International Journal of Clinical and Experimental Neurology, 2017, Vol. 5, No. 1, 1-4
Available online at http://pubs.sciepub.com/ijcen/5/1/1

DOI:10.12691/ijcen-5-1-1

Correlation between Levels of Transforming Growth Factor Beta 1 (TGF-β1) serum with Clinical Outcome on Acute Anterior Circulation Ischemic Strokes

Vivien Puspitasari[1,*], Syarifuddin Wahid[2], Amiruddin Aliah[3], Budhianto Suhadi[4]

[1]Department of Neurology, Faculty of Medicine Pelita Harapan University, Tangerang, Indonesia
[2]Department of Anatomical Pathology, Faculty of Medicine Hassanuddin University, Makassar, Indonesia
[3]Department of Neurology, Faculty of Medicine Hassanuddin University, Makassar, Indonesia
[4]Department of Clinical Pathology, Faculty of Medicine Pelita Harapan University, Tangerang, Indonesia
*Corresponding author: vivien.puspitasari@uph.edu

Abstract *Transforming Growth Factor Beta* (TGFβ) was a major regulatory molecule to suppress the immune response in the inflammatory process. TGFβ was also a growth factor that affects growth, homeostasis, angiogenesis and tissue repair. In the acute phase of stroke, astrocytes were activated and the cells were able to produce anti-inflammatory cytokines such as TGFβ. The purpose of this study was to determine whether there is a correlation between serum levels of TGFβ at acute phase of ischemic stroke and patients' clinical outcomes. The study was conducted in patients with acute anterior system ischemic stroke who came to Siloam Hospital in Tangerang, Indonesia. Blood samples were taken to measure the levels of TGFβ-1serum at ≤ 72 hours and the 3rd day of onset. Clinical severity of stroke assessed using the National Institute of Health (NIH) Stroke Scale at 72 hours, 7th days and 30th days after stroke. The mean serum levels of TGFβ-1 at ≤ 72 hours in the group of subjects with mild NIH Stroke Scale degree was higher than in the group of subjects with moderate/severe NIH Stroke Scale degree ($p = 0.046$). The subjects with elevated levels of TGF-β1 in the acute phase of stroke had better clinical degrees at the 30th day after the stroke, although statistically was not significant ($p = 0.241$). Result of this study showed that TGFβ-1 may act as a neuroprotector against brain tissue damage after ischemic stroke.

Keywords: *ischemic stroke, TGF-β1, stroke outcome, National Institute of Health Stroke Scale*

Cite This Article: Vivien Puspitasari, Syarifuddin Wahid, Amiruddin Aliah, and Budhianto Suhadi, "Correlation between Levels of Transforming Growth Factor Beta 1 (TGF-β1) serum with Clinical Outcome on Acute Anterior Circulation Ischemic Strokes." *International Journal of Clinical and Experimental Neurology*, vol. 5, no. 1 (2017): 1-4. doi: 10.12691/ijcen-5-1-1.

1. Introduction

Globally, stroke is the second leading cause of death above the age of 60 years, and the fifth leading cause of death in people aged 15 to 59 years old. There were 15 million people worldwide suffer of new stroke events that caused nearly six million of deaths and another five million are left permanently disabled. [1] The incidence of ischemic stroke was more than 80% with the rest was hemorrhagic stroke. [2] In Indonesia, stroke was ranked as the first leading cause of death.[3] Stroke was not only attacking the elderly population but also young and productive ones, causing physical and psychological burden for patients, families and communities. Disability from a stroke would have an impact on declining productivity and economic capacity of community as well as the nation. [4]

The mechanism of brain injury in ischemic stroke was a very complex process and associated with an inflammatory process involving neuronal cells, glial cells, endothelial cells, extracellular matrix, and peripheral leukocytes. [5] Activated astrocytes were able to produce anti-inflammatory cytokines such as *Transforming Growth Factor Beta* (TGFβ). TGFβ was a major regulatory molecule for immune response, which may lower the immune response after injury and regulate immune cell phenotype to reduce the inflammatory phase. TGFβ was also a growth factor that affects growth, homeostasis, angiogenesis and tissue repair. [6,7] Intranasal delivery of TGF-β1 in mice after stroke reduces infarct volume and increases neurogenesis in the subventricular zone. [8] According to Dobolyi et al, TGFβ has five dominant neuroprotective effects such as anti-inflammatory, anti-apoptotic, protection against excitotoxicity, triggering angiogenesis and neuronal regeneration. [9]

TGFβ was 25000 dalton's homodimeric protein, which has 3 types of isoforms namely TGFβ1, TGFβ2, and TGFβ3. TGFβ1 was a specific isoform induced by injury. TGFβ was produced and secreted by transformed blood cells such as lymphocytes, monocytes, and platelets. In the brain, all main cells have a TGFβ receptor, but TGFβ signaling mainly increased in post-ischemic astrocytes and microglia. [10,11,12]

In this study, the author intends to explore the role of TGFβ-1 in acute ischemic stroke pathomechanism, and its role in predicting clinical outcomes.

