The Role Of Protective Heat Shock Protein 70 And Proinflammatory Heat Shock Protein 60 Toward The Functional Status Of Acute Thrombotic Ischemic Stroke

Bertha Jean Que, Amiruddin Aliah, Irawan Yusuf, Cahyono Kaelan

Abstract: Clinical experience suggests that the functional status of stroke patients is not directly proportional to the number of risk factors, this means that there are other factors that influence the status of functional role. The aim of this study is to explain the changes in levels of HSP70 and HSP60 associated with changes the functional status of stroke which measured with National Institutes of Health Stroke Scale (NIHSS) in acute thrombotic ischemic stroke. This research is quantitative research is an observational analytic, with a longitudinal observational design (prospective cohort study) and case control. Data was collected by *consecutive sampling.* Examination of serum levels of HSP 70, HSP60 and assessment of NIHSS done in three times at the same time they are the first day (≤ 48 hours), the third day (72 hours) and fifth day (120 hours). There is a significant difference (P <0.05) levels of HSP 60 and HSP 70 between patients with acute ischemic stroke (cases) with normal people (control). Change dynamic level of HSP70, HSP60 and NIHSS, according time of examination there is a significant difference. The first day of HSP 70 levels, the third and fifth, shaped the decline curve according to the NIHSS improvement, while the levels of HSP60 formed a pattern opposite to the NIHSS. Curve levels of HSP70 and HSP60 levels to get to the point value of HSP 60 and HSP70 normal (control). In general, there was no effect of risk factors on extensive infarction, NIHSS, HSP70 and HSP60, except the variable age to HSP70, which in the elderly (70-75 years) levels of HSP70 is higher than other age groups. Changes in levels of HSP70 and HSP60 follow the pattern of change in NIHSS towards improvement. Therefore HSP70 and HSP60 can serve as a prediction for degree of functional the acute thrombotic ischemic stroke. Risk factors are the cause of stroke but do not affect the NIHSS. Age affects levels of HSP 70. In general, HSP60 and HSP70 can be used as a diagnostic and prognostic tool in ischemic thrombotic stroke, but further research is needed to determine the cut-off.

Index Terms: Infarction Thrombotic Stroke, HSP60, HSP70, Risk Factors, NIHSS.

1 INTRODUCTION

Stroke is a disease with high mortality and is the leading cause of disability in adults. In Indonesia, stroke shows the highest mortality rate of 15.4% (Riskesda, 2007)Stroke ranks third cause of death after heart disease and cancer, and is the leading cause of disability in adults. In the United States, an average of 600,000 new patients every year, nearly 30% of death, 20-30% by weight paralyzed and handicapped permanen (Mohrl, 2011). According to the International Epidemiological Studies (World Development Report) there are 4.7 million people die each year because stroke. Stroke is a growing problem in developing countries, 87% of deaths caused by stroke, occurs in many countries with rate income from lower middle (Tuomilehto, 2010). Number of stroke patients in RSUD dr. M. Haulussy Ambon, particularly in the neurological wards (research sites), the number of stroke patients in 2010 were 298 people, that is 223 people (77.16%) of them with ischemic stroke and who died 31 people (13.90%). In 2011 the 248 stroke patients, 178 (71.77%) among patients with ischemic stroke, who died 27 (15.17%). In 2012, 185 people with stroke, in which 125 people (67.57%) with ischemic stroke and 43 people who died (34.40%). From the above data, it appears that in 2012 the mortality rate is high at 34.40%.Mechanisms of brain damage after a stroke is quite complex, making experts still continue to develop theories concerning ischemic brain injury, ie, energy failure, excitotoxicity, accumulation of Reactive Oxygen Species (ROS), programmed cell death and inflammatory processes (Culmsee, 2007). HSP70 protects the brain from a variety of influences including stroke. Although the mechanism is widely known by the chaperone function (protective), HSP70 can also directly inhibit cell death pathways such as apoptosis, necrosis, onkosis and also modulate the response inflamatori (Giffard, 2008). While HSP60 is an important component in maintaining function of mitochondria. In the other, HSP60 is a stressor, suggesting that this protein may also participate in the function instead as housekeeping5,6. HSP60 was also shown to have been found in the circulation of normal people or people with pathological disorders such as aterosklerosis (Tousi, 2007; Stetler, 2010). Stroke is one of the neurological syndromes which is the greatest threat to humans that can lead to disability and death. Epidemiological study of stroke; the study of frequency, distribution and determinants of disease suggests that the study of the various issues related to stroke is still a lot that needs to be studied and researched further. For example, the relative risks of the risk factors are still many who have not been clear. The effect of various pathological processes, multi-factorial, location, type of stroke is also still requires further analysis (Habich, 2007). Based on clinic experience, some of acute thrombotic ischemic stroke patients experience mild paralysis despite of the clinic and laboratory examinations settled more than one risk factor, on

