Original Article

Correlation between Matrix Metalloproteinase-9 (MMP-9) Serum Levels and Alteration of NIHSS Score in Acute Ischemic Stroke

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Abstract

Objective **:** MMP-9 is a proteolytic enzyme that plays a role in stroke pathological process. Its secretion increases rapidly after ischemic stroke onset. It gives a promising biomarker of stroke prognosis.The NIHSS (National Institute of Health Stroke Scale) is a clinical scale for predicting post stroke clinical outcomes. The study aim is to determine the correlation between MMP-9 serum levels and alteration of NIHSS score in acute ischemic stroke.***Material and Methods*** **:**This was a prospective cohort study at Kariadi Hospital Semarang during October 2018 - January 2019. Subjects met the inclusion and exclusion criteria were taken for 5 cc venous blood samples ≤48 hours of stroke onset to examine the MMP-9 serum levels andmeasured alteration of NIHSS scores between the 2nd, 7thand 14th day of stroke onset.***Results*** **:** There were 51 subjects with a mean MMP-9 serum levels of 1.223,24 ng/ml (normal 169-705 ng/ml). Elevated MMP-9 serum levels were correlated with worsening clinical outcomes on 7th and 14th day of stroke onset (p=0.007 ; p=0.005). ROC curve analysis obtained MMP-9 serum levels ≤48 hours of stroke onset with a cutoff point of 899.5 ng/ml, which MMP-9 serum levels above 899.5 ng/ml as a predictor of worsening clinical outcome.***Conclusion*** **:**There was correlation between MMP-9 serum levels ≤48 hours of ischemic stroke onset with clinical outcome on 7th and 14th day of stroke onset.

Keywords: MMP-9 serum levels, alteration of NIHSS score, acute ischemic stroke

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Introduction

The acute blockage of cerebral blood flow may cause many pathological conditions including homeostatic disorders, neuron excitotoxicity, intracellular calcium build-up, peri-infarct depolarization, free radical build-up, lipid peroxidation, and disruption of protein synthesis that trigger irreversible neuronal damage. Dead neuron cells trigger the release of immune response which then stimulate the activation and infiltration of pro-inflammatory cells. The activation of these pro-inflammatory cells will produce cytotoxic substances, such as Matrix Metalloproteinases (MMPs).[1] MMPs can damage the extracellular proteins such as collagen, proteoglycans, elastin, or fibronectin.[2] Among the many MMPs, MMP-9 (Gelatinase B, 92- kDa Collagenase) is the most common in ischemic stroke cases, and its expression increases rapidly after a stroke onset.[3,4] Gelatinase is able to activate a number of pro-inflammatory agents such as chemokine, IL-Iβ (Interleukine-1β), or TNF-α (Tumor Necrosis Factor-α) resulting in damage to type IV collagen. It also can enter the endothelium through the mediation of leukocytes.[2]It is associated with the neuroinflammation process that results in blood-brain barrier damage, fluid leakage, leukocyte infiltration, cerebral edema, and increasing risk of hemorrhagic transformation.[1]MMP-9 also contributes to various complications, for example excitotoxicity, apoptosisand associated with the expansion of cerebral infarction area. Thus, it

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may cause poor clinical output of ischemic stroke patients.[1,5,6] Many studies had been conducted to find blood biomarker as a predictor of clinical outcome in acute ischemic stroke patients in terms of clinical severity.[4, 7]

NIHSS is a clinical scale used to examine stroke patients. It provides useful information in predicting clinical outcomes in post-stroke patients. It is very easy, fast to do, and can be examined by bedside.[8]

The study that determine the correlation between MMP-9 serum levels with the clinical outcome of ischemic stroke, assessed by the alteration of NIHSS score was promising.[4,9]Therefore, we intended to conduct the study.

Material And Methods

This was an observational analytic studywith cohort prospective design. The subjects of this study were 51 patients determined by consecutive sampling (non probability), with acute ischemic stroke who were admitted and hospitalized to the Neurology department ward atKariadi Hospital, Semarang, Central Java, Indonesia; during October 2018 - January 2019. The inclusion criteria were first acute ischemic stroke with an onset of ≤48 hours as evidenced by a non-contrast head CT scan and agreed to participate in the study. Exclusion criteria were patients with hemorrhagic strokes, severe systemic disease (CKD, CHF, chronic liver disease, malignancy), history of drug use that affected MMP-9 levels (tetracycline, minocycline, doxycycline, NSAIDs, statins), peripheral arterial disease, patients receiving thrombolysis therapy. The drop out criteria were patients who die before the 7th and 14th day of stroke onset.

