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The Alteration of Plasma Matrix Metalloproteinase-9 Level after the Addition of Bromelin 500 mg to Standard Therapy of Acute Ischemic Stroke and Its Correlation with Outcome

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Abstract

BACKGROUND: Matrix metalloproteinase-9 (MMP9) expression due to ischemic cause spreading of brain damage. Previous studies have reported that Bromelin was beneficial as anti-inflammation and prevent brain tissue damage.

AIM: This study aimed to determine the alteration of plasma MMP9 level after addition of Bromelin 500 mg to Standard therapy and its correlation with outcome in acute ischemic stroke.

METHODS: This was a preliminary report of a prospective randomised, double-blind study with pre and post-test design, forty-six acute ischemic stroke patients were randomly allocated with Bromelin and Standard groups. Measurement of MMP9 and outcome were performed before and after 14-days treatment.

RESULT: The Bromelin group showed a significant decrement of MMP9 level, from 6.02 ± 0.32 ng/ml before treatment to 5.50 ± 0.94 ng/ml after treatment (p = 0.028). There was a negative correlation between MMP9 level and mRS (r= -0.03; p = 0.905) and a positive correlation toward BI (r = 0.039; p = 0.859), while the Standard group showed increased MMP9 level from 5.82 ± 0.71 ng/ml to 5.91 ± 0.83 ng/ml (p = 0.616) which was correlated insignificantly to outcome.

CONCLUSION: We concluded that the addition of 500 mg Bromelin to standard ischemic stroke therapy reduced MMP9 level significantly and correlated to outcome improvement. However, there is a tight statistical correlation.

Introduction

Ischemic cascade is a complex event and not yet understood entirely, but it can be concluded as bioenergy failure due to focal brain hypoperfusion followed by excitotoxicity, oxidative stress, disruption of blood-brain barrier, microvascular injury, hemostatic activation, post-ischemic inflammation results in cell death, and irreversible dysfunction of neuron cells, glial cells, and endothelial cells [1] [2] [3].

Up until now, the only FDA (The Food and Drug Administration) approved treatment for acute ischemic stroke is a thrombolytic therapy using rt-PA for reperfusion and save the brain tissue from ischemia. This treatment is effective if performed within 3 hours after stroke onset (in the USA), or in Europe within 4.5 hours. Data from the National Institute of Neurological Disorders and Stroke (NINDS) rt-PA Stroke Study revealed that rt-PA treatment given 3 hours after onset could improve clinical outcome and quality of life after 3 months of treatment. But until now, the number of patients who arrived at the hospital under 3 hour after onset is still very low. Also, there are many requirements for this therapy can be performed. In the USA, only 10 % of the patients receive this facility. In the other hand, reperfusion using rt-PA can sometimes cause hemorrhagic transformation which can be dangerous [4] [5] [6] [7].

Inflammation is an important aspect of the pathophysiology of stroke. Recent studies have proved that inflammation and immune response play an important role in a person's vulnerability of having stroke and degree of prognosis, this is due to the extent of brain tissue damage caused by them. The ischemic condition will trigger activation of microglia, acting as a sensor and is a resident immune cell in the central nervous system. But over activation of microglial can be neurotoxic by the release of Reactive Oxygen Species (ROS), Nicotinamide Dinucleotide Phosphate (NaDPH) oxidase, proinflammatory cytokine and induction also activation neurovascular proteinase such as matrix metalloproteinase (MMP), particularly MMP9. After stroke onset, MMP expression becomes uncontrolled, as proteolysis that disrupts the integrity of blood-brain barrier, causing increased permeability of blood-brain barrier leading to brain oedema, neuronal injury, apoptosis/cell death. Other than that, the MMP9 increment will trigger inflammation response through resident cells activation and greater leucocyte infiltration that can cause oedema to worsen. The extent of blood-brain barrier disruption is correlated to type, severity and duration of ischemia [2] [5] [8] [9] [10] [11] [12] [13] [14] [15] [16] [17].