- *Bertha Jean Que, Department of Neurology RSUD dr. M. Haulussy, Jl. dr., Kayadoe, Ambon, Indonesia, PH-+6281343320789. E-mail: quebertha@yahoo.com*
- *Amiruddin Aliah, Department of Neurology Hasanuddin University, Jl. Perintis Kemerdekaan KM. 10 Makassar, Indonesia.*
- *Irawan Yusuf, School of Medicine Hasanuddin University, Jl. Perintis Kemerdekaan KM. 10 Makassar, Indonesia.*
- *Cahyono Kaelan, Department of Neurology Hasanuddin University, Jl. Perintis Kemerdekaan KM. 10 Makassar, Indonesia.*

the other hand there paralyzed stroke patients with more severe risk factors settled relatively fewer. So, in addition to risk factors as the principal cause of stroke, there may be other factors that contribute to severe neurological and clinical severity ischemic stroke patient (Ilyas, 2009). The prevalence, incidence, mortality and morbidity rate of ischemia stroke patients is still high (Misbach, 2011). Prevent the loss of quality of life in post-stroke patients and families also became one of the main objectives in the treatment of stroke (Ilyas, 2009). Hence the need for indicators that can be used to predict the the degree of functional stroke so that patients and families can be prepared from the beginning (Handayani, 2009). By refer to the references statement and some of the statements above, this research is intended to examine more deeply about the protective role of Heat Shock Protein 70 and the effect of pro-inflammatory Heat Shock Protein 60 against Functional Status of Acute Thrombotic Ischemic Stroke, to assess stroke risk factors (Hypertension, Diabetes Mellitus, Dyslipidemia, and smokers) to the area of infarction, levels of HSP 60, levels of HSP 70, and NIHSS.

Research Design

The type of research was quantitative research is an observational analytic, with a longitudinal observational design (prospective cohort study) and control case. Sample cases (stroke) were patients who experienced thrombotic infarction stroke first, then come to the hospital ≤ 48 hours after the attack and proved by CT scan of the head, aged 40-75 years old and were willing to follow the study (informed consent), the exclusion criteria were patients with heart problems or have had TIA (Transient Ischemic Attack). Control samples (non-stroke) were aged 40-75 years, willing to follow the study (informed consent) and exclusion criteria have never experienced a stroke or a seizure disorder. Numbers of sample with stroke (cases) were 37 people and the samples of non-stroke (control) were 25 people. Blood sampling for thrombotic ischemic stroke patient groups (case) were three (3) times, i.e ≤ 48 hours, 72 hours and 120 hours, and for the non-stroke group (control) was 1 (one) time.

Location of Research

This research was done in neurologic ward in RSUD dr. M Haulussy Ambon, because there is amount of stroke patients.

Population and Sample

Research population is all stroke patients in RSUD dr. M. Haulussy Ambon, while research sample is acute trombotic ischemic stroke in neurologic ward, and non stroke sample is normal people. Sample was collected by consecutive sampling.

Data Analysis

Processing and data analysis was done with the help of SPSS software version 17, with a 95% confidence level (α = 0.05) with the following stages: 1). Descriptive statistics, 2). Correlation test with Pearson correlation koofisen, 3). The mean difference test, with the method: Independent Sample T-test, Paired-Sample T test, One Way ANOVA test, and regression.

