Original Article

Association of serum calcium levels with clinical severity of ischemic stroke at the time of admission as defined by NIHSS score: A cross-sectional, observational study

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Abstract

Introduction: Calcium plays an important role in the pathogenesis of ischemic cell damage. Intracellular calcium accumulation leads to neuronal damage by triggering the cycle of cytotoxic events. In this study, the association of serum calcium levels with clinical severity of ischemic stroke as defined by the National Institute of Health Stroke Scale (NIHSS) score was evaluated. **Materials and Methods:** After obtaining ethical approval from the institutional ethics committee, data was collected from 60 ischemic stroke patients, who were divided into two groups of 30 patients each: group 1 with serum ionized calcium less than 4.5 mg/dl and group 2 with serum ionized calcium levels more than 4.5 mg/dl. The stroke severity in the two groups was assessed using the NIHSS score. **Results:** The severity of ischemic stroke according to the NIHSS score was greater in patients with low serum ionized calcium levels compared to the severity of ischemic stroke in patients with normal serum ionized calcium levels. **Conclusion:** Serum ionized calcium certainly plays a role in the pathogenesis of ischemic stroke by influencing the cycle of cytotoxic events that result in ischemic cell death. This study showed that the severity of ischemic stroke was greater in patients with low serum ionized calcium levels compared to patients with normal serum ionized calcium levels.

Keywords: Ischemic stroke, NIHSS score, serum ionized calcium levels, stroke severity

Introduction

Calcium plays a key role in ischemic cell death. Hence the role of calcium ions in conditions like ischemic stroke becomes important. Ischemia depletes adenosine triphosphate (ATP). Without ATP, membrane ion pumps stop functioning and neurons depolarize, allowing intracellular calcium to rise. Depolarization increases neuronal calcium influx. Intracellular calcium accumulation leads to neuronal damage by triggering the cycle of cytotoxic events. Clinical studies suggest that serum calcium levels may be associated with severity of clinical symptoms, prognosis and infarct volume.[1–4] Very few studies exist in literature that studied the association of serum ionized calcium with severity of ischemic stroke. In this study, the association of serum ionized calcium levels with the clinical severity of ischemic stroke as defined by NIHSS score was evaluated.

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Materials and Methods

Study location

Department of Medicine, Vardhman Mahavir Medical College

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& Safdarjung Hospital, New Delhi for a period of 2 years from 01/2016 to 01/2018.

Study design

Observational cross-sectional study.

Sample size

60 cases, 30 in each group.

Study subjects

The study included ischemic stroke patients who satisfies the inclusion criteria and whose diagnosis was confirmed via non-contrast computed tomography (NCCT) brain or magnetic resonance imaging (MRI), if necessary, and admitted in the wards of Safdarjung Hospital.

Inclusion criteria

1. Ischemic stroke patients more than 18 years of age presenting within the first 24 hours of stroke onset
2. Ischemic stroke confirmed by NCCT brain or MRI brain if needed
3. Stroke patients with comorbidities like diabetes, hypertension, hypercholesterolemia,
   Valvular heart disease, prior transient ischemic attack (TIA) and atrial fibrillation.

Exclusion criteria

1. Hemorrhagic stroke as confirmed by NCCT brain
2. Autoimmune diseases
3. Endocrine disorders
4. Malignancy.

Routine investigations

CBC, LFT, KFT, SE, CXR, ECG, Urine routine, Serum calcium and total serum proteins.

Radiological investigations

Echocardiography, NCCT brain, carotid and vertebral artery Doppler, USG abdomen.

Special investigations

Serum ionized calcium levels, MRI brain if necessary.

