



International Journal of Pharma Research and Health Sciences

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## Original Article

# Significance of Ionized calcium in hypertension

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### ARTICLE INFO

### A B S T R A C T

Received: 26 June 2014

Accepted: 14 Aug 2014

**Objective:** We measured the total and ionized serum calcium levels in serum of hypertension patients and compared them with the age and sex matched healthy normotensive controls. We also tried to observe the association of serum calcium (total and ionized with essential hypertension. **Experimental approach:** A total of 47 hypertensives and 28 normal control subjects were selected for the study. Blood pressure was measured and serum total and ionized calcium were estimated in them. Serum total calcium was estimated by O-Cresolphthalein Complexone Method in Semi-Automatic Analyzer. Serum ionized calcium was estimated by Electrolyte Analyzer (Ion Selective Electrode). Statistical analysis was done by SPSS 20 software. **Findings:** The mean serum total calcium level was decreased in hypertension patients than controls but the decrease was not significant ( $p= 0.96$ ). Results also showed the mean ionized calcium level was decreased with advancing age in hypertensive than controls significantly ( $p<0.0001$ ). **Discussion:** Our study have distinctly shown that the mean ionized calcium level is negatively correlated with age in hypertension patients ( $r= -0.87, p<0.0001$ ) but no correlation found in controls. **Conclusion:** Our study suggests, serum ionized calcium has a significant role in pathophysiology of hypertension. Ionized calcium may be estimated in essential hypertension patients to asses the prognosis & calcium supplementation may improve the situation.

**Key words:** Hypertension, total calcium, ionized calcium, age.

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## 1. INTRODUCTION

Presently, essential hypertension is one of the common public health problem which affects all the socioeconomic classes of the population. Often it is asymptomatic, so till diagnosis it can raise the complications like ischemic heart disease, stroke and

other vascular diseases<sup>1</sup>. In the year 2000, 26.4% of the adult population had hypertension and by the year 2025, 29.2% are expected to have this. A total of 1.56 billion people may be affected of it by the year 2025. Approximately 54% of all strokes and 47% of all ischemic heart diseases were attributed to high blood pressure<sup>2</sup>. Hypertension is reported to be fourth contributor to premature death in developed countries and the seventh in developing countries<sup>3</sup>. In India, the prevalence of hypertension is 5.99% and 6.99 % in males and females respectively in urban population and 3.55 and 3.59 % males and females in rural population<sup>1</sup>.

Peripheral resistance is one of the major contributors of arterial pressure. Increased peripheral resistance is a consequence of increase in active tension in the vascular smooth muscles<sup>4</sup>. The calcium ion plays a major role as intracellular second messenger during muscular activity of cardiac and smooth muscle cells. Thus, peripheral vascular resistance is determined by free intracellular calcium concentration. Abnormal calcium metabolism has been identified as one of the significant contributor for essential hypertension by many authors<sup>5</sup>. Furthermore, how the vascular smooth muscle cell responses calcium is critical for vascular tone and blood pressure<sup>6</sup>. Serum free (ionized) calcium is biologically active and tightly regulated by calcium binding hormones whereas serum total calcium is the sum of 3 forms, ionized or free (50%), protein-bound (40%) and soluble form, complexes with anions such as bicarbonate & phosphate. Total calcium is thus greatly influenced by protein concentration, especially Albumin<sup>7</sup>

A variation of results were found in some of the previous studies regarding the link between calcium and blood pressure. Pawade YR et al found the alteration of serum calcium level in essential

hypertension<sup>8</sup>.

Some authors didn't find any difference in serum total calcium between hypertensive and normotensive groups, but serum ionized calcium levels were low<sup>9,10</sup>. A recent study in U.S. showed increased serum total and free calcium levels in hypertensive adults<sup>11</sup>. Elevated basal cytosolic free calcium (ionized) levels and abnormal membrane transport were found in different tissues of hypertensives. Studies also found that the blood pressure levels are directly associated to ionized calcium concentrations in those patients<sup>12,13</sup>. Variations were also observed in extracellular calcium contents of hypertensive patients than controls<sup>14,15</sup>. Jorde R et al observed a highly significant correlation between serum calcium with systolic and diastolic blood pressures<sup>16</sup>.

Under these circumstances, we have estimate the serum total and ionized calcium levels in hypertensive subjects and to compare them with normotensive controls. Furthermore we tried to asses whether any link exists between serum calcium and pathophysiology of hypertension.

## 2. MATERIALS AND METHODS

**Study design-** Cross sectional hospital based study

**Study site-** The study was carried out at the Department of Biochemistry, Calcutta National Medical College and Hospital (CNMCH)

**Duration of study-** The duration of study was six months(01.10.2013-31.03.2014)

**Selection of cases and controls-** A total of 47 hypertensives (28males and 19 females, aged 40 to 60 years) were selected. Informed consent was taken from them. Cases were selected from clinically newly diagnosed hypertensive patients attending the medicine outdoor

patient department of Calcutta National Medical College. 28 (15 male and 13female) age and sex matched healthy control subjects without any family

history of hypertension were also selected for the study, with consent.

