The Correlation of Plasma Malondialdehyde Level Elevation with Infarct Volume on Brain MSCT and BI among Patients with Acute Ischemic Stroke

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***Abstract*—**Stroke is neurovascular impairment, contributing to the forth common cause of death in the world. Furthermore, stroke is the most common cause of disability. Acute ischemic stroke (AIS) were defined as broaden infarct after 48-72 hours of onset. Oxidative stress plays an important role in brain damage after stroke. During oxidative stress, free radicals were released by lipid peroxidation with malondialdehyde (MDA) as the marker. The objective of this research is to analyze the correlation of plasma malondialdehyde level elevation with infarct volume on brain MSCT and BI among patients with acute ischemic stroke. This is an observational study with cross sectional design conducted in Stroke Unit, Dr. Kariadi General Hospital Semarang. Fourty-three patients with AIS within ≤ 72 hours of onset were include in this study. The diagnosis and the infarct volume were confirmed by MSCT. MDA level was measured by using TBARS method. The clinical neurology was assesed by Bartel Index (BI). The statistical and correlation analysis were performed by *Spearman's Rho* test. There are no significant differences in plasma MDA level elevation with infarct volume size. There is significant correlation between infarct volume and BI at admission. There is no significant correlation between plasma MDA level elevation and infarct volume also BI admission. There is no significant correlation between plasma MDA level elevation and infarct volume size. There is significant correlation between infarct volume size and BI admission.

***Keywords*—**Plasma MDA level, acute ischemic stroke, infarct volume, brain MSCT.

I. Introduction

STROKE is a common cause of death and severe disability. In the United States, stroke is the third leading cause of death after cardiovascular disease and cancer. Ischemic strokes account for about 70-80 % of all stroke cases [1]. Expansion period of infarction area is relatively longer, about 48-72 hours after the onset of stroke. The presence of cytotoxic edema within 6 hours after onset is due to failure of energy-adenosine tri phosphate (ATP) in cerebral ischemia and the release of free radicals. Cytotoxic edema then continues to be vasogenic edema and hypodensity appears on the brain CT.

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Brain ischemia causes various cellular and molecular processes leading to neuronal death and permanent brain tissue damage. Brain ischemia begins with a decreased supply of oxygen and glucose and followed by a process such as an increased release of glutamate, acidosis, intracellular calcium. Inflammatory processes then occurs with an increase of cytokine release and migration of leukocytes. Formation of free radicals occurs both during cerebral ischemia and during reperfusion [2].

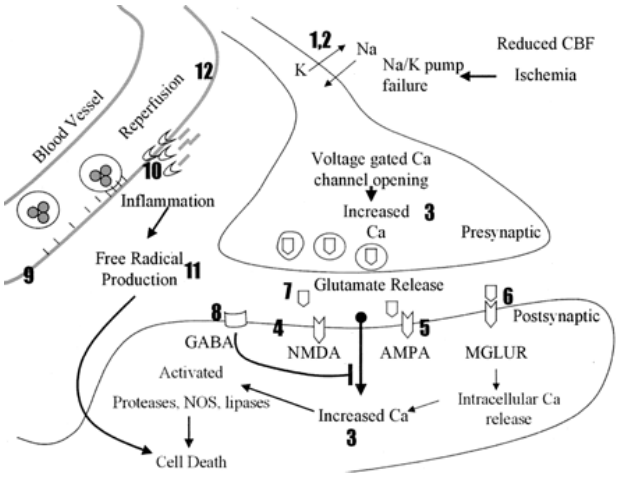

Figure 1 Cascade mechanism of acute ischemic stroke [2]

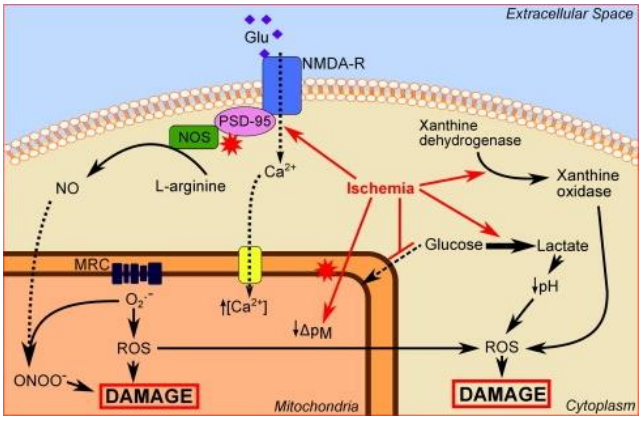

Figure 2 ROS in ischemic stroke [4]