Methodology

Approval from the institutional ethics committee and a written informed consent from all of the participants enrolled in the study was taken. Patients were evaluated by detailed history and clinical examination. The diagnosis of ischemic stroke was confirmed via NCCT brain or MRI brain, if necessary. Their serum ionized calcium levels were measured within the first 24 hours of their stroke incidence. Their stroke severity was measured by the National Institute of Health Stroke Scale (NIHSS) score. Ischemic stroke patients whose diagnosis was confirmed by NCCT brain or MRI brain presenting within the first 24 hours of onset were divided into two groups based on their serum ionized calcium levels: group 1 consisting of ischemic stroke patients with ionized calcium levels less than 4.5 mg/dl; and group 2 consisting of ischemic stroke patients with ionized serum calcium greater than 4.5 mg/dl.

Method

In all included patients, serum ionized calcium levels were measured.

Sample collection

Whole blood of 3–4 ml was collected in gel tubes for measurement of serum ionized calcium. During the sample collection, it was ensured that patients were relaxed and were breathing normally as much as possible. Tourniquets were applied for less than one minute and patients were not allowed to make a fist while sampling. After sample collection, the samples were transported within ten minutes to the biochemistry lab, and analysis for ionized calcium was done within one hour.

Laboratory analysis

The samples brought to the lab were analysed within one hour of collection after clot formation and centrifugation at 3000 rpm for five minutes. Serum ionized calcium levels were then measured by ion selective electrodes (ISE).

Radiological and biochemical investigations

1) Patients over the age of 18 years were included in the study. The presence of ischemic stroke was confirmed by the use of NCCT brain or MRI brain, if necessary.
2) The risk factors for stroke like hypertension, diabetes, coronary heart disease, atrial fibrillation, valvular heart disease and transient ischemic attack were recorded.
3) Systolic blood pressure of more than 140 mmHg and diastolic blood pressure of more than 90 mmHg was considered as hypertension.
4) Lipid profile was used to diagnose hypercholesterolemia wherein serum cholesterol more than 240 mg/dl was considered high.
5) Diabetes was confirmed by ascertaining the use of oral hypoglycaemic agents or insulin prior to the attack of ischemic stroke and also by the fasting and random blood sugar values greater than 126 and 200 mg/dl, respectively, according to ADA guidelines.
6) Routine blood investigations like CBC, LFT, KFT, SE along with total serum proteins to derive the albumin-corrected calcium levels were also measured.
7) Additionally, echocardiography (ECG), carotid and vertebral artery doppler were done for all patients enrolled in the study.
8) USG abdomen and chest X-ray (CXR) were also done to screen for malignancies.

Observation and Results

This study was a cross-sectional, observational study aimed at evaluating the association of serum calcium levels with

the clinical severity of ischemic stroke. The study population consisted of ischemic stroke patients, and it was carried out in the departments of medicine, radio-diagnosis and microbiology at Vardhman Mahavir Medical College & Safdarjung Hospital, New Delhi. The results and observations of the study have been compiled using various statistical tools, wherever applicable, and are as follows:

A total of 60 patients with ischemic stroke were enrolled and profiled as part of the study and they were divided into two groups: group 1 consisting of patients with calcium levels <4.5 mg/dl; and groups 2 consisting of those with calcium levels >4.5 mg/dl [Table 1]. Each group consisted of 30 patients.

Demographic profile of the study population

Age distribution

The age distribution of patients is shown in Table 2. The mean age of patients in group 1 (<4.5 group) was 57 years while the mean age of patients in group 2 (>4.5 group) was 58 years. The difference in age between the two groups was found to be statistically insignificant with $P = 0.834$ [Table 3].

Gender distribution

In the study, 58.33% of the subjects were male and 41.67% were female.

The difference in the distribution of sexes between the two groups was found to be statistically insignificant with $P = 0.693$ [Table 4].

Risk factor distribution

The number of risk factors that patients with ischemic stroke in the two groups had were studied. It was found that the difference in risk factor distribution between the two groups was statistically significant with $P = 0.014$. It means that patients who had low serum ionized calcium levels had more risk factors for ischemic stroke in this study.