Matrix Metalloproteinase-9 is the most common MMP related to stroke event, and many interventional studies have been performed with the objective to inhibit MMP9 showed better clinical outcome. In normal condition, MMP9 expression in brain tissue is minimal to undetected [5] [7] [12] [14].

Naturally, there is inhibition of MMPs by TIMPs. The work of MMPs and their inhibitors are the backgrounds of developing a new therapy for acute ischemic stroke to suppress the ischemic cascade that can spread brain damage so that morbidity and mortality from a stroke can be reduced [18].

A study by Zhao et al. 2006 (cit. Zlokovic 2006) on rats reported that rats that had increased MMP9 showed worse neurological clinical outcome with hemorrhagic complication [18]. The study by Yamashita and Abe, 2011 revealed that edaravone suppress MMP9 expression which protects brain microvascular integrity showed survival improvement and neurological clinical outcome [19]. Lo et al. (cit. Cui et al. 2012) reported that minocycline, a broad spectrum MMP inhibitor can reduce neuronal cell death after ischemia and adding time window for thrombolytic therapy [14].

Bromelin is a proteolytic enzyme (protease) fall under hydrolase, which can break peptide bond, separating proteins and amino acids. In 2006, FDA included Bromelin as a food additive and was a safe substance. Its proteolytic activity makes Bromelin widely used, for example in the food industry, as a supplement, and the substance that prevents browning in apple juice, meat tenderiser, an additive in the cosmetic industry for peeling effect, leather industry for smoothing and washing, and in textile industries [22]. It is accepted as phytotherapy agent and has been accepted as a therapeutic drug for its safety and efficacy. It was first introduced in 1957 (Kelly 1996) for acute inflammation and sports injury. This enzyme has broad-spectrum therapeutic efficacy proven in vitro and in vivo [20] [21] [23] [24] [25] [26] [27].

This enzyme has broad-spectrum therapeutic efficacy, proved in vitro and in vivo, has anti-oedema property, anti-inflammation, antithrombotic, fibrinolytic and malignancy. It's very low toxicity makes this drug save to be used in controlling chronic inflammation diseases [20] [21] [22] [23] [24] [25] [27] [28] [29] [30].

The objective of this study was to determine alteration of plasma MMP9 level in acute ischemic stroke patients after addition of Bromelin 500 mg administered twice a day to standard therapy (aspirin 300 mg once a day) for 14 days, and its correlation to stroke clinical outcome evaluated with modified Rankin Scale (mRS) and Barthel Index (BI).

Material and Methods

This was a double-blind clinical trial with pre and post-test design, performed at Adam Malik General Hospital in Medan, Indonesia, within April 2016 to April 2017, toward acute ischemic stroke patients, diagnosed by Head CT-scan. Inclusion criteria were acute ischemic stroke patients above 18 years old, the first attack and giving consent to be enrolled in this trial. Exclusion criteria were a cardioembolic stroke, brain stem lesion, not having upper gastrointestinal bleeding, and not having history on using anticoagulant drugs, antiplatelet aggregation drugs or anti-inflammation drugs. There were 46 participants who met the criteria, randomised into 2 treatment groups: Standard and Bromelin group, with 23 subjects in each group. All subjects were given acetylsalicylic acid (Aptor) 300 mg once a day as standard therapy for ischemic stroke. In Bromelin group, subjects were given capsule containing Bromelin 500 mg by a dose of 2 times daily for 14 days, while in standard group were given capsule containing dextrin 500 mg (Placebo), also twice daily for 14 days. Dextrin was chosen as placebo due to not having active substance. To meet double-blind criteria, Bromelin and placebo were packaged in the same colour and weight capsules and administered in the same manner also.

All subjects agreed to participate and signed informed consent voluntarily after receiving a detailed description of the study procedures and purposes. The study was approved by the Health Research Ethical Committee of North Sumatera/Adam Malik

General Hospital, Medan, c/o Medical School, University of Sumatera Utara.