Oxidative stress in the brain occurs when the level of polyunsaturated fatty acids (PUFAs) is nearly 50 % of the structure of the brain tissue. PUFA creates oxidative stress in the human biological system through reactive oxygen species (ROS). Many ROS were produced during acute ischemic stroke (AIS), such as free radicals, lipid peroxidation, hydrogen peroxide, malondialdehyde, and others. ROS can cause damage to cells [3]. Neuronal damage in the cytoplasm due to ischemia can lead to an increase in the conversion of xanthine dehydrogenase into xanthine oxidase which will also increase malondialdehyde. Study in mice with global ischemia shows that xanthine oxidase activity increased 5 times after 15 minutes ischemia [3],[4].

Research conducted by Dharap, et al. shows that in the condition of ischemic stroke, there was an expression of changes in noncoding RNA, whose impact is still unclear [5]. Ischemia in the cytoplasm of neurons causes anaerobic glycolysis those results in an accumulation of lactic acid which will increase intracellular acidity as well as the production of ROS. Ischemia in the cytoplasm of neurons causes activation of the receptor N-Methyl-D-aspartate (NMDA) by an increase in glutamate level. Ischemia in the cytoplasm of neurons initially causes membrane depolarization and impaired sodium pump. This will stimulate AMPA-R glutamate to open the Mg plug of NMDA that leads to an influx of Ca^{2+} which will activates adaptor protein postsynaptic density (PSD) -95 and neuronal nitric oxide synthase (nNOS) and iNOS, producing NO from L-arginine. The entry of nitric oxide and Ca^{2+} into the mitochondria via mitochondrial respiratory chain (MRC) causes an increase in Ca^{2+} and generate superoxide (O_2^-) that react with NO produces peroxynitrite radical ($ONOO^-$) which then would lead to mitochondrial damage. Mitochondrial depolarization together with increasing intracellular acidity resulted in increased level of superoxide (O_2^-) conversion to another ROS [6].

Free radicals are highly reactive and tend to react with other molecules, looking for pairs of electrons and turn into a more stable form. Free radicals stimulate the lipase enzyme which will damage the walls of the DNA and change its structure and function leading to death sel [6].

As free radicals are not long lived substances, and have a short half-lives, direct measurement of free radicals was very difficult [7],[8]. Malondialdehyde (MDA) is a marker substance of biological lipid peroxidation and oxidative stress [7]-[9]. Measurement of MDA concentration is widely used as an indicator of oxidative damage on polyunsaturated fats (PUFAs) as well as an indicator of the presence of free radicals [10],[10]. Plasma MDA level of AIS patients within 48 hours of stroke onset is higher than normal and significantly different than control [10],[12]. There are very significant differences in serum MDA level between stroke and TIA compared to the control group, however, serum MDA level were not significantly different in patients with thromboembolic stroke and hemorrhagic stroke patients [13]. Increased lipid peroxide oxidation can lead to increase blood and/or lipid nerve, evident from a significant positive correlation between serum MDA level and serum total cholesterol also serum LDL cholesterol. Brain, which is rich in fat, can also be subject to lipid peroxidation [14].

Cerebral infarct size on Brain CT scan provides important clinical information on stroke cases. Preliminary findings on Brain CT scan in AIS up to six hours showed parenchymal hypodensity occurred because of cytotoxic edema processes by lactic acidosis and cell membrane ion pump failure due to an inadequate supply of ATP and the release of free radicals. After six hours, hypodensity occured because of vasogenic following the loss of tight junctions and endothelial cell integrity [15]. CT scans findings increase to 75 % in the first 48 hours. The peak of the mass effect occurs after 72 hours after ischemic stroke attack [16]. Measurements using a pixel thresholding method based on the signal intensity threshold for both intracranial volume (ICV) and infarction [17]. Beg, et al. found a significant relationship between serum MDA level and lesion size infark [22]

As patients recover from AIS, they are experiencing limitations in their daily activities. Barthel ADL Index instrument (BI) is intended to assess the level of required nursing patient waiter [19].