As shown in Table 5, about 70% of patients in group 1, that is, the low serum calcium group had three or more risk factors for ischemic stroke in this study.

NIHSS score distribution of patient groups

The NIHSS score which is an objective tool was used to quantify the stroke severity of patients in the two groups at the time of admission [Table 6]. It was found that the mean NIHSS score of group 1 (< 4.5 group) was higher than that of group 2 (> 4.5 group). This difference in NIHSS scores between the two groups was found to be statistically significant with $P = 0.0004$, implying that patients who had low serum ionized calcium levels in fact had a more severe stroke [Table 7]. In our study, the patients who had moderate-to-severe stroke and severe stroke according to NIHSS were more likely to be from the group with low serum ionized calcium than the group with higher ionized serum calcium, as is evident from the chart.

Statistical analysis

Categorical variables were presented in number and percentage (%) and continuous variables were presented as mean ± SD and median. Normality of data was tested using the Kolmogorov–Smirnov test. If the normality was rejected, then nonparametric test was used.

Table 1: Distribution of patients according to serum calcium levels

Groups	Serum calcium levels (ionized) mg/dl	Number of cases *n*	Percentage
Group 1	< 4.5 mg/dl	30	50
Group 2	> 4.5 mg/dl	30	50
Total		60	100

Table 2: Distribution of patients according to age

Age	Percentage
≤ 40	5
41-50	30
51-60	20
61-70	33.33
> 70	11.67
Total	100

Table 3: Showing the age distribution of patient groups

Age distribution	Serum ionized calcium (mg/dl)		Total
	< 4.5	>4.5	
≤ 40	7.41%	3.03%	5.00%
41-50	29.63%	30.30%	30.00%
51-60	22.22%	18.18%	20.00%
61-70	33.33%	33.33%	33.33%
> 70	7.41%	15.15%	11.67%
Total	100%	100%	100.00%

Table 4: Showing the sex distribution of patient groups

Sex	Serum ionized calcium levels		Total
	< 4.5 mg/dl	>4.5 mg/dl	
Male	55.56%	60.61%	58.33%
Female	44.44%	39.39%	41.67%
Total	100%	100%	100.00%

Table 5: Showsing risk factor distribution of patient groups

No. of risk factors	Serum ionized calcium levels (mg/dl)		Total
	< 4.5	>4.5	
0	0%	6.06%	3.33%
1	7.41%	27.27%	18.33%
2	22.22%	39.39%	31.67%
3	66.67%	27.27%	45.00%
4	3.70%	0%	1.67%
Total	100%	100%	100.00%

Statistical tests were applied as follows:

1. Quantitative variables were compared using unpaired *t*-test or Mann–Whitney *U* Test (when the data sets were not normally distributed) between the two groups.
2. Qualitative variables were correlated using the Chi-squared test or Fisher's exact test.

A *P* value of < 0.05 was considered statistically significant.

The data was entered in Microsoft Excel spreadsheet and analysis was done using the Statistical Package for the Social Sciences (SPSS) version 21.0.

Discussion

Ischemic stroke remains a major cause of morbidity and mortality and imposes a massive burden on the healthcare system. In this study the role of serum ionized calcium on ischemic stroke was studied.

Calcium plays an important role in the pathogenesis of ischemic cell damage. Ischemic neuronal death engages several terminal pathways including the loss of ionic homeostasis.[5] Intracellular calcium accumulation leads to neuronal damage by triggering the cycle of cytotoxic events. Calcium ions play a role in the multiple mechanisms of cerebral ischemia.[6] For instance, ischemia and hypoxia triggers rapid translocation of calcium from extracellular to intracellular spaces of cerebral tissues.[7,8] Calcium has been studied with regard to its relationship with stroke risk factors and stroke incidence. It has been demonstrated that the calcium levels are decreased in cerebral ischemia. It has also been emphasized that gross brain damage, involving oedema formation and infarction, is enhanced by tissue acidosis and that neuronal damage often showing a pronounced selectivity in localization appears related to disturbed calcium homeostasis and to calcium triggered events such as lipolysis and proteolysis.[6] It has been found that higher serum calcium levels at admission are associated with smaller cerebral infarct volumes among patients with acute ischemic stroke.[9] The impact of both early and delayed calcium levels on clinical outcomes from acute ischemic stroke has also been studied but no significant differences in outcome were noted among early calcium levels.[10]

