ORIGINAL ARTICLE

Bali Medical Journal (*Bali Med J*) 2019, Volume 8, Number 2: 368-372
P-ISSN.2089-1180, E-ISSN.2302-2914

Published by DiscoverSys

Levels of protein C, protein S, and anti-thrombin III in acute ischemic stroke patients at Haj Adam Malik Hospital, Medan

Jenie Erawati Muchti,[1*] Yuneldi Anwar,[2] Adi Koesoema Aman[3]

ABSTRACT

Background: Ischemic stroke is a clinical syndrome with rapid brain function loss due to a disturbance of blood supply to the brain. The role of natural anticoagulants protein C, protein S and antithrombin III (AT III) in ischemic stroke is still unknown. This study aimed to determine the differences in the levels of protein C, protein S and AT III, which were examined on days 1, 3 and 7 of acute ischemic stroke during treatment.

Methods: This was a longitudinal prospective study carried out in Haj Adam Malik General Hospital Medan, from January to May 2018. This study included 21 acute ischemic stroke patients with three times of sampling (day 1, 3 and 7). Protein C, protein S and AT III were examined using the Coatron A4 device. Protein C and AT III were examined using chromogenic assay principles and protein S using clotting assay principles.

Results: Twenty-one patients who participated in this study were 12 men (57.1%) and nine women (42.9%). There was no significant difference in the level of protein C ($p = 0.980$), protein S ($p = 0.680$) and AT III ($p = 0.872$) between day 1, 3, and 7. There was a positive correlation in protein C, protein S and AT III levels between day 1, 3, and 7. There was a negative correlation between protein C and AT III levels on day 3 (r: - 0.498; p: 0.022).

Conclusion: There was no significant difference in the level of protein C, protein S and antithrombin III between day 1, 3, and 7.

Keywords: Acute ischemic stroke, antithrombin III, protein C, protein S.

Cite this Article: Muchti, J.E., Anwar, Y., Aman, A.K. 2019. Levels of protein C, protein S, and anti-thrombin III in acute ischemic stroke patients at Haj Adam Malik Hospital, Medan. *Bali Medical Journal* 8(2): 368-372. DOI:10.15562/bmj.v8i2.1378

[1]Department of Clinical Pathology, Faculty of Medicine, University of North Sumatera/Haj Adam Malik Hospital Medan, Indonesia
[2]Department of Neurology, Faculty of Medicine, University of North Sumatera/Haj Adam Malik Hospital Medan, Indonesia
[3]Division of Haematology, Department of Clinical Pathology, Faculty of Medicine, University of North Sumatera/Haj Adam Malik Hospital Medan, Indonesia

[*]Correspondence to:
Jenie Erawati Muchti, Department of Clinical Pathology, School of Medicine, University of North Sumatera/Haj Adam Malik Hospital Medan, Indonesia
jenie_em@yahoo.com

Received: 2018-11-16
Accepted: 2019-04-01
Published: 2019-08-01

INTRODUCTION

A stroke, also known as a cerebrovascular accident (CVA), is a clinical syndrome with a rapid loss of brain function due to the disruption of blood supply to the brain. It can be caused by blocked or ruptured blood vessels, leading to ischemia (lack of glucose and oxygen supply) due to thrombosis, embolism or bleeding in the brain.[1]

According to the Global Burden of Stroke, stroke is the second most common cause of death (11.8%) of all deaths worldwide, after ischemic heart disease (14.8%). It is also the third most common cause of disability (4.5%) after ischemic heart disease (6.1%).[2] Based on the symptoms and health-workers diagnosis, the highest prevalence of stroke in Indonesia in 2013 was in South Sulawesi (17.9%), followed by Yogyakarta (16.9%), and Central Sulawesi (16.6%). The prevalence of stroke in North Sumatra itself was 6.0%.[3]

Recently, deficits in fibrinolysis or coagulation inhibitors (proteins S and protein C) were found to be important etiologies of ischemic stroke in young people with a frequency range of 13.8% to 16%.[4] The hereditary derivative protein S deficit was found in 12.4% of the population. Protein S is a plasma protein that depends on vitamin K, which potentiates the inactivation of factors Va and Vla by protein C. Hereditary Free Protein S (FPS) deficiency produces a prothrombotic state and has been associated with recurrent venous thrombosis and arterial thrombosis. Recently, FPS deficiency has been recognized as a risk factor for ischemic stroke. Case reports suggest that familial FPS deficiency can play an important role in young patients with cryptogenic stroke.[5]

