related to hypertension. Hypertension is one of the commonest non communicable disease in India with an overall prevalence of 29.8% with a higher prevalence in urban areas. Although our understanding of pathophysiology of hypertension has increased, etiology remains hypothetical . Various studies have shown that essential hypertension is associated with disturbed calcium metabolism like increased cytosolic calcium and decreased serum calcium levels and also increased urinary excretion of calcium in patients with essential hypertension. In this study total serum calcium and corrected serum calcium levels of essential hypertension patients are compared with total and corrected serum calcium of matched normotensive controls.

Aim of the Study: 1. To study the total and corrected serum calcium levels between patients with essential hypertension and normotensive controls.

Materials and Methods: The work was carried out in the out patient department and medical wards of Rajah Muthiah Medical College Hospital, Chidambaram. During October. 2016 to August 2018 it is an cross sectional study with sample size of 100 with 50 newly diagnosed essential hypertension and 50 matched normotensive controls. The present project was approved by the ethical committee

Results: The mean total serum calcium and corrected serum calcium levels in hypertensive patients were comparatively lower than the normotensive group.

Conclusion: There was a negative correlation noted between the total and corrected serum calcium levels in the hypertensive patients. The calcium levels were significantly lower in the hypertensive group.

INTRODUCTION

Hypertension remains the leading cause of death worldwide and one of the world's great public health problems (WHO). Affecting 1 billion people worldwide, systemic hypertension remains the most common, readily identifiable and reversible risk factor for myocardial infarction, stroke, heart failure, atrial fibrillation, aortic dissection and peripheral arterial disease. Because of escalating obesity and population aging in developed and developing countries, the global burden of hypertension is rising and projected to affect 1.5 billion persons, one third of the world's population, by the year 2025. Although our understanding of the pathophysiology of essential hypertension has increased, the etiology still remains hypothetical. Various studies have shown that essential hypertension is associated with disturbed calcium metabolism like increased cytosolic calcium and decreased serum calcium levels and also increased urinary excretion of calcium in patients with essential hypertension.

In this study total serum calcium levels and corrected serum calcium levels of essential hypertension patients is compared and correlated with matched normotensive controls.

Aim of the Study

To study the total and corrected serum calcium levels between patients with essential hypertension and normotensive controls.

MATERIALS AND METHODS

Setting The work was carried out in the out patient department and medical wards of Rajah Muthiah Medical College Hospital, Chidambaram.

Design of the study cross sectional study

Period of the Study October. 2016 to August 2018.
Sample size 100 (50 cases 50 controls)

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Ethical committee approval The present project was approved by the ethical committee.

Inclusion criteria

- ✓ Patients with primary hypertension.
- ✓ Patients whose age was above 18 years were included
- ✓ Both sexes were included.

Exclusion criteria

- ✓ Patients below 18 years.
- ✓ Patients with renal failure
- ✓ Pregnancy.
- ✓ Patients on non-steroidal anti-inflammatory agents, calcium channel blockers, diuretics.
- ✓ Females on oral contraceptive medication.
- ✓ Patients with acute diarhoeal diseases
- ✓ Patients with diabetes
- ✓ Patients with secondary hypertension

Consent

The study group thus identified by the above criteria (inclusion and exclusion criteria) patient was first instructed about the nature of the study. Willing participants were taken up after getting a written informed consent from them.

Study subjects and controls

50 essential hypertensive patients attending the medicine OPD or admitted to the medical wards of Rajah Muthiah Medical College Hospital for the period of two years from October 2016 to august 2018 formed the study group. Controls were selected from those who attended medical outpatient department for minor ailments and healthy volunteers.

Details of the study subjects

All the patients were subjected to detailed history taking, careful physical examination and biochemical analysis to exclude secondary hypertension.

Patient's height and weight were measured the body mass index was calculated using the formula weight / height [m^2]. Patient's hip and waist circumferences were measured.