Table 6: Showing the NIHSS score used to quantify stroke severity

Score	Severity of stroke
0	No stroke symptoms
1-4	Minor stroke
5-15	Moderate stroke
16-20	Moderate to severe stroke
21-42	Severe stroke

Table 7: Showing stroke severity based on NIHSS score in patient groups

Stroke severity	Group		Total
	Serum calcium <4.5 mg/dl	Serum calcium >4.5 mg/dl	
Minor stroke	2 (6.66%)	11 (36.66%)	13 (21.67%)
Moderate stroke	11 (36.66%)	16 (53.33%)	27 (45%)
Moderateo-severe stroke	15 (50%)	3 (10%)	18 (30%)
Severe stroke	2 (6.66%)	0 (0.00%)	2 (3.33%)
Total	30 (100%)	30 (100%)	60 (100%)

In the present study, we took serum ionized calcium levels of 60 ischemic stroke patients presenting in the first 24 hours of incidence of stroke event of both male and female patients. The patients were divided into two groups: one having serum ionized calcium levels <4.5 mg/dl and the other having serum ionized calcium levels >4.5 mg/dl. It was found in the study that patients having serum ionized calcium levels of <4.5 mg/dl in the first 24 hours of stroke incidence had a higher mean NIHSS score and thereby greater severity of stroke.

Age distribution

The total number of patients below the age of 40 was 3 and the total number of patients above the age of 70 was 7. Most patients belonged to the 61–70 age group. No significant difference due to age was found between those who had serum ionized calcium <4.5 mg/dl and those who had serum ionized calcium >4.5 mg/dl.

Gender distribution

Our study included 35 males and 25 females. No significant difference was observed in relation to sex between those who had low serum ionized calcium <4.5 mg/dl and those who had serum ionized calcium >4.5 mg/dl.

Risk factor distribution

In our study, it was found that patients in the low serum calcium group (group 1) had more risk factors for ischemic stroke to begin with. The difference in risk factor distribution between the two groups was found to be statistically significant with $P = 0.014$. But further prospective studies are needed to confirm this association of low serum ionized calcium levels with increased stroke risk factors.

Stroke severity

In our study, it was found that patients, who had low serum ionized calcium levels (< 4.5 mg/dl) and were measured within the first 24 hours of stroke incidence, had a greater severity of stroke as defined by the NIHSS score, that is, calcium had a negative correlation with the clinical severity of ischemic stroke.

These findings were in accordance with those by Ovbiagele *et al.*[10] who observed that the severity of stroke was lesser in patients who had higher delayed (72–96 hours) serum calcium. Similar findings were reported by Guven *et al.*[11] where they found that NIHSS score was higher in the group with low serum calcium levels. Another study about infarct volume by BH Buck *et al.*[9] reported that higher serum calcium levels on admission were associated

with smaller cerebral infarct volumes among patients with acute ischemic stroke. Two studies were conducted in India also, one by Gupta A, *et al*.[12] which reported that 24–48-hour calcium levels had a strong correlation with severity and functional outcome in acute ischemic stroke patients and that higher serum calcium levels were associated with lesser severity of ischemic stroke. Another Indian study by Borah M, *et al*.[13] found that a negative correlation was found between serum calcium levels and infarct size in acute ischemic stroke. In contrast, one study by Chung JW, *et al*.[14] found that elevated serum calcium levels were associated with a poorer short-term outcome and a greater risk of long-term mortality after acute ischemic stroke. Similarly, another study[7] found that higher serum calcium levels were associated with increased severity of ischemic stroke and vice versa.

