Low Adjusted Serum Calcium Level as a Predictor of Poor Outcome in Patient With Acute Ischemic Stroke

Agus Suryawan¹, AABN Nuartha², Thomas Eko Purwata³, DPG Purwa Samatra⁴, I Putu Eka Widyadharma⁵

Neurology Department of Udayana University / Sanglah General Hospital, Denpasar

Abstract: 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, however the relationship of Adjusted serum calcium levels and the pathways involved in ischemic injury is unclear. The aim of this study is to determine whether low Adjusted serum calcium levels can be used as a predictor of poor outcome in acute ischemic stroke patient. This is a prospective cohort study of acute ischemic stroke patients admitted to Sanglah General Hospital from December 2016 until February 2017. Adjusted serum calcium level was obtained ≤72 hours from onset. Outcome was classified as poor and good, according to the National Institutes of Health Stroke Scale (NIHSS) score taken on admission and 7 days after the onset. Statistical analysis was performed using Chi-square. A total of 60 patients were enrolled and met the criteria. Subject's characteristic described by sex, age, onset, stroke type, serum adjusted calcium level, first and second NIHSS score. Serum adjusted calcium level mean was lower in poor outcome group (8.78 ± 0.07) than subjects in the good outcome (9.05 ± 0.06). Chi-square analysis revealed lower Adjusted serum calcium levels accompanied by poor outcomes statistically significant (RR = 3.2; 95% CI = 1.34 to 7.62; p = 0.007). Multivariate analysis revealed lower Adjusted serum calcium levels as an independent predictor of poor outcome in acute ischemic stroke (RR = 6.47; 95%IC = 1.69 to 24.72; p = 0.006). Low Adjusted serum calcium level is an independent predictor of poor outcomes in patients with acute ischemic stroke.

Keywords: adjusted calcium, poor outcome, acute ischemic stroke.

1. Introduction

Stroke is a major problem in both developed and developing countries. Stroke, as the leading cause of global death and disability, has affected more than 700,000 people in America.[1] The overall incidence of stroke in Asia is 116-483/100,000 per year.[2] Stroke often results in disability and causes emotional distress and economic problems for patients and their families.[3]

Calcium is an essential element for various biological processes from fertilization to death. The serum calcium is divided into three fractions: 50% of calcium ions in the active form, 40% bound to serum proteins, principally albumin and 10% bound to anions such as bicarbonate and citrate.[4] Calcium plays an important role in the pathogenesis of ischemic cell death. Accumulation of intracellular calcium causes the death of neurons that form the basic pathomechanism of ischemic stroke driven by exotoxicity. [4-6] Measurement of total calcium level is affected by level of total protein, especially albumin. Hypocalcemia often occurs due to a decrease in the fraction bound to albumin, although active calcium levels may be normal.[4,7] Hypoalbuminemia is commonly found in patients with stroke, giving a predictor of poor outcomes.[4] Alteration of protein levels, can cause changes in total calcium without affecting ionized calcium physiologically and clinically, thus, the total calcium serum adjustment to albumin is very important when trying to determine the value of normal calcium.[8-10] For this reason, the adjusted calcium is a better parameter to evaluate the effect of calcium on the cell when direct ionized calcium level measurement are is available.[10]

This study aims to determine whether low Adjusted serum calcium level can be used as a predictor of poor outcome during treatment in patients with acute ischemic stroke.

2. Subject and Methods

Study Design

We used observational prospective cohort in subjects with acute ischemic stroke. Samples were taken with consecutive non-random sampling method. This research was conducted in the Department of Neurology Faculty of Medicine, University of Udayana/Sanglah General Hospital, Denpasar, from December 2016 - February 2017.

