International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2015): 78.96 | Impact Factor (2015): 6.391

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

Agus Suryawan[1], AABN Nuartha[2], Thomas Eko Purwata[3], DPG Purwa Samatra[4], I Putu Eka Widyadharma[5]

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 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 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 Amerikca.[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 is 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 1: Baseline characteristic of study subjects

Variable		low ASC level (n=30)	normal ASC level (n=30)
Age (years)	Mean ± SD	55.67 ± 12.57	60.83 ± 13.24
Sex	Male	22 (55%)	18 (45%)
	Female	8 (40%)	12 (60%)
Onset (hours)	Median (min-max)	8 (1-72)	9,5 (2-70)
Stroke type	Thrombus	16 (45.7%)	19 (54.3%)
	Emboli	14 (56%)	11 (44%)
NIHSS I	Median (min-max)	6 (4-21)	4,5 (3-15)

ASC: Adjusted serum calcium

Subjects with poor outcomes were more frequent in the low Adjusted serum calcium levels (n=16; 76.2%) than in the normal level group (n=5; 23.8%). The results of the bivariate analysis showed that low Adjusted serum calcium level and history of dyslipidemia in acute ischemic stroke subject significantly increased to have poor outcome (Table 2).

Based on the 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 (tabel 3).

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

Variable		Poor outcome (n=21)	Good outcome (n=39)	RR (95%CI)	*p*
Low ASC Level	Yes	16 (76.2%)	14 (35.9%)	3,2(1.34-7.62)	0.007*
	No	5 (23.8%)	25 (64.1%)		
Sex	Male	14 (66.7%)	26 (66.7%)	1.000 (0.48-2.08)	1.000
	Female	7 (33.3%)	13 (33.3%)		
Stroke type	Thrombus	12 (57.1%)	23 (59.0%)	0.952 (0.48-1.91)	1.000
	Emboli	9 (42.9%)	16 (41.0%)		
History of hypertension	Yes	17 (81.0%)	33 (84.6%)	0.85 (0.36-1.99)	1.000
	No	4 (19.0%)	6 (15.4%)		
History of dyslipidemia	Yes	9 (42.9%)	32 (82.1%)	0.35 (0.18-0.68)	0.005*
	No	12 (57.1%)	7 (17.9%)		
History of diabetes mellitus	Yes	2 (9.5%)	8 (20.5%)	0.526 (0.15-1.91)	0.468
	No	19 (90.5%)	31 (79.5%)		
History of smoking	Yes	4 (19.0%)	12 (30.8%)	0.65 (0.26-1.64)	0.501
	No	17 (81.0%)	27 (69.2%)		
History of alcohol consumption	Yes	2 (9.5%)	1 (2.6%)	2.00 (0.83-4.82)	0.576
	No	19 (90.5%)	38 (97.4%)		

* statistically significant

Table 3: Multivariate analysis

Variable	RR (95%CI)	p
ASC level	6.47 (1.69-24.72)	0.006*
History of dyslipidemia	0.14 (0.38-0.55)	0.005*

* statistically significant

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

calcium measurement. Hypocalcemia often occurs due to a decrease in the fraction bound to albumin, although active calcium levels may be within normal levels. Hypoalbuminemia is commonly found in stroke patients thus, hypocalcemia 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 and 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 Ca^{2+}-sensing current non-selective channels (IcsnSC) 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 messenger, but can also act as an external ligand, and that extracellular calcium ion may be the important first messenger.[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 Vauthey 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 oxysterols, 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

[1] Gribkoff, V.K., Starrett, J.E., Dworetzky, S.I., Hewawasana. P., Boissard, C.G., Cook, D.A., Frantz, S.W., Heman, K., Hibbard, J.R., Huston, K., Johnson, G., Krishnan, B.S., Kinney, G.C., Lombardo, L.A., Meanwell, N.A., Molinoff, P.B., Myers, R.A., Moon, S.L., Ortiz, A., Pajor, L., Piwschi, R.L., Post-Munson, D.J., Signor, L.J., Srinivas, N., Taber, M.T., Thalody, G., Trojnacki, J.T., Wiener, H., Yeleswaram, K., Yeola, S.W. Targeting Acute Ischemic Stroke with Calcium Sensitive Opener of Maxi-K Potassium Channels. Nature Medicine. 2001.7(4):471-477.