A modest positive association between cardiovascular disease and elevated serum calcium concentrations was reported by Rohrmann S *et al*.[15] In their study, Larsson SC *et al*.[16] reported that a genetic predisposition to higher serum calcium levels was associated with increased risk of coronary artery disease (CAD) and myocardial infarction. Clarifying the exact pathophysiological mechanism that may underlie these clinical observations has been challenging, especially because it is unclear whether serum Ca++ level exerts a primary effect on ischemic stroke or if it reflects an epiphenomenon of ischemic stroke severity.[17] Animal studies have shown that Ca++ movement from serum to brain occurs primarily via the choroid plexuses,[5] and when neurons and glia are exposed to lipid peroxidation, their intracellular structures lose their protection from the extracellular space and a Ca++ sink is created. As a result, more calcium is extracted from the blood into the brain. In order to pull Ca^{++} from the serum, the gradient must be sufficient to reduce the content of Ca^{++} in the serum.[5] It is thought that total neuronal cell Ca^{++} content may increase to 150% of control or more.[5] Furthermore, the finding of more substantial decreases in calcium levels of ischemic stroke patients than of transient ischemic attack and controls may also support this hypothesis.[18] However, whether the amount would be sufficient to change the serum levels to the degree noted in our study is unknown.

A comparison of MR images quantifying the extent of brain injury or measurement of Ca^{++} concentration in the cerebrospinal fluid[6] versus serum Ca^{++} levels would provide insight as to whether greater cerebral damage is associated with lower Ca++ levels. Calcium channel blockers are extensively evaluated in acute stroke with hope that stemming excessive cellular calcium influx caused by ischemia might prevent neuronal injury.

Interestingly, the potential role of serum calcium as a clinical prognosticator is not limited to ischemic stroke. Studies of general medical conditions, particularly among the critically ill, have shown that those with hypocalcaemia tend to be more severely ill, and have higher mortality rates than those with normocalcemia.[15,16,19,20] We did not have information on medical complications after the strokes in trial, and so it is conceivable that the Ca^{++} levels we observed may have been influenced by the subsequent development of intercurrent illnesses.

Strengths of the study

One of the main advantages of the study was that it measured the ionized calcium levels which is the physiologically active form of calcium. To the best of our knowledge, there are only very few studies published in literature which have evaluated the association between serum ionized calcium levels and ischemic stroke severity. Since our study found an association between low serum ionized calcium levels and increased severity of ischemic stroke, it may help primary care physicians to be vigilant about potentially catastrophic consequences of low serum ionized calcium levels and make timely referrals and other interventions to reduce morbidity and mortality from ischemic stroke.

Limitations of the study

The study has its limitations in that it was not a prospective study. The study could not exclude the possibility that unmeasured confounding may explain some of our findings. Finally, we lacked brain imaging data to study the association between infarct size and serum ionized calcium level.

Conclusion

In conclusion, it can be said that the severity of ischemic stroke increases with decreasing serum ionized calcium levels. Because of this association, serum ionized calcium can be used as a prognostic marker for ischemic stroke patients. It also raises the possibility of using calcium supplements to reduce the risk or severity of ischemic stroke. Further randomized controlled trials are needed to confirm this potential benefit of calcium supplements. In a country like India where the morbidity, mortality and the expenditure associated with ischemic stroke is high, the potential ability of a routine blood test like serum calcium levels to provides important prognostic information with regard to ischemic stroke severity cannot be understated.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published, and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

Key messages

Calcium plays a key role is ischemic cell death. Low serum ionized calcium levels may be an indicator of increased severity of ischemic stroke. Increased intracellular calcium can trigger apoptotic pathways and contribute to cell death.

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Conflicts of interest

There are no conflicts of interest.

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