2. Research Methods

The research was conducted during the period of August 2014 - January 2015 using a longitudinal cohort design.The rated of clinical outcome was assessed after 1 month post-stroke. The study population was all patients with anterior system ischemic stroke who comes within 72 hours after onset and treated at Siloam Hospitals Lippo Village. Patients with a history of previous stroke, thrombolysis therapy, impaired liver function, impaired renal function, Congestive Heart Failure, Acute Myocardial Infarct, or pneumonia were excluded from the study. The diagnosis of anterior system acute ischemic stroke was done by using head DW-MRI. Venous blood sampling was taken from subjects who met the inclusion criteria to measure levels of TGF-β1 serum using Human TGF-β1 Immunoassay Quantikine® (R & D Systems, Inc., USA). Blood sampling performed at ≤ 72 hours of onset and 7^{th} days post stroke and the clinical degree was assessed at ≤ 72 hours of onset, 7^{th} days and 30^{th} days post stroke using the NIH Stroke Score. This study was granted an etichal approval from Mochtar Riyadi Institute of Nanotechnology Ethic Committee No. 082 / MRIN-EC / 09/2014. Informed consents from all subjects were collected prior to data collection.

3. Result

Sixty four subjects were obtained the inclusion criteria. There were 46 men (62%) and 18 women (29%). Among these subjects, 16 persons were examined twice for their levels of TGF-β1; at 72 hours and 7^{th} days of stroke onset. The samples' age was ranged from 37 to 89 years old with mean age was58.78 ± 11.31. The majority age group was 50-59 years old(46.8%). Most of subjects (93.75%) had hypertension. Clinical classification of LACI (lacunar infarct anterior circulation) and moderate initial clinical degree (NIHSS score 4-15) with an average score of 5.2 ± 3.74 was found in 76.56% and 59.4% of all subjects respectively. Table 1 and Table 2 shows the general characteristics of subjects in this study.

The clinical degree of acute stroke patients was measured using the NIH Stroke Scale with mild clinical grade classification if NIH Stroke Scale was 0-3, moderate clinical grade if NIH Stroke Scale was 4-15, and severe clinical grade if NIH Stroke Scale was 16-42. Clinical degrees were measured within 72 hours, 7^{th} days of onset and the 30^{th} days of onset. 59.4% of total subjects came up with moderate NIH Stroke Scale (4-15). On the 30^{th} day, as many as 85.9% of subjects had mild NIH Stroke Scale (0-3).

Spearman correlations test showed that levels of TGF-β1 which taken at 72 hours of onset was not significantly associated with clinical degrees on the 30th day. However, if scores of NIH Stroke Scales was classified into mild, moderate and severe and comparative T test was done then group with mild clinical degree at 30^{th} day had a significantly higher mean levels of TGF-β1 at ≤ 72 hours (mean 53467.82 ± 10834 pg/L) compared to group with moderate to severe clinical degree (mean 44277.67 ± 4560 pg / L) with significance p = 0.046. In addition, the results showed consistently negative correlation between the levels of TGF-β1 with clinical degrees of NIH Stroke Scales. TGF-β1 levels on the 7th dayon the 7th day of onset showed a negative correlation that was almost meaningless with NIH Stroke Scale scores at the 30^{th} days. (Table 3)

Table 1. Characteristic of Subjects

Variable	N	Percentage (%)
Gender		
Man	46	71.88
Woman	18	28.13
Age's Group		
30-39	1	1.56
40-49	10	15.63
50-59	30	46.88
60-69	10	15.63
70-79	11	17.19
80-89	2	3.13
BMI* Category		
Underweight	1	1.56
Normal	36	56.25
Overweight	20	31.25
Obesity	7	10.94
Diabetes		
Yes	17	36.96
Hipertension		
Yes	60	93.75
Dyslipidemia		
Yes	53	88.33
Smoking		
Yes	17	73.44
Atrial Fibrillation		
Yes	5	7.81
Type of Anterior Circulation Stroke (Bamford)		
LACI**	49	76.56
Non-LACI***	15	23.43

* BMI: Body Mass Index
** LACI: Lacunar Circulation Infarcts
*** Non LACI: Non Lacunar Circulation Infarcts, consisting of TACI (Total Anterior Circulation Infract) and the PACI (Partial Anterior Circulation Infarct).