Antithrombin III (AT III) were found to be significantly low at all time points after stroke. Protein C values increased significantly at admission and were normal at subsequent measurements. Protein S values were normal at admission but significantly decreased later. Natural anticoagulant values did not correlate with the etiology of stroke, stroke risk factors, or neurological values. However, the value of AT III at admission showed a significant correlation with stroke severity and disability at three months of onset. Natural anticoagulant values do not predict the recurrence of ischemic stroke.[7]

The prevalence of protein C, protein S and AT III deficiency have been reported in Chinese adults with cerebral ischemia. The overall value of thrombophilia is 27%. Protein S deficiency is the most common disorder, which is followed by protein C with protein S deficiency, and protein C deficiency. However,

protein S deficiency is more common in young adults. The positive correlation between protein C and AT III was found in patients with normal protein C activity but not in those with protein C deficiency. There was no correlation between AT III and protein S. The odds ratio of protein C and protein S were 5.29 and 2.86 respectively. Therefore, the inability of antagonist thrombin by the Protein C / S protein axis can be related to the occurrence of cerebral ischemia in the Chinese population. Antithrombin seems only to show minor roles.[8]

Protein C, protein S, and AT III seem to have important roles in the pathogenesis of ischaemic stroke. Based on that, this study aimed to determine the difference in the level of protein C, protein S and AT III between day 1, 3 and 7 of acute ischemic stroke during treatment.

METHODS

The study was conducted with the longitudinal prospective method. It was carried out from January to May 2018 in the Clinical Pathology Department, Faculty of Medicine, University of North Sumatra / Haj Adam Malik General Hospital Medan in collaboration with the Neurology Department, Faculty of Medicine, University of North Sumatra / Haj Adam Malik General Hospital Medan. The samples in this study were all acute ischemic stroke patients in Haj Adam Malik Hospital Medan who met the inclusion criteria; the inclusion criteria were patients with an acute ischemic stroke, confirmed by a head CT scan, who signed the informed consent to participate in this study. The exclusion criteria were patients with liver disease and those who were on anticoagulant drug therapy.

Protein C, protein S, and AT III levels were measured three times, on day 1, 3, and 7. They were examined using the Coatron A4 device. Protein C and AT III were measured using the principle of the chromogenic assay, while protein S was measured using the principle of clotting assay.

The difference in protein C, protein S and AT III levels between day 1, 3 and 7 were analyzed using the repeated ANOVA test for data that were normally distributed. The Friedman test was used for data that were not normally distributed. The correlation in protein C, protein S and AT III levels between day 1, 3 and 7 were analyzed using the Pearson correlation test for normally distributed data, while the Spearman correlation test was used to analyze data that were not normally distributed. Data analysis was done using a computer statistics program. It was considered significant if the p-value was < 0.05.

RESULTS

In this study, there were 21 acute ischemic stroke patients, with 12 men (57.1%) and 9 women (42.9%). The complete general characteristic of patients can be seen in Table 1.

As shown in Table 2, there was no significant difference in protein C, protein S, and AT III levels between day 1, 3 and 7 with p-values of 0.980, 0.680, and 0.872 respectively.

As shown in Table 3, there was a positive correlation in protein C levels between day 1 and 3, day 1 and 7, and day 3 and 7. Likewise, there was also a positive correlation in protein S and AT III levels between day 1 and 3, day 1 and 7, and day 3 and 7.

As shown in Table 4, there was a positive correlation between the level of protein C and protein S in day 1, 3, and 7, yet the results were not statistically significant.

Table 1 General characteristics of acute ischemic stroke patients

Parameter	N = 21
Age, years (Mean ± SD)	56.9 ± 14.04
Gender	
Male	12 (57.1%)
Female	9 (42.9%)
Blood Pressure, mmHg (Mean ± SD)	
Systole	150.48 ± 25.98)
Diastole	87.14 ± 11.02
History of disease, n (%)	
Hypertension	10 (47.6%)
Diabetes Melitus (DM)	1 (4.8%)
Hypertension dan DM	4 (19.0%)
No Hypertension and DM	6 (28.6%)

Table 2 The difference in protein C, protein S, and AT III levels between day 1, 3, and 7

Variable	Measurement			P
	Day 1	Day 3	Day 7	
Protein C (Mean ± SD)	86.5 ± 28.79	86.72 ± 28.13	85.03 ± 32.6	0.980*
Protein S (Mean Rank)	33.67	33.19	29.14	0.680**
AT III (Mean Rank)	33.43	32.10	30.48	0.872**

*: Repeated ANOVA test; **: Friedman test; ***: Significant p-values < 0.05

Table 3 Correlation of protein C, protein S, and AT III between day 1 and 3, day 1 and 7, and day 3 and 7