Patients who met the inclusion and exclusion criteria received informed consent; measured NIHSS score of 2nd day of stroke onset (K_0), NIHSS score of 7th day of stroke onset (K1) and NIHSS score of 14th day of stroke onset (K_2). Thus, measuredthe alteration of NIHSS scores between 2nd with 7thday (K_{1-0}), and between 2nd with 14th day (K_{2-0}). As much as 5 cc of venous blood sampling was taken ≤48 hoursstroke onset for measured MMP-9 serum levels. Examination of MMP-9 serum levels at Prodia Clinical LaboratorySemarang, used the ELISA (Enzyme Linked Immunosorbent Assay) method with the Quantikine®ELISA Human MMP-9 reagent kit (R&D Systems, Inc., Minneapolis, USA) stain: DMP900, Lot: P176247. The calibration standard range was 0.313-20 ng/mL, with detection limits ≤0.156 ng/mL, 100x dilution factor, measurements using Microplate Reader Bio-Rad Model 680 (USA) instruments with Microplate Manager ver 5.2.1 software (Bio-Rad Laboratories Inc., CA, USA), with a normal range in healthy subjects was 169-705 ng/mL.

The data were analyzed with SPSS for Windows version 22. Hypothesis testing of MMP-9 serum levels with alterations of the NIHSS score on a numerical scale, initially the Kolmogorov Smirnov test was carried out to see the normality of the data, because the data distribution was not normal then it was followed by the Spearman Rank correlation test.The cutoff point for MMP-9 serum level ≤48 hours of stroke onset as a predictor of clinical outcome in ischemic stroke patients was measured by a Receiver Operating Characteristic (ROC) curve prediction model test.

Results

Fifty one patients as subjects study follow the procedures. The characteristicof subjects shown in table 1.

Table 1. The characteristic of subjects

Variable(n=51)	F	%	Mean ± SD	Median (min-max)
Sex				
Male	28	54.9		
Female	23	45.1		
Age				
<65 years	32	62.7		
≥65 years	19	37.3		
DM				
Yes	18	35.3		
No	33	64.7		
Hypertension				
Yes	41	80.4		
No	10	19.6		
BMI				
Non obese	37	72.5		
Obese	14	27.5		
Number and extent of infarct				
Single lacunar	4	7.8		
Multiple lacunar	40	78.4		
Single territory	7	13.7		
MMP-9 serum level ≤48 hours of stroke onset			1.223±719.72	1.035 (219 - 2928)
Normal (169-705 ng/ml)	12	23.53		
High (>705 ng/ml)	39	76.47		
NIHSS				
K_0 (2nd day onset)			7.47±3.512	7 (3 s/d 15)
K_1 (7th day onset)			6.86±3.715	6 (1 s/d 15)
K_2 (14th day onset)			5.88±3.675	4 (1 s/d 13)
Δ NIHSS				
K_{1-0}			-0.61±1.429	-1 (-3 s/d 4)
K_{2-0}			-1.59±1.824	-2 (-5 s/d 4)

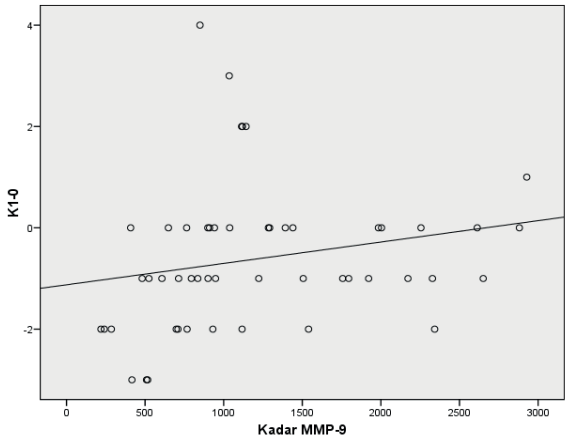
The characteristic subjects obtained that most of subjects were male (54.9%), (62.7%) were aged <65 years. There were (80.4%) suffering from hypertension, (35.3%) suffering from diabetes, (27.5%) suffering from obesity. The highest number and area of infarction were multiple lacuners (78.4%). The mean MMP-9 serum levels ≤48 hours of stroke onset was higher than the normal value, about 1,223.24±719.72 ng/ml, with a range of 219-2,928 ng/ml (median 1.035 ng/ml), and increased MMP-9 serum levels was present in majority of subjects (76.47%). The mean NIHSS score on 2nd day of stroke onset was 7.47±3.512, 7th day was 6.86±3.715, and 14th day was 5.88±3.675. The mean alterations of NIHSS score between 2nd and 7th day (K_{1-0}) was -0.61±1.43, the mean alterations of NIHSS score between 2nd and 14th day (K_{2-0}) was -1.59±1, 82.