Research Results

Table 1. shows significant differences (p <0.05), HSP60 levels and HSP 70 levels according time of examination (≤ 48 hours, 72 hours, 120 hours) between patients of acute ischemic stroke (cases) with normal (control). From Table 2. have shown the levels of HSP70 (≤ 48 hours, 72 hours, 120 hours) at an old age (70-75 years) is higher than in the younger age group (40-70 years). From Table 3. We could see that patients with one (1) risk factor is significantly different from patients with 2 (two) risk factors, but not significantly different for patients with 3 (three) or more risk factors for widespread suffering infarction. Referring to Figure 1, can be explained that the test is based on sampling time (day 1, day 2, and day 3) movement pattern of decreased levels of HSP70 formed according NIHSS (status of functional stroke), while the levels of HSP 60 form the decrease pattern refers to the NIHSS. But the direction of movement of HSP 60 and HSP 70 stroke (cases) to the normal value of HSP 60 and HSP 70 (control) in accordance with the degree of functional improvement of stroke test sample. Referring to Figure 2. (a) and (b) so, the associated characteristics of HSP60 levels with NIHSS and HSP70 levels with the NIHSS. Association of HSP60 levels with NIHSS can be explained that when the value of HSP60 levels is 1.222 so, the score of NIHSS is 3,541, so it is a sequential value the levels of HSP60 is 1138, NIHSS is 4,946 and HSP60 value is 1.109, NIHSS is 5,108; then it shows a pattern the downward movement of the association, which in the beginning when high NIHSS score, the lower the value of HSP 60 levels (inversely). It means getting better degree of functional decline in patients characterized by NIHSS score, the higher the value of HSP 60 levels. Association of HSP70 levels with NIHSS can be explained that when the value of the HSP70 by 0.210 so, the value of NIHSS is 5,108, so it is a sequential value HSP70 levels by 0189, NIHSS is 4,946 and the value of HSP70 levels by 0173, NIHSS is 3,541. It shows the pattern of movement between the levels of HSP 70 association with NIHSS is directly proportional, so that when the value of NIHSS moving up the value of the HSP 70 levels will move up, and vice versa. It means the possibility of a protective role of HSP 70 levels moves so well that NIHSS all the samples of the research improved; marked by the decreasing value of NIHSS (zero is the smallest value of NIHSS, meaning the patient is normal). Figure 3. shows graphic tendency of NIHSS and the ratio of HSP70/HSP60 toward the sampling time of stroke with a score mean (NIHSS and ratio of HSP70/HSP 60). NIHSS score looks likely to decline while the value of the ratio HSP 70/HSP 60 also form a pattern that tends to decline following stroke time of blood sampling. This mean that, when the NIHSS decreased, which gives an indication of the structural refinement of stroke, so, the ratio of HSP 70/HSP 60 that is an anti-inflammatory to proinflammatory has also tends to decreased toward point of normal ratio (0.098-control).

Discussion

The results of the analysis shows significant difference (p <0.05), sample stroke of HSP60 and HSP70 and non-stroke, mean levels of HSP60 and HSP70 levels change significantly when a person suffered a stroke in this case the acute thrombotic ischemic stroke. Since the beginning HSP was found as the protein to be triggered by stress, HSP70 will increase followed ischemic-reperfusion injury in some place in the brain. On the other hand, HSP60 is an important component in maintaining the function of mitochondria. In addition to the constitutive function of chaperon mitochondria,

HSP60 is a stressor, this is suggesting that this protein may also participate in the function instead as housekeeping. HSP60 is also shown to have been found in circulation of normal people or people with pathological disorders such as atherosclerosis (Tousi, 2007; Kimura, 2012). By the time the first day of the test sample, third, and fifth, the movement of HSP70 levels form a pattern decreases NIHSS (degree of functional stroke), while the levels of HSP 60 form a pattern opposite to the NIHSS. But the direction of movement of HSP60 and HSP70 stroke (cases) to the value of HSP60 and HSP70 normal (control) in accordance with the degree of functional improvement of stroke test sample. HSP70 response (anti-inflammatory) large above normal values, at the beginning of thrombotic ischemic stroke attack, indicating the condition of HSP70 beyond optimal conditions (eustress) due to excessive stress, whereas HSP60 (proinflammatory) under the normal value indicates aggressiveness than working conditions HSP70 as a proinflammatory excessive, HSP60 also shows conditions that are beyond the optimal conditions (eustress) due to the decrease in the stress experienced by the cell HSP60, but in a state of distress (Chrousos, 2009).
Risk factors suffered by a patient more than one, does not guarantee it will lead to a more comprehensive infarction patients who have a risk factor, in other words, that the area of infarction is not directly proportional to the number of risk factors. Similarly, NIHSS score, levels of HSP 70 and HSP 60 levels, which does not have a significant mean difference, meaning extensive infarction does not have any significant impact on the value of NIHSS, levels of HSP 70, and HSP 60 levels. The existence of significant differences between the age groups 70-75 years age group to another at variable levels of HSP 70 (anty-inflammatory), showed that in the elderly will increased the levels of oxidative stress and the accumulation of protein denaturation than at a young age, so to recover, level of HSP70 should be increased (Pardue, 2007).