Data Collection

Patients with acute ischemic stroke with onset ≤ 72 hours and ≥ 25 years old who were willing to sign informed consent included into this study. The exclusion criteria were: patients with stroke who were not confirmed by a brain CT scan; clinical symptoms of posterior circulation; history of prior stroke; history of other brain disorders; history of acute myocardial infarction; history of blood transfusions; history of malignancy; stroke patients with impaired parathyroid hormone and thyroid hormone, hypercalcemia, impaired liver function and kidney function; acute infectious disease; autoimmune disease; and pancreatitis. On admission, the total calcium and albumin serum levels were measured, and using these data the Adjusted serum calcium level was calculated. The NIHSS score was used to measure the severity of each subject, and was taken on admission and day-7th of the treatment.
Laboratory Methods
Adjusted calcium level was calculated by using the formula: adjusted calcium (mg / dl) = calcium serum (mg / dl) + 0.8 [4- albumin serum (g / dl)].[9-12] The total calcium serum and albumin serum levels are measured using the Cobas 501 in the Clinical Pathology Laboratory of Sanglah Hospital. The measurement was done once in each subject, that was when subject was admitted to hospital and the measurement should not exceed the 72 hours from the onset of stroke.

Statistical Analysis
Adjusted serum calcium levels divided into two subgroups: low (adjusted serum calcium levels <8.9 mg/dl) and normal (8.9 to 10.1 mg/dl). Outcomes are grouped into poor outcome (defined as increase in the NIHSS score ≥2 points or death occurred during treatment), and good outcome (defined as no change of, or decrease of, or increase of the NIHSS by one point). Analysis was done using SPSS 20 for windows. Bivariate analysis was done using Chi-Square with continuity correction. The level of significance is expressed by p<0.05 and the relative risk (RR) with 95% confidence interval (CI). To determine the influence of other factors as predictors of the outcome, we also included multivariate analysis using nominal regression method. The study was approved by the Ethic Committee of Udayana University-Sanglah General Hospital Denpasar.

3. Result
A total of 60 subjects with acute ischemic stroke were included in the study. The mean age in the group of low serum adjusted calcium levels are 55.67 ± 12.57 years, lower than those in the normal level group (60.83 ± 13.24 years). Embolism-type stroke appeared more in the low Adjusted serum calcium group (n=14; 56.0%) than the normal level group (n=11; 44.0%). The basic characteristic of the subjects are displayed in Table 1.

<table>
<thead>
<tr>
<th>Variable</th>
<th>low ASC level (n=30)</th>
<th>normal ASC level (n=30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>Mean ± SD</td>
<td>55.67 ± 12.57</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td>Male 22 (55%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Female 8 (40%)</td>
</tr>
<tr>
<td>Onset (hours)</td>
<td>Median (min-max)</td>
<td>8.1 (1-72)</td>
</tr>
<tr>
<td>Stroke type</td>
<td></td>
<td>Thrombus 16 (45.7%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Emboli 14 (45%)</td>
</tr>
<tr>
<td>NIHSS I</td>
<td>Median (min-max)</td>
<td>6 (4-21)</td>
</tr>
</tbody>
</table>

Results of the multivariate analysis, it was found that Adjusted serum calcium level and history of dyslipidemia were statistically significant as independent risk factors for outcome of patients with acute ischemic stroke (table 3).

Table 1: Baseline characteristic of study subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>Poor outcome (n=21)</th>
<th>Good outcome (n=39)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low ASC Level</td>
<td>Yes 16 (76.2%)</td>
<td>14 (35.9%)</td>
</tr>
<tr>
<td></td>
<td>No 5 (23.8%)</td>
<td>25 (64.1%)</td>
</tr>
<tr>
<td>Sex</td>
<td>Male 14 (66.7%)</td>
<td>26 (66.7%)</td>
</tr>
<tr>
<td></td>
<td>Female 7 (33.3%)</td>
<td>13 (33.3%)</td>
</tr>
<tr>
<td>Stroke type</td>
<td>Thrombus 12 (57.1%)</td>
<td>23 (59.0%)</td>
</tr>
<tr>
<td></td>
<td>Emboli 9 (42.9%)</td>
<td>16 (41.0%)</td>
</tr>
<tr>
<td>History of hypertension</td>
<td>No 17 (81.0%)</td>
<td>33 (84.6%)</td>
</tr>
<tr>
<td></td>
<td>Yes 4 (19.0%)</td>
<td>6 (15.4%)</td>
</tr>
<tr>
<td>History of dyslipidemia</td>
<td>No 12 (57.1%)</td>
<td>7 (17.9%)</td>
</tr>
<tr>
<td></td>
<td>Yes 9 (42.9%)</td>
<td>32 (82.1%)</td>
</tr>
<tr>
<td>History of diabetes mellitus</td>
<td>No 19 (90.5%)</td>
<td>31 (79.5%)</td>
</tr>
<tr>
<td></td>
<td>Yes 2 (9.5%)</td>
<td>8 (20.5%)</td>
</tr>
<tr>
<td>History of smoking</td>
<td>No 17 (81.0%)</td>
<td>27 (69.2%)</td>
</tr>
<tr>
<td></td>
<td>Yes 2 (9.5%)</td>
<td>1 (2.6%)</td>
</tr>
</tbody>
</table>