Variable	r	p
Protein C*		
Day 1 and 3	0.791	0.001***
Day 1 and 7	0.685	0.001***
Day 3 and 7	0.836	0.001***
Protein S*		
Day 1 and 3	0.719	0.001***
Day 1 and 7	0.779	0.001***
Day 3 and 7	0.871	0.001***
AT III**		
Day 1 and 3	0.786	0.001***
Day 1 and 7	0.709	0.001***
Day 3 and 7	0.887	0.001***

*: Pearson test; **: Spearman's test; ***: Significant p-values < 0.05

Table 4 Correlation between protein C and protein S

Day	r	p
Day 1	0.335	0.138
Day 3	0.216	0.348
Day 7	0.275	0.227

* Spearman's test; ** Significant p-value < 0.05

Table 5 Correlation between protein C and AT III

Day	r	p
Day 1	- 0.135	0.507
Day 3	- 0.498	0.022**
Day 7	- 0.329	0.145

* Spearman's test; ** Significant p-value < 0.05

Table 6 Correlation between protein S and AT III

Day	r	p
Day 1	0.190	0.409
Day 3	- 0.056	0.810
Day 7	- 0.135	0.561

* Spearman's test; ** Significant p-values < 0.05

As shown in Table 5, there was a negative correlation between protein C and AT III levels in day 3.

There was no correlation between protein S and AT III levels on day 1, 3, and 7 as shown in Table 6.

DISCUSSION

This study consisted of 21 patients, 12 men (57.1%) and 9 women (42.9%), who were diagnosed with acute ischemic stroke. Haapaniemi et al. reported that more men (72.7%) than women (27.3%) experienced mild and moderate stroke.[7] Sofyan et al. stated that the incidence of stroke was greater in men (52.0%) than women (48.0%).[9] This could happen because men are more likely to be at risk of stroke as the incidence of stroke in women increases in postmenopausal age. Before menopause, women are protected by the estrogen hormone, which increases high-density lipoproteins (HDL) that play an important role in preventing the process of atherosclerosis.[10]

In this study, the average age of patients with acute ischemic stroke was 56.9 ± 14.04 years. Hapaaniemi et al. reported that the average age of patients with acute ischemic stroke was 60.2 ± 11.4 years.[7] Abdullah et al. showed that the average age of patients with acute ischemic stroke was 64.5 ± 15.8 years and reported that deaths caused by stroke increased along with age.[11] The frequency of stroke increases along with age is due to the aging process, where all organs of the body, including blood vessels in the brain, experience deterioration in function. Blood vessels become inelastic, especially in the endothelial part, which thickens in the intima part, resulting in the narrowing of the blood vessel lumen, leading to decreased cerebral blood flow.[12]

The mean systolic blood pressure in the subjects was 150.48 ± 25.98 mmHg, and the diastolic blood pressure was 87.14 ± 11.02 mmHg. Hypertension is the main risk factor for ischemic and hemorrhagic strokes. In this study, the highest history of disease accompanying ischemic stroke was hypertension (47.6%). According to Juan et al., hypertension increased the risk of stroke twice than normal people.[13] Similarly, Sorganvi et al. reported that hypertension increased the risk of stroke 3.8 times than normal people.[14] Hypertension is a potential risk factor for stroke because it can cause the rupturing of cerebral blood vessels or cause narrowing of cerebral blood vessels. The rupture of cerebral blood vessels results in cerebral hemorrhage, while the narrowing of the brain blood vessels interferes with blood flow to the brain, causing the death of brain cells.[15]

In this study, we found no significant difference in the level of protein C between day 1, 3, and 7. Haapaniemi et al. examined protein C on

admission, one week, one month and three months and found that protein C increased significantly at admission but was low at all points after stroke.[7]

We found no significant difference in the level of protein S between day 1, 3, and 7. Haapaniemi et al. reported that protein S was normal at admission but significantly decreased later.[7] There was no significant difference in the level of AT III between day 1, 3, and 7 in this study. Haapaniemi et al. found that AT III levels were low at all time points.[7] There was a positive correlation in protein C, protein S, and AT III levels between day 1, 3, and 7. This finding indicates that in acute ischemic stroke, protein C, protein S, and AT III levels decrease on day 1, 3, and 7.