[2] Suwanwela, N.C., Poungvarin, N. Stroke Burden and Stroke Care System in Asia. Neurology India, 2016.64: 46-51

[3] Kulshreshtha, A, Anderson, LM. Stroke in South Asia: A Systematic Review of Epidemiologic Literature from 1980-2010. Neuroepidemiology, 2012; 32: 123–129.

[4] Appel, S.A., Molshatzki, N., Schwammenthal, Y., Merzeliak, O., Toashi, M., Sela, B.A., dan Tanne, D. Serum Calcium Levels and Long-Term Mortality in Patients with Acute Stroke. Cerebrovasc Dis. 2011.31:93–99.

[5] Bouachet, T dan Henley, J.M. Calcium as an Extracellular Signalling Molecule: Perspectives on the Calcium Sensing Receptor in the Brain. C.R. Biol, 2005.328(8): 691-700.

[6] Guven, H., Çilliler, A.C., Koker C., Sarikaya, S.A., Comoglu, S.S. Association of Serum Calcium Levels with Clinical Severity of Acute Ischemic Stroke. Acta Neurol. Belg. 2011.111: 45-49.

[7] Sava, L., Pillai, S., More, U., Sontakke, A. Serum Calcium Measured; Total Versus Free (Ionized) Calcium. Indian Journal of Clinical Biochemistry. 2005.20(2): 158-161.

[8] Ovbiagele, B., Starkman, S., Teal, P., Lyden P., Kaste M., Davis S.M., Hacke W., Fierus M., Saver J.L. Serum Calcium as Prognosticator in Ischemic Stroke. Stroke. 2008.9: 2231-2236.

[9] Sofronescu, A.G. Serum Calcium. Editors: Staros E.B. Available from: http://emedicine.medscape.com/article/2087447-overview. 2013.

[10] Chung, J.W., Ryu, W.S., Kim, B.J., Yoon, B.W. Elevated Calcium after Acute Ischhemic Stroke: Associated with a Poor Short-Term Outcom and Long-Term Mortality. Journal of Stroke. 2015.17(1): 54-59.

[11] Pain, RW., Rowland, K.M, Phillips, P.J, Duncan, M.B. Current "Corrected" Calcium Concept Challenged. British Medical Journal. 1975.4:617-619.

[12] Payne, R.B., Margarete, C., Morgan, D.B. Interpretation of Serum Total Calcium: Effects of Adjustment for Albumin Concentration on Frequency of Abnormal Values and on Detection of Change in the Individual. Journal of Clinical Pathology. 1979.32:56-60.

[13] Calvi, L.M; Bushinsky, D.A. When is it appropriate to order an ionized calcium? J Am Soc Nephrol. 2008.19: 1257–1260.

[14] Ganti, L., Gilmore, R.M., Weaver, A.L., Brown, R.D. Prognostic Value of Complete Blood Count and Electrolyte Panel During Emergency Departement Evaluation for Acute Ischemic Stroke. Hindawi Publishing Corporation. 2013.

[15] Gupta, A., Dubey, U., Kumar, A., Singh S. Correlation of Serum Calcium Levels with Severity and Functional Outcome in Acute Ischemic Stroke Patients. International Jounal of research in Medical Sciences. 2015.3(12):3698-3702.

[16] Kasundra, G.M., Sood, I., Bhushan, B., Bohra, G.K., Supriya, P.S. Clinico-Radiological Correlation Between Serum Calcium and Acute Ischemic Stroke. Internasional Journal of Advanced Medical and Health Research. 2016.1(2): 69-74.

[17] Reid, I.R., Bolland, M.J., Avenell, A., Grey, A. Cardiovascular effects of calcium supplementation. Oasteoporosis Int. 2011.22:1649-1658.

[18] Vauthey, C., de Freitas, G.R., van Melle, G. Devuyst, G., Bogousslavsky, J. Better outcome after stroke with higher serum cholesterol level. Neurology. 2000.54:1944-1949.