4. Discussion
Acute ischemic stroke patients who have poor outcomes were found more in the low adjusted calcium levels group (n=16; 76.2%) than in the normal level group (n=5; 23.8%). Chi-square analysis with continuity correction have shown ischemic stroke patients with low adjusted calcium levels are 3.2 times more likely to have poor outcomes compared to those with normal adjusted calcium levels (p = 0.007). These findings are consistent with the study by Ganti et al. (2013) in which routine laboratory parameters such as calcium was a predictor of early mortality after acute ischemic stroke with a relative risk (RR) of 2.9 (CI 95% = 1.4-5.9).[14] Apple et al. (2007) found that lower total calcium level was associated with higher mortality rates of all-cause mortality and poor outcome after 1 month and 1 year.[4] Gupta et al. (2015) found an independent association between levels of calcium to the severity of the stroke and functional outcomes.[15] On the contrary, Kasundra et al. (2014) found that high calcium level was associated with a better prognosis and recovery after acute ischemic stroke (except in the posterior circulation stroke), and a higher calcium levels was associated with a more small size infarction.[16]

Adjusted serum calcium level was chosen as predictor, rather than total calcium serum or ionized calcium, for the fact that level of total protein, especially albumin, affect the total

Table 2: Bivariate analysis of factors that affected outcome in acute ischemic stroke patients

<table>
<thead>
<tr>
<th>Variable</th>
<th>Poor outcome (n=21)</th>
<th>Good outcome (n=39)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low ASC Level</td>
<td>Yes 16 (76.2%)</td>
<td>14 (35.9%)</td>
</tr>
<tr>
<td></td>
<td>No 5 (23.8%)</td>
<td>25 (64.1%)</td>
</tr>
<tr>
<td>Sex</td>
<td>Male 14 (66.7%)</td>
<td>26 (66.7%)</td>
</tr>
<tr>
<td></td>
<td>Female 7 (33.3%)</td>
<td>13 (33.3%)</td>
</tr>
<tr>
<td>Stroke type</td>
<td>Thrombus 12 (57.1%)</td>
<td>23 (59.0%)</td>
</tr>
<tr>
<td></td>
<td>Emboli 9 (42.9%)</td>
<td>16 (41.0%)</td>
</tr>
<tr>
<td>History of hypertension</td>
<td>No 17 (81.0%)</td>
<td>33 (84.6%)</td>
</tr>
<tr>
<td></td>
<td>Yes 4 (19.0%)</td>
<td>6 (15.4%)</td>
</tr>
<tr>
<td>History of dyslipidemia</td>
<td>No 12 (57.1%)</td>
<td>7 (17.9%)</td>
</tr>
<tr>
<td></td>
<td>Yes 9 (42.9%)</td>
<td>32 (82.1%)</td>
</tr>
<tr>
<td>History of diabetes mellitus</td>
<td>No 19 (90.5%)</td>
<td>31 (79.5%)</td>
</tr>
<tr>
<td></td>
<td>Yes 2 (9.5%)</td>
<td>8 (20.5%)</td>
</tr>
<tr>
<td>History of smoking</td>
<td>No 17 (81.0%)</td>
<td>27 (69.2%)</td>
</tr>
<tr>
<td></td>
<td>Yes 2 (9.5%)</td>
<td>1 (2.6%)</td>
</tr>
</tbody>
</table>