Conclusion

1). Levels of HSP60 and levels of HSP70 stroke sample (cases) different with non-stroke samples (control). 2). Based on the test sampling time (day 1, day 3, and day 5), the movement of levels of HSP70 formed decreased pattern according to the status of functional stroke(NIHSS), while the levels of HSP60 formed a pattern opposite to the NIHSS. But the direction of movement of HSP60 and HSP70 stroke (cases) to the normal value of HSP60 and HSP70 (control) in accordance with the degree of functional improvement of stroke test sample. 3). Extensive infarction is not directly proportional to the number of risk factors that affects the patient, and also did not provide a significant impact on the value of NIHSS, levels of HSP70 and HSP60 levels. 4). In the elderly (70-75 years) HSP 70 is higher in the elderly due to increased levels of oxidative stress and the accumulation of protein denaturation than young age. In general, HSP60 and HSP70 can be used as a diagnostic and prognostic tool in ischemic thrombotic stroke, but further research is needed with variant outcome patients and to determine the cut-off.

Bibliography

[1] Mohr, J.P., Wolf, P.A., Grotta, J.C., et al, 2011. STROK. Pathophysiology, Diagnosis, and Management. Fifth edition. Elsevier Saunder.

[2] Tuomilehto, J., Mahonen, M., Sarti, C. 2010. Basic Epidemiology of stroke and Risk Assessment, in Text book of Stroke Medicine. Cambridge University Press 2010.

[3] Culmsee, T., Krieglstein, J. 2007. Ischemic Brain Damage After Stroke: New Insights into Efficient Therapeutic Strategies. International Symposium on Neurodegeneration and Neuroprotection. EMBO reports (2007) 8, 129-133.

[4] Hossmann, K.A., Heis, W.D. 2010. Neuropathology and pathophysiology of stroke, in Text book of Stroke Medicine. Cambridge University Press 2010.

[5] Giffard, R.G., Han, R.Q., Emery, J.F. 2008. Regulation of Apoptotic and Inflammatory Cell Signaling in Cerebral Ischaemia-The complex Roles of Heat Shock Protein 70. *Anesthesiology*, 2008 August, 109(2):339-348.

[6] Stetler, R.A., Gan, Y., et al. 2010. Heat Shock Protein: Cellular and Molecular Mechanisms in the Central Nervous System. *Progress in Neurobiology* 92 (2010) 184-211.

[7] Tousi, A.S.,Steptoe A., et al. 2007. Plasma Heat Shock Protein 60 and Cardiovascular Disease Risk: the role of psycosocial, genetic, and Biological Factors. *Cell Stress & Chaperones* (2007) 12 (4) 384-392

[8] Habich, C., Burkart, V. 2007. Interaction of Heat Shock Protein 60 with Innate Immune Cells, in Heat Shock Protein volume 1. Published by Springer.

[9] Misbach, J. 2011. STROKE. Diagnosis, Pathophysiology, Management. Group of Stroke Study. PERDOSSI.

[10] Ilyas, M., et al. 2009. The Role of Computed Tomography Scan (CT Scan) in Predicting Outcome of Patients with Acute Ischemic Stroke. Indonesian Journal of Medical Science. Volume 1 No. 6 October 2009 p. 309-321.

[11] Handayani, D.Y.,Dewi, D.E. 2009. Quality of life in Post-Stroke Patients and Families. Psycho Idea, Tahun 7 No 1, Februari, 2009.

[12] Kimura, A., et al. 2012. Elevated Anti Heat Shock Protein 60 Antibody titer is Related to White Matter Hyperintensities. *Journal of Stroke and Cerebrovascular Disease*, vol 21, No.4(May), 2012, pp 305-309.

[13] Chrousos, G.P. 2009. Stress and Disorders of the Stress System. *Endocrinol* 5, 374-381 (2009).