Patients were informed to refrain from smoking or drinking tea or coffee for at least thirty minutes before measuring blood pressure. Then blood pressure was measured using the following guidelines.

Guidelines for measuring blood pressure

Conditions for the Patient

Posture

Sitting postures are usually adequate for routine follow-up. Patient should sit quietly with back supported for five minutes and arm supported at the level of heart.

Circumstances

- ✓ No caffeine for preceding hour
- ✓ No smoking for preceding 30 minutes.
- ✓ No exogenous adrenergic stimulants like phenylephrine in nasal decongestants or eye drops for papillary dilation
- ✓ A quite, Warm setting.

Equipment

Cuff Size

The bladder should encircle and cover 2/3rd of the arm length.

Technique

Number of readings

- ✓ On each occasion, take at least two readings, 1minute apart.
- ✓ For diagnosis, obtain at least three sets of readings a week apart.
- ✓ Initially, take pressure in both arms, if pressure differs, use arm with higher pressure.

Performance

- ✓ Inflate the bladder quickly to a pressure 20 mm of Hg above the systolic, as recognised by the disappearance of the radial pulse.
- ✓ Deflate the bladder 3 mm of Hg every second.
- ✓ Record the Korotkoff phase V (disappearance) .
- ✓ If Korotkoff sounds are Weak, have the patients raise the arm, open and close the hand 5 to 10 times, after which the bladder should be inflated quickly.

Recording

Note the pressure, patient position, the arm, cuff size (e.g., 140/90, seated, right arm, large adult cuff).

Calcium Estimation

Calcium was measured by aresanzo III method

The principle of this method is that calcium bind specifically with arsenazo III at an acidic pH to form a blue - purple coloured compound. The intensity of the colour of the compound is directly proportional to the amount of calcium present in the sample. In the calcium estimation kit, there will be a standard sample with measured calcium of 10 mg/dl with which a standard stock solution(S) has to be prepared. The serum of patient is noted as test sample (T). A solution with distilled water is also prepared and branded as blank (B). Now the absorbance (Abs) of standard(S) and test(T) are measured against the blank(B) by absorption spectrophotometry at 650 nm(Red) in room temperature within one hour and the readings to be noted. This method has a linearity upto 15 mg/dl.

Serum Calcium (mg/dl) = [Abs of (T) / Abs of (S)] x10.

Corrected Serum Calcium Level

Corrected Serum Calcium Level = Serum Calcium(mg/dl) + { 0.8 [4.0 - Serum albumin (g/dl)]} In this study the correction was done for albumin using the above formula and corrected serum calcium levels were obtained.

Estimation Of Serum Albumin

Albumin BCG Method

The principle of this method is that albumin bind with the dye bromocresol green (BCG) in a buffered medium to form a green coloured complex. The intensity of the colour of the complex formed is directly proportional to the amount of albumin present in the sample. In the albumin estimation kit, there will be a standard sample with measured albumin of 4g/dl with which a standard stock solution(S) has to be prepared. The serum of patient is noted as test sample (T). A solution with distilled water is also prepared and branded as blank (B). Now the absorbance (Abs) of standard(S) and test

(T) are measured against the blank(B) by absorption spectrophotometry at 628 nm(Red) and the readings to be noted. This method has a linearity upto 7 g/dl.

Serum Albumin (g/dl) = [Abs of (T) / Abs of (S)] x 4.

Statistical Analysis

Data was entered in Microsoft excel spread sheet and analysed statistically using Statistical packages for the social sciences [SPSS- 21]. Results were considered significant if the 'p' value was below 0.05.

The following tests were used for statistical analysis

1. Independent sample 't' test for quantitative variables.
2. Mann Whitney 'U' test for the nominal data.
3. Pearson correlation coefficients.
4. Analysis of variance (ANOVA).

Conflict of interest

There was no conflict of interest.