Based on the background, the researchers feel that it is needed to scientifically prove the relationship between increasing plasma MDA level with infarct volume Brain MSCT and BI at AIS. The purpose of this study was to analyze the relationship between increasing of plasma MDA level with infarct volume Brain MSCT and BI at AIS.

II. METHOD

This research was an observational study with a cross-sectional design conducted in Stroke Unit, Dr. Kariadi General Hospital Semarang. Subjects were Indonesian, determined by consecutive sampling, and qualified analysis after recording AIS patients which comfirmed by Brain MSCT examination. The sample size was calculated using the formula for the sample size to estimate the correlation coefficient (r) 0.5 with a significance level of 95 % and power 90 %, taking into account the possibility of drop out by 10 %, so the sample size was 43 respondents.

Inclusion criteria include patients within their first exposure to AIS, both men and women, aged $\geq$ 45 years with onset at admission $\leq$ 72 hours. Exclusion criteria were acute coronary heart disease, atrial fibrillation ECG recording and acute cardiac decompensation, stroke recurrent infarction. Subjects who met both inclusion and exclusion criteria then fill in a questionnaire, followed by anamnesis as well as neurological examination.

Neurological examination includes Brain MSCT, MDA blood level measurement, and BI assesment on admission. Brain MSCTs were conducted in the department of Radiology Dr. Kariadi General Hospital Semarang using *pixel thresholding technique* and were analyzed by using Leica Q500

MCP. First the pixel thresholding technique was based on thresholds in signal intensity for both ICV and infarct. Each intracranial pixel with attenuation lower than bone was assigned to the ICV. For each slice, this threshold in signal intensity was determined by the investigator and depended on the density scale settings of the CT scan and the attenuation of the infarct. Second is the manual tracing technique and finally is the stereological counting grid. Finally All measured areas were multiplied by the slice distance to obtain the total volumes. The observer is the radiologist who had an experience in the field of neurology and performed the measurements independently. To test the intraobserver variability, all scans were reassessed by one author after all first measurements had been completed. The size of the infarct volume was measured in cm^3 and converted into mm^3, then categorized into three categories, Small, Medium and Large. Brain MSCT confirmed the territorial infarction as local hypodensity, multiple infarcts. For measuring MDA level of TBARS using a modification of the thiobarbituric acid method (applichem) and tricloroacetic acid (applichem). BI assessment was performed at admission.

Patients willing to participate in this study signed informed consent. Ratio scale data such as MDA level and infarct volume size will be expressed as mean and standard deviation if the data was normally distributed or median if the distribution is not normal. Nominal and ordinal scale data is expressed as frequency distributions and proportion.

Data analysis begins with a comparative test of plasma MDA level with infarct volume, and BI confounding factors using *Chi-square* or *Fisher exact* test and *Kolmogorov-Smirnov* test if the scale of ordinal variables and *independent sample t test* when data were normally distributed and homogeneous. The data is not homogeneous; the *Mann-Whitney* test (for numeric variable scale) is used. Hypothesis test used in this study is *Spearman's correlation* test to determine the correlation coefficient between the peripheral blood plasma MDA level and the size of the infarct volume.

III. Result

Overview of the demographic characteristics of the subjects according to the factors is shown in **TABLE I**. The mean age of subjects was 57.84 ± 9.40 with similar proportion of male and female subjects.

AIS infarct volume has a normal distribution medium infarct size (p = 0.186) and large infarct size (p = 0.392) with homogeneous data variance (p = 0.257) no significant difference between age and infarct volume size category. No significant difference in the proportion between the sexes with infarct volume size category.

Most of the subjects have hypertension as the risk factor for stroke. Data on **TABLE III** shows that there were no significant correlation between the characteristics of risk factors (smoking, hypertension, heart disease, diabetes mellitus, and dyslipidemia) with AIS infarct volume size category.

TABLE IV describes the characteristics of the vital signs, and laboratory examinations were grouped by the size of acute ischemic stroke infarct volume. Normality and variance of the data was then tested by using the *Shapiro-Wilk* and *Levene's Test*, and found that there was no significance between total cholesterol HDL, LDL difference HDL cholesterol level and infarct volume size category.