Table 2. Characteristic of Subjects

Variable	n	Mean±SB	Min	Max
Age (years)	64	58.78 ± 11.31	37	89
MAP* (mmHg)	64	111.26 ± 14.28	84	145.33
BMI	64	25.11 ± 3.88	17.6	35.6
TGF-β1 at 72 hours	64	53017.08 ± 10861.86	32768	93724
TGF-β1 on day-7	16	49245.06 ± 11678.93	28791	73804

* MAP: Mean Arterial Pressure.

Table 3. Correlation of 72 hours and 7th day biomarker levels to clinical degree on the 30th day

Biomarker	N	Biomarker vs Clinical degree on the 30th day	
		R	P
TGF-β1 at 72 hours vs NIHSS at 72 hours	64	-0.104	0.413
TGF-β1 at 72 hours vs NIHSS on the 7th dayon the 7th day	64	-0.110	0.388
TGF-β1 at 72 hours vs NIHSS on the 30th day	64	-0.123	0.331
TGF-β1 on the 7th dayon the 7th day vs NIHSS on the 30th day	16	-0.485	0.057

* Spearman test with significance P <0.05.

Table 4. Mean NIH Stroke Scale values in biomarker levels group based on increase / decrease criteria

Biomarker Changes	NIHSS on the 7th day			NIHSS on the 30th day		
	n	Mean±SD	p	n	Mean±SD	p
			TGFB			
Elevated	9	4.66 ± 3.9	0.8441	9	2.77 ± 3.3	0.2413
Decreased	7	4.28 ± 3.59		7	1.14 ± 1.34	

* Paired t-test was used to see the differences between mean to significance level if p- ≤ 0,05.

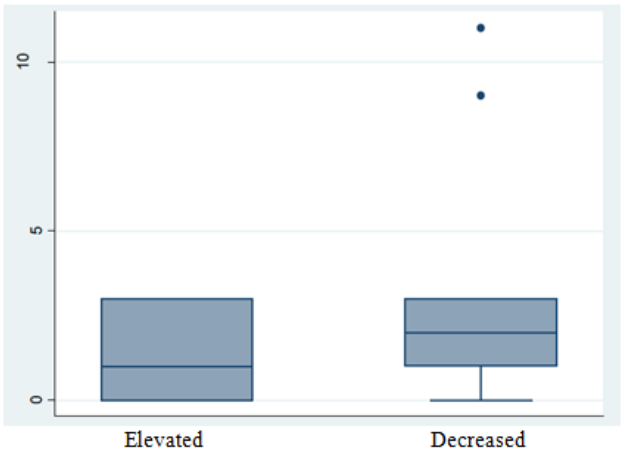

Figure 1. The mean NIH Stroke Scale in the group has increased and decreased level of TGF-β1

Changes in 72 hours biomarker levels versus 7th day biomarker levels might be categorized into "increasing" and "decreasing", then using t-test the differences between NIH Stroke Scale onon the 7th dayon the 7th day compared to 30th days would be seen. Based on these categories, the groups with increased TGF-β1 levels had milder clinical degrees than the groups with decreased TGF-β1 levels (See Table 4). After the Spearman correlation test, there was no significant correlation between changes in the TGF-β1 levels at 72 hours and 7th day observation to NIH Stroke Scale on the 30th day with r = 0.228 and p = 0.396.

4. Discussion

In this study, there were 64 subjects who met the inclusion criteria with mean age of 57.78 years. This finding was in accordance with aresearch on 2009 in Indonesia who had mean age for all cases of stroke were 58.8 years. Most risk factors were hypertension (93.75%), followed by dyslipidemia (88.33%) and smoking (73.44%). Other studies in Indonesia done by Kusuma (2009) and Yudiarto (2014) also showed that hypertension was the most risk factor, followed by diabetes and dyslipidemia. [3,13] When viewed from the type of stroke, most patients (76.56%) in this study came up with a clinical syndrome of LACI. (Table 1)

TGFβ-1 was one of the cytokines that acted as anti-inflammatory and neurotrophic factor. TGFβ-1 plays a role in neuroplasticity of the brain to repair injured brain tissue post-stroke. On 2012 and 2013, Yoo Dobolyi showed that the TGFβ-1 had a neuroprotective effect, which suppresses inflammation and reduces infarct volume in experimental animals. [9] By using t-test, this study found that mean levels of TGF-β1 on the 30th day in the group with mild clinical degree (mean 53467.82 ± 10834 pg / L) was significantly higher than in the group with the moderate-severe clinical degree (mean 44277.67 ± 4560 pg / L) with significance p = 0.046. Spearman correlation test results showed that levels of TGF-β1 at 72 hours of onset was not significantly associated with clinical degree on the 30th day, but has consistently shown a negative correlation between the levels of TGF-β1 with clinical degrees NIHSS. TGF-β1 levels taken on the 7th dayon the 7th day of onset showed a negative correlation which almost meaningless with NIHSS score on the 30th day post-stroke (r = - 0.485 and p = 0057) (See Table 3). The results of this study supported the hypothesis of the role TGF-β1 as a protective factor and improve clinical outcomes post stroke.