[14] Pardue, M. et al. 2007. Macrophages and the Stress Respons, in Heat Shock Proteins: Potent Mediators of Inflammation and Immunity. Vol. 1, editor Alexzander A.A. Asea.

Appendices

Table 1. *Demographic characteristics of the variable sample of stroke (cases) and non-stroke (control)*

No.	Parameter	Kasus (n=37)				Kontrol (n=25)				P
		Frek	Mean	±	SD	Frek	Mean	±	SD	
1	Umur (tahun)		56.740	±	7.450		53.291	±	8.746	0.100
2	Kelompok Umur:									
	1) 40,00-49,99	7			(18.92%)*	11			(44.00%)*	
	2) 50,00-59,99	20			(54.05%)*	8			(32.00%)*	
	3) 60,00-69,99	7			(18.92%)*	4			(16.00%)*	
	4) 70,00-75,00	3			(8.11%)*	2			(8.00%)*	
3	Jenis Kelamin									
	1) Laki-laki	9			(24.30%)*	8			(32.00%)*	
	2) Perempuan	28			(75.70%)*	17			(68.00%)*	
4	Merokok	8			(21.50%)*	4			(16.00%)*	
5	Luas Infark (cm^2)		0.0839	±	0.108					
6	Glukosa Puasa (mg/dL)		131.081	±	66.605		102.080	±	40.993	0.057
7	Cholesterol Total (mg/dL)		221.162	±	50.249		204.520	±	39.667	0.170
8	Tekanan Darah									
	1) Tekanan Darah_≤ 48 jam		2.784	±	1.109		1.000	±	0.000	0.000
	2) Tekanan Darah_72 jam		2.622	±	1.210					0.000
	3) Tekanan Darah_120 jam		2.000	±	1.080					0.000
9	NIHSS									
	1) NIHSS_≤ 48 jam		5.108	±	1.729					
	2) NIHSS_72 jam		4.946	±	2.185					
	3) NIHSS_120 jam		3.541	±	1.994					
10	HSP60 (ng/mL)									
	1) HSP60_≤ 48 jam		1.109	±	0.171		1.281	±	0.098	0.000
	2) HSP60_72 jam		1.138	±	0.175					0.000
	3) HSP60_120 jam		1.222	±	0.259					0.270
11	HSP70 (ng/mL)									
	1) HSP70_≤ 48 jam		0.210	±	0.160		0.124	±	0.019	0.010
	2) HSP70_72 jam		0.189	±	0.119					0.009
	3) HSP70_120 jam		0.173	±	0.106					0.027
12	Rasio HSP70/HSP60									
	1) ≤ 48 jam		0.194	±	0.129		0.098	±	0.019	0.000
	2) 72 jam		0.169	±	0.087					0.000
	3) 120 jam		0.144	±	0.066					0.001

Table 2. *Characteristics of HSP70 based on group age when taking the sampling of stroke (cases)*

Kelompok Umur/HSP	Mean	±	SD	Kelompok Uji				ρ
HSP70_≤ 48 jam								
40.00-49.99	0.152	±	0.047	1)	40.00-49.99	vs	50.00-59.99	0.918
50.00-59.99	0.190	±	0.073	2)	40.00-49.99	vs	60.00-69.99	0.954
60.00-69.99	0.189	±	0.060	3)	40.00-49.99	vs	70.00-75.00	0.001

Kelompok Umur/HSP	Mean	±	SD	Kelompok Uji			ρ
70.00-75.00	0.527	±	0.472	4)	50.00-59.99	vs 60.00-69.99	1.000
				5)	50.00-59.99	vs 70.00-75.00	0.001
				6)	60.00-69.99	vs 70.00-75.00	0.004
HSP70_72 jam							
40.00-49.99	0.150	±	0.044	1)	40.00-49.99	vs 50.00-59.99	0.973
50.00-59.99	0.169	±	0.049	2)	40.00-49.99	vs 60.00-69.99	0.875
60.00-69.99	0.190	±	0.056	3)	40.00-49.99	vs 70.00-75.00	0.002
70.00-75.00	0.419	±	0.355	4)	50.00-59.99	vs 60.00-69.99	0.962
				5)	50.00-59.99	vs 70.00-75.00	0.002
				6)	60.00-69.99	vs 70.00-75.00	0.011
HSP70_120 jam							
40.00-49.99	0.138	±	0.035	1)	40.00-49.99	vs 50.00-59.99	0.961
50.00-59.99	0.157	±	0.041	2)	40.00-49.99	vs 60.00-69.99	0.947
60.00-69.99	0.164	±	0.038	3)	40.00-49.99	vs 70.00-75.00	0.001
70.00-75.00	0.387	±	0.322	4)	50.00-59.99	vs 60.00-69.99	0.998
				5)	50.00-59.99	vs 70.00-75.00	0.005
				6)	60.00-69.99	vs 70.00-75.00	0.005