Financial Support **nil**

RESULTS

Table 1 Comparison of Age Between the Groups

Age	A		B		T	p
	Mean	S.D	Mean	S.D		
	56	9.28	55.06	10.86	0.46	0.643

It is inferred from the Table 1 that, mean age of the study group was 56 ± 9.28 years whereas the mean age of the control group 55.06 ± 10.86 years. The obtained 't' value was 0.46 with the corresponding **'P' = 0.643 > 0.05.** Therefore, there was no significant difference in the age between the groups, both groups were homogenous with respect to age.

Table 2 Gender Distribution and Comparison

Gender	A		B		Mean Whitney 'U' Tent 'Z'	P
	N	%	N	%		
Male	23	46	26	52		
Female	27	54	24	48	0.597	0.550
Total	50	100	50	100		

The gender comparison is presented in the Table.2 It is inferred that, in group 'A', 46% were male and 54% were females. In group 'B' 52% were male and 48% were females. Z= 0.597, **P=0.550 > 0.5.** There was no significant difference in the distribution of gender between the groups.

Table 3 Symptoms in the Study Group

Symptoms	N	%
Headache	5	10
Giddiness	12	24
Combination	6	12
Easy Fatigability	2	4
No	25	50
Total	50	100

It is observed from the table 3 that, majority of the study patients 50% had no symptoms. Giddiness was observed in 24% of the study patients, headache was the feature in 10% and the combination of the symptoms was observed in 12%.

Table 4 Personal Habits and the Family History

	A		B		MANN WHITNEY 'U'	
	N	%	N	%	Z	P
Smoking	14	28	16	32	0.434	0.664
Alcohol	13	26	16	32	0.658	0.511
Family History	18	36	16	32	0.420	0.674

It is inferred from the table 4 that, in group A, 28% were smokers, 26% were alcoholic and family history was positive in 36% of the patients. In groups 'B' smokers were 32% and alcoholics were 32% and the positive family history was noted in 36%. The obtained 'P' value were **0.664, 0.511** and **0.674** for smoking, alcohol and family history respectively and which was greater than 0.05. Therefore, there was no significant difference in the smoking alcohol and family history between the groups.

Table 5 Comparison of Life Style Between the Groups.

Life Style	A		B		Mann Whitney 'U' Test	
	N	%	N	%	Z	P
Sedentary	25	50	24	48		
Non Sedentary	25	50	26	52	0.199	0.842
Total	50	100	50	100		

It is inferred from the Table 5 that, 50% was leading the sedentary life style in group A and 48% was the sedentary nature of life style in the group B. The difference in the life style between the groups was statistically insignificant, **Z=0.199, P=0.842.>0.05.**

Table 6 BMI Distributions Between the Groups.

BMI	A		B	
	N	%	N	%
<25	25	50	23	46
>25	25	50	27	54

It is inferred from the table 6 that, 50% of the patients in group A had BMI > 25 and 54% in group 'B' had BMI > 25.

Table 7 Comparisons of BMI Between the Groups.

BMI	A		B		MannWhitney 'U' Test	
	Mean	S.D	Mean	S.D	T	P
	24.39	3.64	24.89	3.74	0.693	0.490

The mean BMI in the group 'A' was 24.39 ± 3.64 and it was 24.89 ± 3.74 in the control group, **t=0.693, P= 0.490 > 0.05.** The difference in the BMI was statistically insignificant between the groups.

Table 8 Comparison of Blood Pressure Between the Groups

BP	A		B		Mann Whitney 'U' Test	
	N	%	N	%	Z	P
Systolic	168.40	14.47	111.72	6.88	25.01	0.001
Diastolic	100.16	8.68	71.16	6.31	19.10	0.001

The Mean systolic blood pressure in the group 'A' was **168.40 ± 14.47** and the mean diastolic blood pressure in the group 'A' was **100.16 ± 8.68.** The mean systolic and diastolic blood pressure in the group 'B' was **111.72 ± 6.88** and **71.16 ± 6.31** respectively. There was a significant difference in the systolic, **t=25.01, p=0.001** and the diastolic, **t=19.10, p=0.001** blood pressure between the groups.