According to the theory of blood clotting, autocatalytic processes in small amounts of enzyme formed in each reaction will cause large amounts of enzymes in subsequent reactions. AT III plays a vital role in the control mechanism. It inhibits thrombin activity, F. XIIa, F. XIa, F. Xa, F. IXa, F. VIIa, plasmin and kalikrein. Protein C activates F. Va and F. VIIIa. Protein C is circulated in an inactive form and is activated by thrombin in the presence of thrombomodulin cofactors released by endothelial cells. The active protein C break F. Va and F.VII, and it becomes the inactive form in the presence of protein S cofactor.[16]

There was no correlation between the levels of protein C and protein S, protein C and AT III, and protein S and AT III. This can be caused by several factors, such as the shorter duration of sampling and the unavailability of preliminary data on the results of protein C, protein S and AT III examination. Reduced protein C levels were affected by deficiencies of Activated Protein C (APC) complex, which resulted in F. Xa and F. IXa activation and hypercoagulation. The prevalence of heterozygous protein C deficiency is 1 in 200 – 300.[17] Likewise, homozygous protein S deficiency disorders are usually present in neonates.[18]

AT III deficiency is rarely associated with stroke. During focal cerebral ischemia, changes in the hemostatic system, platelet activation, inhibitor activators, and plasminogen activators occur, especially in the acute phase when thrombin activity increases and fibrinolytic activity is depressed. The conversion of prothrombin to active thrombin is a key event in the coagulation cascade. Thrombin is a serine protease that is an important component in the coagulation cascade. Direct infusion of large doses of thrombin into the animal brain causes infiltration of inflammatory cells, the proliferation of mesenchymal cells, the formation of scar tissue, brain edema, and seizures. Thus, early edema formation involves activation of the production of the coagulation cascade and thrombin. AT III may have other functions besides its role in inhibiting clotting. AT III suppresses leukocyte infiltration and subsequent tissue damage in endotoxin vascular injury or ischemic-reperfusion injury. The recent development of immunochemistry tests has enabled the detection of breakdown product from fibrin formation to fibrinolysis. Among other things, the complex Thrombin – Antithrombin III (TAT) reflects the activation of thrombin and fibrin formation, while D-dimer is a marker of plasmin and fibrinolysis activity. Thrombin works on a variety of physiological substrates (fibrinogen, Protein C, platelets, etc.) and is inhibited by AT III, producing inactive proteinase/inhibitor complexes (complex thrombin-antithrombin III (TAT)).[19]

The pathological changing process of protein C to APC is catalyzed by the Thrombomodulin (TM) complex, so the APC-TM complex is the best parameter to assess protein C.[20]

Evaluating stroke patients in hypercoagulable conditions depends on a positive test result. A positive result will change the intended therapy. Hypercoagulability studies are carried out when one or more of the following criteria are met: (1) several risk factors for arteriosclerosis, (2) recurrent thrombosis, (3) strong family history of thrombosis, and (4) the onset of thrombosis at a young age. In this situation, someone might consider getting a test for all hypercoagulation conditions. Conditions that must be immediately tested for protein S, protein C, and AT III were veins or arterial thrombosis occurred in patients under 45 years, recurrent thrombosis without precipitating factors, thrombosis in unusual locations, positive family history of thrombosis, thrombosis during pregnancy, skin necrosis caused by warfarin (protein S, protein C) or resistance to heparin (AT III). Additionally, these tests must be done within two months after stroke and at least two weeks after warfarin-free. The tests must be repeated for confirmation, and family members must also be tested.

CONCLUSION

In this study, there was no significant difference in the levels of protein C, protein S and AT III between day 1, 3, and 7. There was a positive correlation in the levels of protein C, protein S, and AT III between day 1, 3, and 7. There was no correlation between the levels of protein C and protein S, protein C and AT III, and protein S and AT III. Further research with a longer time spans are needed. It is hoped that this study can be useful for the initial assessment of the homeostasis state in acute ischemic stroke patients.

ETHICAL CLEARANCE

This study was approved by the Ethics Committee of the Faculty of Medicine, University of North Sumatra, Medan with a registration number: 647/DEP/KEPK FK USU-RSUP HAM/2017.

CONFLICT OF INTEREST

The authors declare that they have no competing interest regarding the manuscript.

FUNDINGS

The authors were responsible for the study funding from PT. Sejahtera Anugrah Medika.

AUTHOR'S CONTRIBUTION

Adi and Yuneldi were responsible for data analysis and conceptual framework. Jenie was responsible for gathering the samples, data analysis, and statistical analysis.