Table 3. *Result of Different Test Many Stroke Risk Factors (can modified) toward Extension infarction, HSP60 levels ≤ 48 hours, HSP70 levels ≤ 48 hours and NIHSS ≤ 48 hours in stroke samples (cases)*

Uraian	Kelompok Faktor Risiko (FR) Strok	Mean	±	SD	Kelompok Uji			ρ
Luas Infark	FR = 1	0.030	±	0.043	1)	FR = 1 vs	FR = 2	0.015
	FR = 2	0.146	±	0.138	2)	FR = 1 vs	FR ≥ 3	0.749
	FR ≥ 3	0.060	±	0.072	3)	FR = 2 vs	FR ≥ 3	0.076
HSP60 ≤ 48 jam	FR = 1	1.026	±	0.176	1)	FR = 1 vs	FR = 2	0.087
	FR = 2	1.171	±	0.140	2)	FR = 1 vs	FR ≥ 3	0.434
	FR ≥ 3	1.112	±	0.180	3)	FR = vs	FR ≥	0.631

Uraian	Kelompok Faktor Risiko (FR) Strok	Mean	±	SD	Kelompok Uji			ρ
					2		3	
HSP70 ≤ 48 jam	FR = 1	0.215	±	0.072	1) FR = 1	vs	FR = 2	0.990
	FR = 2	0.224	±	0.248	2) FR = 1	vs	FR ≥ 3	0.914
	FR ≥ 3	0.188	±	0.071	3) FR = 2	vs	FR ≥ 3	0.839
NIHSS ≤ 48 jam	FR = 1	4.455	±	1.695	1) FR = 1	vs	FR = 2	0.350
	FR = 2	5.429	±	1.505	2) FR = 1	vs	FR ≥ 3	0.448
	FR ≥ 3	5.333	±	1.970	3) FR = 2	vs	FR ≥ 3	0.989

Keterangan :

FR = 1 : Sebanyak 1 (satu) faktor risiko yang dapat diubah, diderita oleh sampel strok

FR = 2 : Sebanyak 2 (dua) faktor risiko yang dapat diubah, diderita oleh sampel strok

FR ≥ 3 : Sebanyak 3 (tiga) atau lebih faktor risiko yang dapat diubah, diderita oleh sampel strok