Table 9 Comparison of Blood Urea Between the Groups.

Blood urea	A		B		T	P
	Mean	S.D	Mean	S.D		
	25.92	4.33	27.52	4.48	1.82	0.07

It is inferred from the Table 9 that the mean blood urea in the study group was 25.92 ± 4.33 whereas it was 27.52 ± 4.48 in the controls. **t= 1.82, p= 0.07.** The difference was statistically insignificant.

Table 10 Comparison of Serum Creatinine Between the Groups

Serum Creatinine	A		B		T	P
	Mean	S.D	Mean	S.D		
	0.74	0.15	0.77	0.26	0.894	0.374

It is observed from the Table 10, that the mean serum creatinine in the study group was 0.74 ± 0.26 in the controls. **t = 0.894, p=0.374.** The difference was statistically insignificant.

Table 11 Comparison of T. Sr. Calcium Between the Groups.

TS calcium	A		B		t	p
	Mean	S.D	Mean	S.D		
	8.69	0.48	9.70	0.79	7.65	0.001

It is observed from the Table 11 that, the mean serum calcium was comparatively lower in the study group, **8.69 ± 0.48** than in the controls**, 9.70 ± 0.79. t= 7.65, p=0.001.** The difference was statistically significant.

Table 12 Comparison of Serum Phosphate Between the Groups

Serum phosphate	A		B		t	p
	Mean	S.D	Mean	S.D		
	3.58	0.37	3.77	0.38	2.62	0.010

It is inferred from the Table 12 that, the average serum phosphate level was comparatively lower in the study group, **3.58 ± 0.37** than in the controls, **3.77 ± 0.38** and the difference was statistically significant**, t=2.62, p=0.01 < 0.05.**

Table 13 Comparison of Serum Albumin Between the Groups

Serum albumin	A		B		t	p
	Mean	S.D	Mean	S.D		
	4.06	0.31	4.08	0.36	0.267	0.790

It is inferred from the Table 13 that, the mean serum albumin was **4.06 ± 0.31** in the study group and it was **4.08 ± 0.36** in the controls**. t = 0.267, p = 0.790.** The difference was statistically insignificant.

Table 14 Comparison of Corrected Serum Calcium Between the Groups

C.Serum Calcium	A		B		t	p
	Mean	S.D	Mean	S.D		
	8.66	0.55	9.65	0.80	17.27	0.001

It is inferred from the Table 14 that, the average blood calcium for the study group was comparatively less, **8.66 ± 0.55** than the control group, **9.65 ± 0.80** and the difference was statistically significant. **t=17.27, p=0.001.**

Table 15 The relationship between systolic blood pressure and the Sr.Calcium

Systolic BP	R	P
Total Sr. Calcium	-0.604	0.001
Corrected Sr. Calcium	-0.614	0.001

The relationship between the systolic blood pressure and the Sr.Calcium was performed by the pearson's correlation coefficients. It is inferred form the Table 15 that, the correlation between the Total. Sr.Calcium and the systolic BP was negative and it was statistically significant, **r = -0.604, p = 0.001.** Likewise, negative and significant correlation was observed between the systolic blood pressure and the corrected Sr. Calcium, **r = -0.614, p = 0.001.** Hence, as systolic blood pressure value was more, the corresponding total and corrected Sr.Calcium was less and Vice-Versa.

Table 16 The relationship between the diastolic blood pressure and the serum calcium

Diastolic BP	R	P
Total Sr. Calcium	-0.562	0.001
Corrected Sr.Calcium	-0.574	0.001

The relationship between the diastolic blood pressure and the total Sr.Calcium and corrected Sr.Calcium was performed by the pearson's correlation coefficients. It is inferred from the Table 16 that, there was significant negative correlation was observed between the total Sr.Calcium and the diastolic blood pressure**, r = -0.562, p = 0.001** and the diastolic blood pressure and the corrected Sr. Calcium, **r = -0.574, p=0.001**. Therefore, as the diastolic blood pressure was higher, the corresponding Sr.calcium was less and vice- Versa.