A study done by Doyle in 2010 which using mice as a model for acute stroke showed an increased TGFβ signaling from the first day with peak on the 7.5th day. The signal increasing was particularly visible on astrocytes. [14] However, previous studies mentioned that TGF-β1 serum levels in patients with acute stroke up to day 14 did not show a significant difference with the control. This study showed no significant difference between average levels of TGF-β1 at ≤ 72 hours and on the 7th day (p = 0384) with lower mean on the 7th day. This may be explained that the increaseing signal of TGFβ-1 in astrocytes at acute phase of stroke did not hav an impact to the increasing serum levels of TGFβ. It may take more than 7 days or more than 14 days to cause an increasing serum level of TGF-β1. So, the neuroprotective role of

TGF-β1 was likely to be occured after the acute phase of stroke.

The weakness of this study was it included subjects with extensive infarct volume. NIH Stroke Scale severity on admission may influence the clinical outcomes on the 30th day after stroke. Therefore, we need further research with large number and more homogeneous samples. The role of physical exercise and psychological factors in post-stroke patients was also needed to be considered because it might affect the clinical outcome.

5. Conclusion

This study showed the average levels of TGFβ-1 taken at ≤ 72 hours post stroke were significantly higher in the group of subjects with mild NIHSS compared to moderate/severe NIHSS group on the 30th day post-stroke ($p = 0.046$). In the group of subjects who have elevated levels of TGFβ-1 post-stroke, the mean of NIHSS was lower (mean 1.14 ± 1.34) compared to the group of subjects with decreased levels of TGFβ-1 (mean 2.77 ± 3.3). It may support the hypothesis that TGFβ acts as a neuroprotective agent against brain tissue damage after ischemic stroke.

References

[1] World Health Organization [Internet]. Global burden of stroke. [Cited 2014 Aug 10]. Available from: http://www.who.int/cardiovascular_diseases/en/cvd_atlas_15_burden stroke.pdf.

[2] Go AS, Mozaffarian D, Roger VL. (2013). Heart Disease and Stroke Statistics-2013 Update: A Report from the American Heart Association. *Circulation.* 127:e6-e245.

[3] Kusuma Y, Venketasubramanian, Kiemas LS, Misbach J. (2009). Burden of stroke in Indonesia. Int J Stroke, 4(5): 379-80.

[4] Truelsen T., Bonita R. (2003). Advances in Ischemic Stroke Epidemiology. In: Barnett HJM, Bogousslavsky, Meldnun H. *Advances in Neurology Ischemic Stroke.* Lipincott Williams and Wilkin pp. 1-11.

[5] Brea D, Sobrino T, Ramos-Cabrer P, et al. (2009). Inflammatory and neuroimmunomodulatory changes in acute cerebral ischemia. Cerebrovasc Dis , 27, pp. 48-64.

[6] Iadecola C, Anrather J. (2012). The immunology of stroke: from mechanism to translation. Nat Med, 17(7): 796-808.

[7] Ceuleman A, Zgvac T, Koojman R. (2010). The dual role of the neuroinflammatory response after ischemic stroke: modulatory effects of hypothermia. *Journal of Neuroinflammation*, 7(74): 1-18.

[8] Ma M, Ma Y, Yi X. (2008). Intranasal delivery of transforming growth factor-beta 1 in mice after stroke reduces infarct volume and increases neurogenesis in the subventriculat zone. BMC Neuroscience, 9: 117.

[9] Dobolyi A, Vincze C, Pal G, et al. (2012). The Neuroprotective Functions of Trasnforming Growth Factor Beta Proteins. Int J Mol Sci, 13, pp. 8219-8258.

[10] Boche D, Cunningham C, Gauldie J, Perry VH. (2003). Transforming Growth Factor- β1 -Mediated Neuroprotection Against Excitotoxic Injury in Vivo. J Cereb Blood Flow Metab, 23 (10): 1174-1182.

[11] Beck H, Plate KH. (2009). Angiogenesis after cerebral ischemia. Acta Neuropathol, 117: 481-496 .

[12] Hamby ME, Sofroniew MV. (2010) Reactive Astrocytes as Therapeutic Targets for CNS disorders. *Neurotherapeutics*, 7(4): 494-506.

[13] Yudiarto F, Machfoed M, Darwin A, Ong A, et all. 2014. Indonesia Stroke Registry. Neurology, 82(10) supplement S12.003.

[14] Doyle KP, Cekanaviciute E. Maner L, Buckwater MS. (2010). TGFβ signaling in the brain increases with aging and signals to astrocytes and innate immune cells in the weeks after stroke. Journal of Neuroinflammation, 7(62): 1-13.