Based on the results (table 2), the hypothesis that there is a significant correlation between MMP-9 serum levels ≤48 hours of stroke onset with alteration NIHSS score in 2nd and 7th day of stroke is proven (*p* 0.007) with a correlation coefficient 0.374.

Table 2. Statistical test of the correlation between MMP-9 serum levels and alteration of NIHSS score between 2nd and 7th day of stroke onset

Variable		n	Correlation coefficient*(r)*	p**
MMP-9 serum level	Δ NIHSS K_{1-0}	51	0.374	0.007[j]

Statistical analysis using Spearman's test, ** significant when p <0.05



MMP-9 serum levels

Figure 1. Correlation between MMP-9 serum levels ≤48 hours of stroke and alteration of NIHSS score between 2nd with 7th day of stroke onset (K_{1-0})

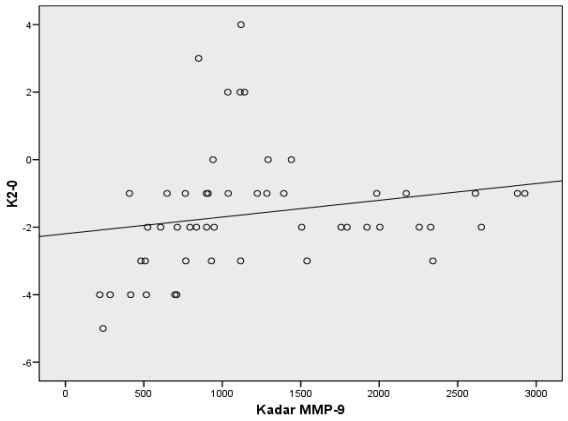
Figure 1 showed that increasing MMP-9 serum levels ≤48 hours of stroke onset there was a tendency for an increasing NIHSS score on the 7th day of stroke onset, this indicates a worsening clinical outcome.

Based on the results (table 3), the hypothesis that there is a significant correlation between MMP-9 serum levels ≤48 hours of stroke onset with alteration NIHSS score in 2nd and 14th day of stroke is proven (*p* 0.005) with a correlation coefficient 0.386.

Table 3. Statistical test of the correlation between MMP-9 serum levels and alteration of NIHSS score between 2nd with 14th day of stroke onset

Variable		n	Correlation coefficient*(r)*	p**
MMP-9 serum level	Δ NIHSS K_{2-0}	51	0,386	0.005[j]

Statistical analysis using Spearman's test, ** significant when p <0.05



MMP-9 serum levels

Figure 2. Correlation between MMP-9 serum levels ≤48 hours of stroke and alteration of NIHSS score between 2nd with 14th day of stroke onset (K_{2-0})

Figure 2 showed that increasing MMP-9 serum levels ≤48 hours of stroke onset there was a tendency for an increasing NIHSS score on the 14th day of stroke onset, this indicates a worsening clinical outcome.

Discussion

Bloodbiomarkers are beginningtoshow a role in determiningthe diagnosis, management, and prognosis ofischemic stroke patients. Althoughimagingtestplaythebiggestrole in determiningtherapy, blood biomarkers may

havean important role when imagingis not available, orwhen making predictionsaboutfuture stroke complications and recurrentstroke events. Many biomarker examinations have been investigated, andlevelsthat are higherthan normal in thepathophysiologyof stroke are consistently associated with worse outcomes.[10]