Blood samples were collected in vacutainer tubes. Serum separation was isolated by centrifugation at 1000 g over 15 minutes. The serum samples should always be pre-diluted 1:5 (100 µl serum + 400 µl water) and stored at 2-8^{o}C until the time of analysis.

Matrix metalloproteinase-9 concentrations in plasma samples were analysed using MMP9 human ELISA Assay Kit (EA 100106-Origene) according to manufacturers' instructions and Chemwell 2910 analyser. Limit of detection of MMP9 human ELISA Assay Kit (EA 100106-Origene) is 31 pg/ml or 0.3 µg/ml.

The plasma MMP9 level and outcome measurement were performed twice, before and after treatment. The outcome was determined using modified Rankin Scale (mRS) and Barthel Index (BI). To evaluate alteration of the MMP9 level before and after treatment, paired t-test was used and to determine the correlation between plasma MMP9 level and mRS and BI score, Pearson correlation test was used. All data were expressed as mean ± S.D. P < 0.05 was considered to be significant.

Results

The total subject was 46 participants. Bromelin group was consisted of 23 subjects, with 17 male (73.9 %), while the standard group was also comprised of 23 subjects, with 14 male (60.9 %). Mean of age in the Bromelin group was 56.04 ± 10.13 year, while in standard was 60 ± 12.6 year, and there was no significant difference between group (p = 0.583). Subject's demographic characteristics are presented in Table 1.

Table 1: Demographic Characteristics of Studied Groups

Characteristics	Total	Bromelin 500 mg	Standard Drug
N (%)	46 (100%)	23 (50%)	23 (50%)
Mean Age ± SD	58.20 ± 11.48	56.04 ± 10.13	60 ± 12.60
Gender			
Male (%)	31 (67.4%)	17 (73.91%)	14 (60.87%)
Female (%)	15 (32.6%)	6 (26.09%)	9(39.13%)
Marital Status			
Married (%)	46 (100%)	23 (50%)	23 (50%)
Single (%)	0 (0%)	0 (0%)	0(%)
Ethnicity			
Bataknese	21 (45.7%)	9 (39.13%)	12 (52.17%)
Karonese	5 (10.9%)	4 (17.39%)	1 (4.35%)
Javanese	15 (32.6%)	8 (34.78%)	7 (30.43%)
Melayunese	2 (4.3%)	0 (0.00%)	2 (8.70%)
Acehnese	1 (2.2%)	1 (4.35%)	0 (0.00%)
Padangnese	2 (4.3%)	1 (4.35%)	1 (4.35%)
Hypertension			
Yes	37 (80.4%)	18 (78.26%)	19 (82.61%)
No	9 (19.6%)	5 (21.74%)	4 (17.39%)
Diabetes Mellitus			
Yes	8 (17.4%)	4 (17.39%)	4 (17.39%)
No	38 (82.6%)	19 (82.61%)	19 (82.61%)
Hypercholesterolemia			
Yes	37 (80.4%)	21 (91.30%)	16 (69.57%)
No	9 (19.6%)	2 (8.70%)	7 (30.43%)
Smoking			
Yes	19 (41.3%)	10 (43.48%)	9 (39.13%)
No	27 (58.7%)	13 (56.52%)	14 (60.87%)

N= number of patients; S.D= Standard Deviation.

There were 23 subjects on the Bromelin 500 mg group, measured their MMP9 level, mRS score and BI score, before and after treatment. Since the test of normality showed normal distribution data, paired t-test was performed to determine the difference of MMP9 level, mRS, and BI score before and after treatment, with the level of significance p < 0.05.

The result of this study on MMP9 after Bromelin 500 mg administration showed significant decrement of the MMP9 level. The mean of MMP9 before treatment was 6.02 ± 0.32 ng/ml and after treatment was 5.50 ± 0.4 ng/ml, where the decrement was statistically significant (p = 0.028) (Table 2).