RESULTS

- ✓ A total of 100 individuals were selected . They were divided into two groups, group A and group B.
- ✓ Group A was the study group which included 50 newly detected essential hypertensive patients . Group B was the control group which included 50 normotensive individuals.
- ✓ Age and gender was homogenous between the groups.
- ✓ There was no significant difference in the personal habits like smoking and alcohol between the groups.
- ✓ There was no significant difference in the positive family history between the groups
- ✓ The mean BMI in the group A was 24.39 ± 3.64 and it was 24.89 ± 3.74 in group B. there was no significant difference in the BMI between the groups.
- ✓ There was no significant difference in the blood urea , creatinine, and serum albumin between the groups .
- ✓ The mean systolic blood pressure of group A and group B were **168.40 ± 14.47 mmHg** and **111.72. ± 6.88 mmHg** respectively while the mean diastolic blood pressure were **100.16 ± 8.68 mmHg** and **71.16 ±6.31 mmHg** respectively.
- ✓ The mean total serum calcium and corrected serum calcium levels in group A were **8.69 ± 0.48 mg/dl** and **8.66 ± 0.55 mg/dl** while the mean total serum calcium and corrected serum calcium levels in group B were **9.70 ± 0.79mg/dl** and **9.65 ± 0.800 mg/dl** respectively. The calcium levels were significantly lowered in group A when compared with group B.

DISCUSSION

Systemic hypertension remains the most common, readily identifiable and reversible risk factor for myocardial infarction, stroke, heart failure, atrial fibrillation, aortic dissection and peripheral arterial disease.

Evidence is growing that calcium physiology is altered in essential hypertension, but whether this is a secondary association or a causal relationship is unresolved. Intracellular calcium ions are known to have direct effects on peripheral vascular tone and it has been reported in various trials that hypertensive persons have increased concentrations of intracellular free calcium that decrease to normal levels with antihypertensive treatment.

Total and Corrected Serum Calcium Levels

Various epidemiological studies stated that the calcium status of humans with essential hypertension and genetic animal models of hypertension is characterized by low serum total and ionized calcium concentration, increased intracellular calcium, increased urinary calcium excretion, and increased parathyroid hormone (PTH) concentration.

This study used total serum calcium and corrected serum calcium, the latter is an alternative but not a substitute to serum ionised calcium.

In this study the mean total serum calcium and corrected serum calcium levels in group A(cases) were **8.69 ± 0.48 mg/dl** and **8.66 ± 0.55 mg/dl** while the mean total serum calcium and corrected serum calcium levels in group B(controls) were **9.70 ± 0.79 mg/dl** and **9.65 ± 0.80 mg/dl** respectively. Statistical analysis revealed that the total and corrected serum calcium levels were significantly lowered in essential hypertensives when compared to their matched normotensive controls ($P < 0.001$ and $P < 0.001$ respectively).This observation is supported by some of the following studies According to K. Sudhakar *et al.*[1], The mean total serum calcium levels were significantly ($p<0.01$) decreased in males and females in hypertensive group when compared with normotensive controls. In the first-degree relatives also the total serum calcium levels were significantly decreased ($p <0.01$) when compared with the controls.

AR Folsom *et al*., [2] studied the serum calcium fractions in essential hypertensive and matched normotensive subjects. In their study he observed hypertensive subjects had lower mean serum levels of ultrafilterable calcium ($p = 0.01$), ionized calcium ($p = 0.09$), and complexed calcium ($p= 0.04$) and higher levels of protein-bound calcium ($p = 0.07$). Calculated serum concentrations of complexed calcium was significantly lower in hypertensive subjects ($p = 0.04$), while protein bound calcium concentrations was higher ($p = 0.07$). Serum phosphorus and albumin concentrations, as well as estimated dietary calcium intake, were not different between the two groups.