**Inclusion criteria:** Subjects between the age group of 40 -60 years were selected. Samples from cases were collected before institution of any hypertensive treatment. The criteria for diagnosis of hypertension were systolic pressure of >140 mm of Hg and diastolic pressure of >90 mm of Hg.

**Exclusion criteria:** Hypertensive patients who were already on anti-hypertensive treatment were excluded from the study. Study subjects were examined systematically to exclude any disease (Secondary hypertension) or factors known to cause or associated with hypertension. Subjects on drugs like steroids, oral contraceptive pills, and thyroxin were excluded from the study. Similarly, subjects with any underlying condition or taking any drug known to alter serum calcium levels or calcium supplementations have been excluded from the study.

**Ethical Clearance:** Before commencement of the work, Ethical Clearance was obtained from the Institutional Ethics Committee, according to the Helsinki Declaration. Written informed consent was taken from cases and control subjects.

#### Methods for analysis of test parameters

**Blood pressure measurement<sup>17</sup>:** Blood Pressure of the study subjects were measured in sitting posture. The instrument used was mercury sphygmomanometer (cuff size 12.5x40 cms).Systolic and diastolic blood pressures were taken according to the criteria. Two readings were taken from them with a difference of 5 minutes. The average reading was recorded.

**Serum total<sup>18</sup> and ionized calcium<sup>19</sup>:** Total 5 ml of blood was taken (without using tourniquete) from the study subjects with 12 hours fasting condition and divided into two parts. One part is kept in a sterile plain vial for estimation of total calcium while the second

part was kept in a pre heparinized vial for estimation of ionized calcium. The samples were analyzed within 30 mints of collection. The estimation of serum total calcium was done in Semi automatic Analyzer (TRANSASIA ERBA CHEM-5 Plus). The principle was based on O-Cresolphthalein Complexone Method (crest biosystems).Serum ionized calcium was analysed by 9180 Electrolyte Analyser.Principle based on Ion Selective Electrode (Roche Diagnostics GmbH MannheimGermany).

**Statistical analysis:** Done by using SPSS 20 Software.

### 3. RESULTS

Results showed that there is a significant increase in mean diastolic and systolic blood pressure in hypertensive case group than normotensive control Serum total and ionized calcium are decreased in hypertensive group than normotensive control group but the decrease is significant only in serum ionized calcium but not in serum total calcium. Statistical analysis also shows there is a negative correlation exists between serum ionized calcium and advancing age in hypertensive cases.

**Table 1: Distribution of B.P among the case and control group**

	Systolic blood pressure(mmHg)	Diastolic blood pressure(mmHg)
	Mean ±SD	Mean ±SD
Control Group(n=28)	120.78±5.25	82.07±4.97
Case Group	157.97±16.97	94.85±9.33
p-value	<0.0001	<0.0001

### 4. DISCUSSION

Results of our study have shown that the mean serum total calcium level is decreased in hypertension patients than controls but decrease is not significant (Table-2).Result also shows the mean ionized calcium level has decreased with advancing age but no such

correlation found in controls. Our study have distinctly shown that the mean ionized calcium level is negatively correlated with advancing age in hypertension patients (figure1). Some authors also found that the serum ionized calcium was decreased significantly in hypertensives<sup>20, 21</sup> which corroborates our study.

Some researches have studied only serum total calcium levels and found it to be lowered in hypertensive subjects<sup>22, 23</sup>. Booloo Sharma et al showed serum calcium levels to be low in hypertensives<sup>24</sup>. In a recent study by Hazari M A et al there was no significant difference in serum calcium levels between normotensive and hypertensive groups<sup>25</sup>. Takale LR shows significant decrease of free and total calcium levels in hypertensives when compared to controls<sup>26</sup>.

Some authors recently found a link between serum total and ionized calcium with essential hypertension<sup>27</sup>, furthermore they indicated the role of serum calcium in hypertension.

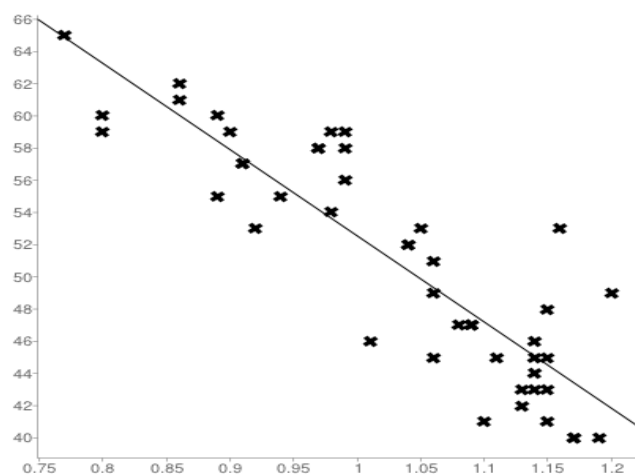
In our results, hypertensives showed lower values of serum calcium than normotensives. [Table 2], but the difference is statistically non-significant. (P=0.96).