Table 3: Multivariate analysis

<table>
<thead>
<tr>
<th>Variable</th>
<th>RR (95%CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>History of diabetes mellitus</td>
<td>0.14 (0.38-0.55)</td>
<td>0.005*</td>
</tr>
</tbody>
</table>

* statistically significant
calcium measurement. Hypocalcemia often occurs due to a
decrease in the fraction bound to albumin, although active
calcium levels may be within normal levels. Hypoalbuninemia is commonly found in stroke patients thus,
hypoalbuminemia may occur concurrently.[4-7] About half of the
calcium in serum is bound to serum proteins, primarily
albumin. Thus, changes in protein levels can cause changes in
total calcium without affecting the ionized calcium
physiologically and clinically. Therefore, the total calcium
level adjustments to albumin is very important when trying to
determine the normal value. For this reason, the adjusted
calcium is a better parameter to evaluate the effects of
calcium in the cell when the measurement of ionized calcium
levels is not directly available.[10] In outpatient setting,
estimated ionized calcium level by calculating the total
calcium and albumin remain practical and cost-effective.
Ionized calcium is superior in identifying disorders of
calcium in patients who received citrate blood transfusion;
in critically ill patients. Patients with end stage chronic kidney
disease (CKD), hyperparathyroidism and hypercalcemia of
malignancy were excluded from this research.[13]

Serum calcium level is maintained within a narrow range through a series of feedback mechanisms involving
the parathyroid hormone and active vitamin D metabolite 1,25-
dihydroxyvitamin D, composed of integration signal between the
parathyroid glands, kidney, intestines, and bone.[4] Calcium plays a physiological role in some pathomechanism
of cerebral ischemia. Metabolism of calcium during and immediately after the transient period of ischemia affect the
cascade of events that causes later neuronal injury.[8,10,15]

Decreased blood flow in the brain under 10-12 ml/100g/min causes infarction, almost regardless of the duration. CBF 6-8
ml/100g/min causes depletion of ATP, an increase in extracellular potassium, increased intracellular calcium, and
cellular acidosis, and always leads to signs of necrosis histologically. Free fatty acid (as phospholipases) is activated
damages neuronal phospholipid membranes. The accumulation of prostaglandins, leukotrienes, and free radicals, leads to intracellular protein and enzyme
denaturation.[16] In ischemic condition, the release of glutamate from neurons and glia activates N-methyl-D-
aspartate (NMDA) receptors and triggers a rapid translocation of calcium from the extracellular to intracellular
compartment in brain tissue. Animal studies have shown displacement of serum calcium in the brain cells mainly
occurs through the choroid plexus, and when neurons (and/or
glial cells) were exposed to lipid peroxidation, there will be loss of intracellular structure protection from the extracellular
compartment and decreased levels of serum calcium. As a
result, more calcium is extracted from the blood to the brain.

In order to extract calcium from the serum, the gradient must
be sufficient to reduce serum calcium levels. It is estimated
that total calcium levels of neuronal cells could be increased
to 150% or more from normal. In addition, the findings of a
decrease in calcium level was greater in patients with
ischemic stroke compared with transient ischemic attacks or
control group can also support the hypothesis.[8,10,15]