Higher MMP-9 serum levelsin the acute phase of ischemic stroke was associated with an increased risk of mortality (OR 1.29 95% CI 1.01-1.66) and major disability (OR 1.12 95% CI 1.01-1.23). Thus, MMP-9 serum levelscould be one of the important prognosis predictors in ischemic stroke.[3] In this study,76.47% had high MMP-9 serum levels≤48 hours of stroke onset. The mean MMP-9 serum level was 1.223.24 ± 719.723 ng / ml, higher than its normal value of 169-705 ng / ml. This is consistent with previous study which stated that the increasing of MMP-9 activity was seen in human brain tissue at two days after cerebral infarction.[5]

Stroke causes disruption of blood supply and oxygen in the brain, so stroke not only needs to be handled in post event, but also needs to be understood the cause of the occurrence by molecular. Inflammation is correlated with secondary injury mechanism in acute ischemic stroke.[11]Many studies conclude that MMP-9 serum levels that circulate in the acute phase of stroke were related to infarct volume and severity of stroke.[12]The results of this study were in accordance with previous studies, which reported that MMP-9 serum level ≤48 hours of stroke onset significantly correlated withthe alteration of

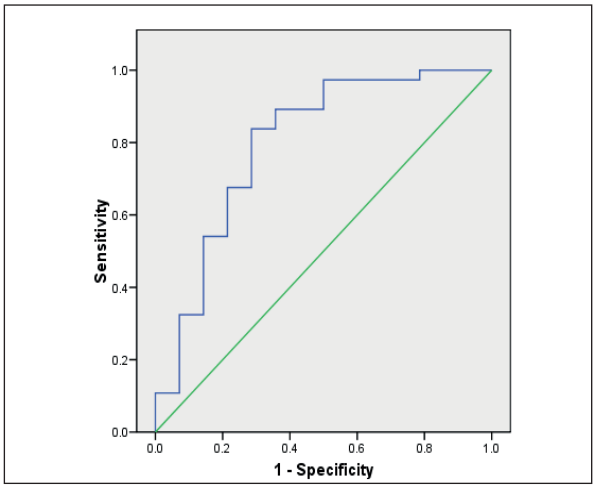


Figure 3. ROC curve analysis related to the efficacy of MMP-9 serum levels for identifying clinical outcomes in acute ischemic stroke

NIHSS score; where there was a tendency for an increase in the NIHSS score on the 7th and 14th day follow-up of stroke onset by increasing MMP-9 serum level ≤48 hours of stroke onset.

AUC	Asymp. Sig.	Sensitivity	Specificity	Cut-off point
0.797	0.001	0.730	0.714	899.5

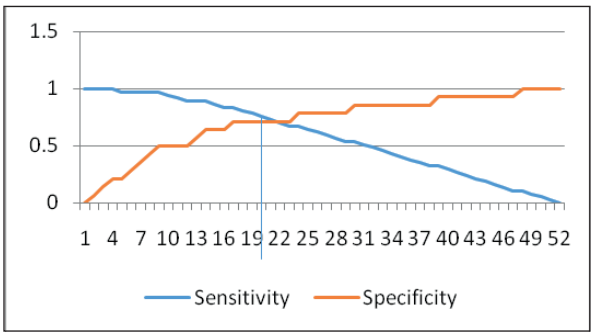


Figure 4. MMP-9 serum levels related to clinical output in acute ischemic stroke

Based on the analysis of the ROC curve, the optimal cut-off point for MMP-9 serum levels on the 21st axis is 899.5 ng / ml, which can identify the clinical outcome of acute ischemic stroke patients. MMP-9 serum level above 899.5 ng / ml is a predictor of worsening clinical outcomes, and AUC value of 79.7% with a sensitivity of 73.0% and specificity of 71.4% (95% CI 0.643 - 0.952) indicated that the results of the above prediction model was statistically quite good.

The limitations of this study was blood sampling only performed once on ≤48 hours of stroke onset and was not repeated either on the 7th or 14th day of stroke onset, so that it can be seen the difference in MMP-9 serum levels was correlated with the clinical outcome of acute ischemic stroke patients. Measurement of biomarkers as a predictor of clinical outcome in this study is only one type (MMP-9 serum levels), so it is necessary to examine another panel of potential biomarkers related to the neuroinflammatory process in acute ischemic stroke, such as MMP-2, MMP-13, TNF-α, IL-1β, etc.

Conclusion

Increasing of MMP-9 serum levels ≤48 hours of stroke onset are significantly correlated with worsening of NIHSS score between 2nd , 7th and 14thday of stroke onset in acute ischemic stroke.

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