**Table 2: Distribution of Serum ionized and total calcium among the case and control group.\*indicates significant p<0.05.**

	Serum Ionized calcium(mmol/l) Mean ±SD	Serum total calcium(mmol/l) Mean ±SD
Control Group(n=28)	1.157±0.108	2.375±0.287
Case Group(n=47)	1.032(4.12±0.115)	2.372±0.267
p-value	<0.0001*	0.96

The correlation study of age with ionized calcium level indicates a negative correlation between the two

parameters in hypertensive case group (r= -0.87, p<0.0001) but no significant correlation of advancing age with ionized calcium exists in normotensive controls(r= -0.34, p = 0.86) Folsom et al<sup>28</sup> Kazushi Tsuda et al<sup>29</sup> and Markus Koschet et al<sup>30</sup> found the similar trends. K. Sudhakar et al<sup>31</sup> documented the significant decrease of total serum calcium levels in hypertensives and their first degree relatives. These observations may be influenced by the dietary habit and genetic pools of the study subjects.



**Fig 1:-correlation between age and ionized calcium in hypertensive case group**

We observed that the serum ionized calcium is significantly low in hypertensives in comparison to normotensives.(P<0.001).

Similar results were observed by David A et al<sup>14</sup>, and Folsom et al<sup>28</sup>. Andreas Hvarfner et al<sup>32</sup> and Barbagallo et al<sup>33</sup> have also observed the same trend. Furthermore Mario Barbagallo et al have done the correlation and regression analysis of serum ionized calcium with age in the hypertensive patients and in the normotensive controls and observed the same trend with us. Some studies have shown the altered physiology of calcium in essential hypertension. Membrane transport and intracellular calcium concentrations have a major role in function of cardiac and vascular smooth muscle

Furthermore, in hypertensive animal models, the smooth muscle is hyper-responsive to changes in extracellular calcium concentrations and that the vascular membrane permeability to calcium is increased<sup>26</sup>.

Although there are some evidence of positive correlations between blood pressure levels and serum total calcium<sup>16,34,37</sup>. But the essential hypertensive subjects had lower serum ionized calcium concentrations even when the total calcium levels were similar<sup>14,28</sup>. In agreement with them we found, decrease of serum total calcium in hypertensives but the decrease is not significant. Furthermore we observed ionized calcium falls significantly in them. This may be explained by the fact that total calcium is greatly influenced by protein concentration, especially albumin<sup>7</sup>.

Some studies hypothesized that, due to abnormal membrane transport of calcium in hypertensives, the serum ionized calcium level is observed low, but the same is increased intracellularly resulting arteriolar vasoconstriction in hypertension<sup>15, 38-43</sup>. This disequilibrium in ionized calcium concentration may be the consequences of altered sodium calcium membrane transport in smooth muscles, observed by Blaustein<sup>27</sup>. Actually these observations are partly influenced by some other factors, the effect of calcium on blood pressure is influenced by sodium, potassium, magnesium, parathyroid hormone and renin concentrations. The relation between serum sodium, potassium with calcium concentration is quite surprising, since 24 hrs sodium excretion was negatively correlated with serum calcium, whereas potassium excretion correlated positively<sup>44</sup>. Increasing the extracellular ionized calcium concentration can cause the membrane stabilization, resulting relaxation of vascular smooth muscles, found by some authors<sup>45,46</sup>. Some authors have shown that

persons with low calcium diet are more prone to develop hypertension<sup>8</sup>. Calcium supplementation was also found to stabilize the blood pressure in hypertensives<sup>47</sup>.

In summary of these discussions it can be stated that the serum ionized calcium has a significant role in pathophysiology of essential hypertension.

## 5. CONCLUSION

In our study, we have found the significant decrease of serum ionized calcium in hypertensives when compared to control subjects, whereas serum total calcium didn't follow the same trend. Furthermore, we have also shown that ionized calcium is negatively correlated with age in hypertensive patients but no such correlation was observed in normotensive controls. Our study suggests to conclude that lowered serum ionized calcium can accelerate the hypertension and aging process which may be improved by calcium supplementation.

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**Conflict of interest**

There is no conflict of interest between authors

**Funding**

This research was funded by Government of West Bengal, Deptt of Health along with other reagents and kits required for Biochemistry Dept, Calcutta National Medical College