Calcium influx into cells via NMDA receptor is a major
pathway for delayed cell death and ischemia associated
excitotoxicity. The other pathways such as transient receptor
potential channel (TRP) and non-selective cation channels
cause ion imbalance that may escalate during ischemia and
play roles in calcium-mediated neuronal death. Replacing
extracellular calcium levels after a period of low calcium
levels known to cause a paradoxical increase in intracellular
calcium levels. This shows that TRP channels are likely to contribute to the calcium paradox and delayed neuronal death
after ischemic stroke. Ion calcium influx into cells via
NMDA receptors and voltage-dependent calcium channels
could potentially degrade extracellular calcium and this
reduction causes disinhibition of Ca2+-sensing current non-
selective channels (IscsSC) and then the membrane
depolarization and more calcium influx. On the other hand, a
decrease in extracellular pH during ischemia, activate the
acid-sensing ion channels (ASIC). ASIC activation is
triggered by stretching the membrane, the release of
arachidonic acid, lactate production or decrease in
extracellular calcium levels in a condition that occurs in
the ischemic neurons and causes calcium influx. Moreover,
there’s a theory that minimal increase in extracellular calcium
may influence intracellular second messengers by Ca-sensing
receptors (CaR) and may initiate antiapoptotic pathway. This
shows that calcium ion not only act as an intracellular second
message, but can also act as an external ligand, and that
extracellular calcium ion may be the important first
message.[6]

The relationship between calcium intake and the incidence of
ischemic stroke is still a controversy. High calcium diets have
been linked with a decreased risk of stroke. In addition to the
hypotensive effects of calcium, calcium also decreases
platelet aggregation and lowering plasma cholesterol
levels.[6] Study in normal elderly woman and in patients with
renal impairment showed calcium supplements increase the
risk of cardiovascular disease, increased risk of myocardial
infarction by 27-31% and increased risk of stroke by 12-
20%. Increased cardiovascular risk with calcium supplements
are consistent with epidemiological data related to high
calcium levels in the circulation for cardiovascular disease in
the normal population. There are several possible
pathophysiological mechanisms for this effect, including the
effect of vascular calcification, the function of blood vessel
cells, and blood clotting.[17]

The study also found a significant relationship between
history of dyslipidemia with stroke outcomes, which shows a
history of dyslipidemia as an independent factor for better
outcomes of acute ischemic stroke. These results are similar to
studies conducted by Vauhey et al. (2000) in which patients with high cholesterol levels had 2.2 times lower risk
of death (p = 0.002) and 2.1 times lower risk for poor
outcomes in the first 1 month (p <0.001) compared to
patients with normal cholesterol levels. After adjusting for
confounding variables, multivariate analysis showed a high
cholesterol level remains an independent predictor of good
outcome (OR = 0.48; CI 0.34 to 0.69, p <0.001). Cholesterol
oxidation generates oxyesters, although this is still toxic to
the cell, but less dangerous than free radicals. This suggests,
cholesterol can work as a buffer, neutralizing the proportion
of free radicals, preventing the expansion of the lesion, and
improve the cell healing capacity.[18]
The strength of this study was that the design used was prospective cohort and as such, multiple factors could be analyzed as predictors of poor outcome in patients with acute ischemic stroke. Additionally, multivariate analysis was done to control other variables that may contribute to the outcome. The weaknesses of this study were the fact that we skipped other outcome predictors such as size of infarction, lesion location and collateral vessels due to the limited diagnostic tools used. Also, we did not consider variables such as history of hypertension, history of dyslipidemia, heart disease such as atrial fibrillation or coronary heart disease, and diabetes, were already well-controlled or not. This comorbidity differences also means that some patients who received additional therapy for their condition may have affected the results. The other weaknesses were the age and sex were not matched early on in the study, and the adjusted levels of calcium measurement was only carried out once, so that the possible reduction in the adjusted levels of calcium could not be excluded.

5. Conclusion

Based on these results, we can conclude that low adjusted calcium levels in patients with acute ischemic stroke is an independent risk predictor, with 6.47 times more likely to have poor outcomes than subjects with normal serum adjusted calcium levels. We suggest that regular evaluations of adjusted serum calcium levels is carried out upon hospital admission in patients with acute ischemic stroke in an attempt to predict the occurrence of a bad outcome, so a more targeted management can be done to increase potential outcomes of stroke. We also suggest further research on the relationship between adjusted serum calcium levels with the outcome of acute ischemic stroke patients with consideration for the aforementioned weaknesses. Further research is also needed to study the relationship between dyslipidemia and each lipid components with acute ischemic stroke outcomes.

References