REFERENCES:

1. Shaikh MK, Makhija P, Baloch ZAQ, Mughal MF, Devrajani BR, Shaikh S, Das T. C-Reactive Protein in Patients with Ischemic Stroke. *World Appl Sci J*. 2011; 15(9): 1220 – 4.
2. Feigin VL, Norrving B, Mensah GA. Global Burden of Stroke. *Circ Res*. 2017; 120(3): 439 – 448. DOI: 10.1161/CIRCRESAHA.116.308413.
3. Kementerian Kesehatan RI. Badan Penelitian dan Pengembangan Kesehatan. Riset Kesehatan Dasar. Riskesdas 2013. Jakarta: Kementerian Kesehatan RI; 2013.
4. Assogba K, Belo M, Apetse KM, Sonhaye L, Balogou KAA. Protein S Deficiency and Ischemic Stroke. *Global J Med Res: A Neur Nerv Sys*. 2014; 14(2): 11-2.
5. Mayer SA, Sacco RL, Hurlet-Jensen Ann, Shi T, Mohr JP. Free protein S deficiency in acute ischemic stroke. A case-control study. *Stroke*. 1993; 24(2): 224 – 7.
6. Hooda A, Khandelwal PD, Saxena P. Protein S deficiency: Recurrent ischemic stroke in young. *Ann Indian Acad Neurol*. 2009; 12(3): 183 – 4. DOI: 10.4103/0972-2327.56319.
7. Haapaniemi E, Tatlisumak T, Soinne L, Syrjälä M, Kaste M. Natural anticoagulants (antithrombin III, protein C, and protein S) in patients with mild to moderate ischaemic stroke. *Acta Neurol Scand*. 2002; 105(2): 107-114.
8. Chen WH, Lan MY, Chang YY, Chen SS, Liu JS. The prevalence of protein C, protein S, and antithrombin III deficiency in non-APS/SLE Chinese adults with noncardiac cerebral ischemia. *Clin Appl Thromb Hemost*. 2003; 9(2): 155-162.
9. Sofyan AM, Sihombing IY, Hamra Y. Hubungan Umur, Jenis Kelamin, dan Hipertensi dengan Kejadian Stroke. *Medula J*. 2013; 1(1); 24-30.
10. Price S, Wilson L. Patofisiologi : Konsep Klinis Proses - Proses Penyakit. Jakarta: EGC; 2006.
11. Abdullah A, Ssefer V, Ertugrul U, Osman E, Esref A, Ugur CM et al. Evaluation of serum oxidant/antioxidant balance in patients with acute stroke. *J Pak Med Assoc*. 2013; 63(5): 590 – 593.
12. Misbach J. Jannis J. Diagnosis Stroke. Dalam: Soertidewi L & Jannis J, Stroke, Aspek Diagnostik, Patofisologi, Manajemen. Jakarta: Badan Penerbit FK UI; 2011: 57-84.
13. De Jesus Llibre J, Valhuerdi A, Fernandez O, Llibre JC, Porto R, Lopez AM et al. Prevalence of stroke and associated risk factors in older adults in Havana City and Matanzas Provinces, Cuba (10/66 population-based study). *MEDICC Rev*. 2010; 12(3): 20 – 26.
14. Sorganvi V, Kulkarni M, Kadeli D. Atharga S. Risk Factors for Stroke: A Case Control Study. *Int J Curr Res Rev*. 2014; 6(3): 46 – 52.
15. Kulshreshtha A, Anderson LM, Goyal A, Keenan NL. Stroke in South Asia: a systematic review of epidemiologic literature from 1980 to 2010. *Neuroepidemiology*. 2012; 38(3): 123 – 9. DOI: 10.1159/000336230.
16. Farida Oesman & Rahajuningsih D Setiabudy. Fisiologi Hemostasis dan Fibrinolisis. Hemostasis dan Trombosis. Jakarta: FK UI; 2009: 1-15.
17. Tiong IY, Alkotab ML, Ghaffari S. Protein C deficiency manifesting as an acute myocardial infarction and ischaemic stroke. *Heart*. 2003; 89(2): E7.
18. Bose K, Saha S, Das KK, Kundu C. A rare cause of acute ischaemic stroke due to combined deficiency of protein C and protein S in an infant. Sri Lanka J Child Health. 2017; 46(2): 172 – 174. DOI: 10.4038/sljch.v46i2.8276.
19. Piazza O, Scarpati G, Cotena S, Lonardo M, Tufano R. Thrombin antithrombin complex and IL-18 serum levels in stroke patients. *Neurol Int*. 2010; 2(1): e1. DOI: 10.4081/ni.2010.e1.
20. Castellino FJ, Ploplis VA. The protein C pathway and pathologic processes. *J Thromb Haemost*. 2009; 7 Suppl 1: 140 – 5. DOI: 10.1111/j.1538-7836.2009.03410.x.
21. Moster ML. Coagulopathies and arterial stroke. *J Neuroopthalmol*. 2003; 23(1): 63 – 71.