Table 2: Plasma MMP9 Level (ng/ml) and Outcome

	MMP9		P	mRS		P	BI		p
Drugs	Pre	Post		Pre	Post		Pre	Post	
Bromelin 500mg	6.02± 0.32	5.50± 0.94	0.028*	3± 1.107	2± 1.340	0.604*	50± 27.83	60± 30.60	0.002*
Standard Drugs	5.82± 0.71	5.91± 0.83	0.616*	3± 0.949	3± 1.033	0.002*	60± 23.45	75± 22.55	0.001*

* = Paired T-test

The result of mRS after 500 mg Bromelin administration showed unsignificant decrement, where mRS score before treatment was 3 ± 1.107 and after treatment was 2 ± 1.34 (p = 0.604) (Table 2).

The study result on BI score after Bromelin 500 mg administration showed a significant increment, where before administration the score was 50 ± 27.83 and after administration was 60 ± 30.6 with p = 0.002 (Table 2).

The result on the MMP9 level in the standard group showed unsignificant increment. The mean of MMP9 before treatment was 5.82 ± 0.71 ng/ml and after treatment was 5.91 ± 0.83 ng/ml, this was not statistically significant (p = 0.616) (Table 2).

This study result on mRS score in standard group revealed changes, where before treatment, the mRS score was 3 ± 0.949 and after treatment was 3 ± 1.033 (p = 0.002) (Table 2).

The result of this study on BI score after standard therapy showed significant increment, where BI score before treatment was 60 ± 23.45 and after treatment was 75 ± 22.55, with p = 0.001 (Table 2).

Using Pearson correlation test between decrement of MMP9 and mRS after Bromelin 500 mg administration, there was a negative correlation but not statistically significant (r =-0.03; p = 0.905) (Table 3)

Table 3: Correlation of MMP9 Decrement and Outcome

	mRS		BI	
	r	p	r	P
MMP9 Decrement Level	-0.03	0.905**	0.039	0.859**

Pearson correlation test between decrement of MMP9 level and increment of BI score showed positive unsignificant correlation (r = 0.039; p = 0.859) (Table 3)

Discussion

Matrix metalloproteinase-9 (MMP9) is a family of proteolytic enzyme, involved in the breakdown of extracellular matrix during tissue remodelling [7]. The action of the MMPs on the basal lamina and tight junction proteins (TJPs) in endothelial cells is the final common pathway for the opening of the BBB, which allows cells to enter the central nervous system and attack invading organisms [31]. During a stroke, it attacks the extracellular matrix around the blood vessels and neurons, facilitating neural cell death. MMP-9 disrupts the blood-brain barrier in the early phase following cerebral ischemia, leading to leakage, leukocyte infiltration, brain oedema, and haemorrhage [7].

This study found that plasma MMP-9 level to be high in the two groups of patients. In Bromelin groups mean of the MMP-9 level were 6.02 ± 0.32 and the Standard group were 5.82 ± 0.71. Expression of MMPs in the adult brain is very low to undetectable [32]. This study proved that expression of MMP-9 is upregulated in the brain in response to ischemic stroke. This is by the study by Heo *et al*. 2003 (*cit.* Abdelnaseera *et al*. 2015), found that MMP -9 serum level on admission was significantly higher in stroke patients compared with the control group [33].

In this study, the addition of Bromelin 500 mg to standard therapy showed significant decrement of plasma MMP9 level (p 0.028), the increment of mean BI score (p = 0.002) and unsignificant decrement of mRS score (p = 0.604), proved that clinically there was outcome improvement. This concluded that MMP9 decrement could improve clinical outcome. Study of Lindsell *et al*. 2005 (*cit.* Abdelnaseera *et al*. 2015) showed there was an association between higher serum levels of MMP-9 and more severe stroke, supported the role of MMP-9 as an independent predictor of clinical severity of ischemic stroke in the acute stage [33]. Previous several studies reported that treatment with MMP inhibitors or MMP neutralising antibodies decreases infarct size and prevents BBB breakdown after focal ischemic stroke [31].

We concluded that the addition of 500 mg Bromelin to standard ischemic stroke therapy reduced MMP9 level significantly and correlated to outcome improvement. However, there is a tight statistical correlation.

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