Faktor Risiko : Dislipidemia, Diabetes Melitus, Hipertensi, dan Perokok

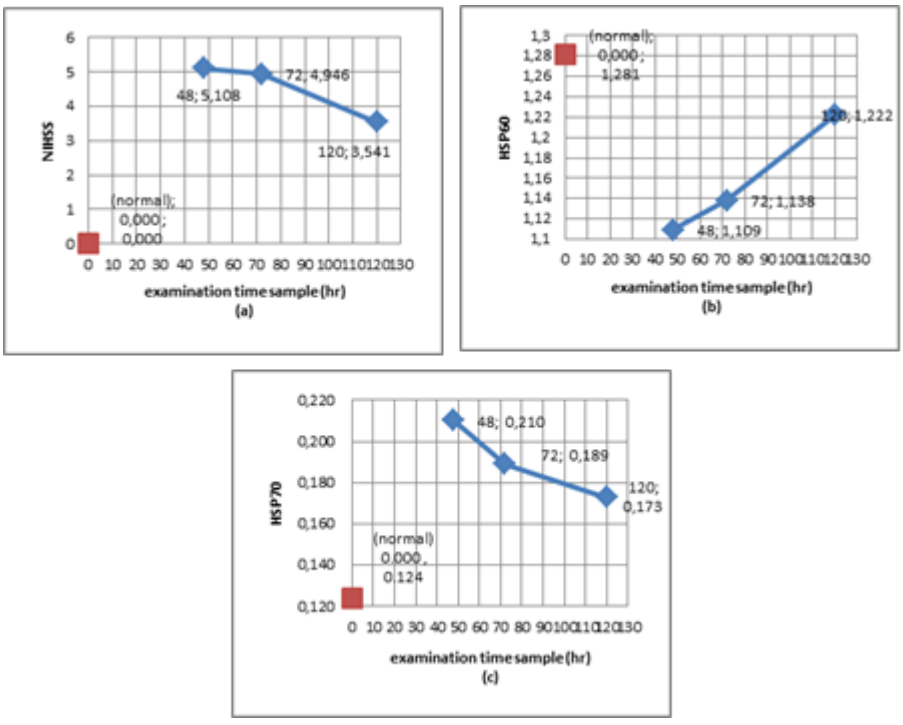

Figure 1. *Profile NIHSS, HSP 60 and HSP 70*

when taking the sampling of stroke (cases)

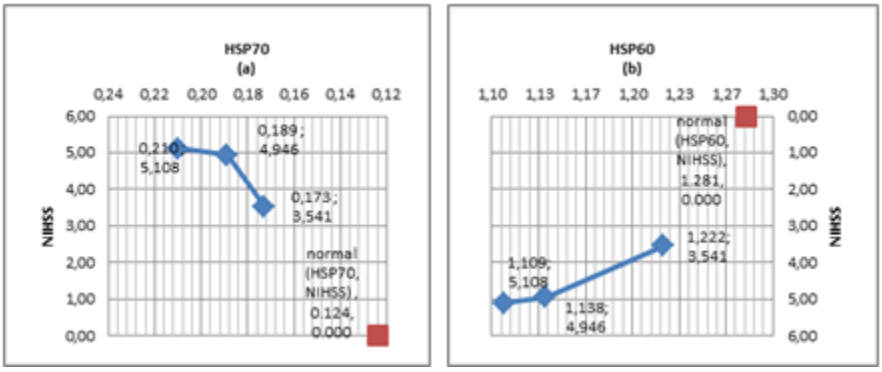

Figure 2. *Association graphs of HSP60 and HSP70 toward NIHSS*

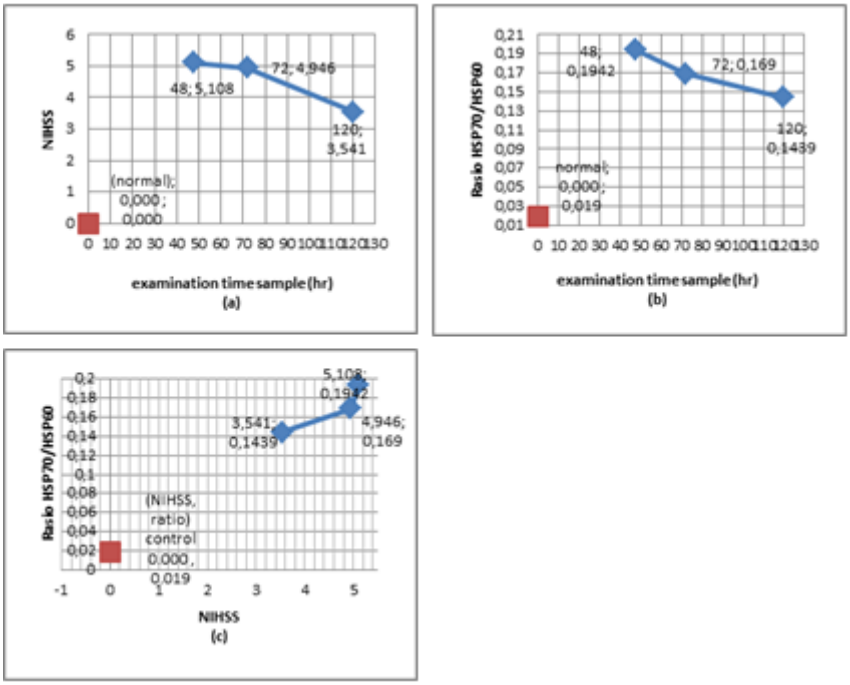

Figure 3*. Charts NIHSS Tendency and ratio of HSP70/HSP60 toward Sampling Time (a, b) and the Ratio Association of HSP70/HSP60 toward NIHSS (c)*