Fu Y, Wang S., *et al*.,[3] in their study suggested that that the hypertensive group consistently demonstrated a significant decreased activity of ATPase studied, with significantly lower plasma calcium and higher cytosolic calcium levels when compared with those in normotensive group ($P < 0.01$ or $P < 0.05$, respectively). No significant differences were found in either plasma Mg^{2+} or intracellular Mg^{2+} level between the two groups.

Strazzullo P *et al*.,[4] studied several of the biochemical abnormalities of calcium metabolism and were able to detect a significant reduction in total serum calcium levels in hypertensive subjects, although unable to detect a significant reduction in serum ionized calcium levels. This study also reported total and fractional urinary calcium excretion were elevated in subjects with essential hypertension.

Erne P, Bolli P., *et al*.,[5] in their study on "correlation of platelet calcium with blood pressure: effect of antihypertensive therapy" reported a decrease in the serum total calcium concentration in essential hypertensive patients. Touyz, R.M.,*et al*.,[6] also reported a decrease in the serum total calcium concentration in essential hypertensive patients.

Some investigators; McCarron DA.,[7] and Resnick LM, Laragh JH.,*et al*.,[8] also noted that, compared with normotensive subjects, essential hypertensive subjects had lower serum ionized calcium concentrations even when total calcium levels were similar.

Wright GL, Rankin GO.,[9] in their study on concentrations of ionic and total calcium in plasma of four models of hypertension noted a lower serum ionized and total serum calcium concentrations in spontaneously hypertensive rats(SHR).

Several other investigators have reported positive associations between blood pressure levels and concentrations of serum total calcium which is in contrast with the present study. Harlan WR., *et al*., [10] Rolf Jorde., *et al*.,[11] and Kesteloot H., *et al*.,[12] observed a direct correlation between serum total calcium concentrations and arterial pressure. This seemingly paradoxical observation is likely secondary to a failure of the investigators to correct for the hemoconcentration that attends human hypertension. This failure results in an artifactual increase in total calcium values because of an increase in serum protein (albumin) concentration, the fraction to which most calcium is bound.

Correlation of Total and Corrected Serum Calcium Levels With Various Subsets of Study Group (Essential Hypertensive Group

According to AR Folsom[2] *et al*. and Staessen J, Sartor F, *et al*., [13] serum total calcium was similar in both the sexes and no significant difference noted.

According to C Brot., *et al*.,[14] noted that there was no difference in serum ionized calcium between smokers and non-smokers in their study. J. Sundsfjord., R. Jorde., *et al*.,[15] in an observation of tromso study, showed a positive association of serum calcium with body mass index (BMI) in both sexes that persisted after correcting for other variables in a multiple regression model. Physical activity (lifestyle) had no significant association with serum calcium. In females alcohol consumption was negatively, and cigarette smoking was positively associated with serum calcium.

Limitations of the Study

- ✓ The sample size was small.
- ✓ This being a cross sectional study follow up was not done.
- ✓ Serum ionised calcium, cytosolic calcium, urinary calcium, serum parathormone levels, serum renin levels, and serum Vit D_3 levels were not measured due to constraints.
- ✓ Calcium supplementation was not attempted in the patients due to ethical reasons.

Summary

The present study aimed at comparing the total and corrected serum calcium levels in newly detected essential hypertensive patients with matched normotensive controls. It also aimed at correlating the total and corrected serum calcium levels with systolic and diastolic blood pressure in newly detected essential hypertensive patients. With rigid criteria individuals, 50 essential hypertensives cases and 50 normotensive controls were selected and enrolled in the study.

The total and corrected serum calcium levels were found to be significantly lowered in cases when compared to controls. Also a significant negative correlation between the calcium levels and systolic and diastolic blood pressure was noted.

CONCLUSION

The total and corrected serum calcium levels were significantly lowered in newly detected essential hypertensive patients when compared to normotensive controls.

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