TABLE I CHARACTERISTIC SUBJECTS ACCORDING TO DEMOGRAPHIC FACTOR

Variable		N (%)
Age (Mean ± SD)	57.84 ± 9.40	
Gender	Males	22 (51.2 %)
	Females	21 (48.8 %)
Hypertension	Yes	31 (72.1 %)
	No	12 (27.9 %)
Diabetes mellitus	Yes	11 (25.6 %)
	No	32 (74.4 %)
Smoking	Yes	15 (34.9 %)
	No	28 (65.1 %)
Dyslipidemia	Yes	25 (58.1 %)
	No	18 (41.9 %)

TABLE II COMPARATIVE TEST OF DEMOGRAPHIC FACTOR WITH INFARCT VOLUME SIZE

Variable		Infarct Volume AIS				p	Odds Ratio (95 % CI)
		Medium (n = 5)		Large (n = 38)			
Age (mean ± SD)		57.80 ± 5.02		57.84 ± 9.88		0.993#	
		n	%	n	%		
Gender	Females	3	60	18	47.4	0.664*	0.600 (0.090-4.01)
	Males	2	40	20	52.6		

#independent sample T test

*Fisher exact test

TABLE III PROPORTIONAL COMPARATIVE TEST OF PLASMA MDA ELEVATION RISK FACTORS WITH INFARCT VOLUME CATEGORY

Variable		AIS Infarct Volume				p	Odds Ratio (95 % CI)
		Medium (n = 5)		Large (n = 38)			
		n	%	N	%		
Smoking	Yes	2	40	13	34.2	1.000*	1.282 (0.190-8.663)
	No	3	60	25	65.8		
Hypertension	Yes	3	75	25	65.8	1.000*	1.560 (0.147-16.527)
	No	1	25	13	34.2		
Cardiovascular disease	Yes	1	20	6	15.8	1.000*	1.333 (0.126-14.099)
	No	4	80	32	84.2		
Diabetes Mellitus	Yes	3	75	20	52.6	0.613*	2.700 (0.257-28.341)
	No	1	25	18	47.4		
Dyslipidemia	Yes	4	80	21	55.3	0.380*	3.238 (0.330-31.743)
	No	1	20	17	44.7		

*Fisher exact test

TABLE IV MEAN COMPARATIVE TEST VITAL SIGN AND LABORATORY EXAMINATION WITH INFARCT VOLUME CATEGORY

Variable	AIS Infarct Volume		*p*	Odds Ratio (95 % CI)
	Medium (n = 5)	Large (n = 38)		
	(mean ± SD)	(mean ± SD)		
Systolic blood pressure	147.00± 19.24	153.42 ± 26.87	0.609#	(123.12-170.88) (144.59-162.25)
Diastolic blood pressure	87.00 ± 9.74	91.45 ± 14.47	0.588∞	(74.90-99.10) (86.69-96.20)
One-time blood sugar	240.00 ± 252.07	155.45±137.12	0.532∞	(72.99-552.99) (110.38-200.52)
Fasting blood sugar	158.80 ± 51.19	140.11 ± 74.27	0.272∞	(95.24-222.36) (115.69-164.52)
2-hour postprandial blood sugar	234.40 ± 84.63	181.89 ± 89.37	0.129∞	(129.32-339.48) (152.52-211.27)
Cholesterol	175.60± 45.62	201.03± 37.86	0.175#	(118.95-232.25) (188.58-213.47)
Triglyceride	203.60 ± 113.94	147.50 ± 68.09	0.118#	(62.12-345.08) (125.12-169.88)
HDL	53.60 ± 50.09	40.55 ± 8.62	0.569∞	(8.59-115.80) (37.72-43.39)
LDL	136.40 ± 59.68	126.29 ± 34.66	0.577#	(62.30-210.50) (114.90-137.68)

#independent sample T test

∞Mann-Whitney test

Transformation of the data and re-tested normality of the data using *Shapiro Wilk* and homogeneity of variance test using *Levene's test* obtained a normal distribution and homogeneous variance of data for variables systolic blood pressure and triglyceride level. Then, the carried out *independent sample T test* found no difference between systolic blood pressure and triglyceride level with infarct volume size category.

As for variable diastolic blood pressure, one time blood sugar level, fasting blood sugar level, and 2-hour postprandial blood sugar data transformation and re-tested normality of the data using *Shapiro-Wilk* and homogeneity of variance test using *Levene's test* obtained abnormal distribution and the variance of data is not homogeneous, so the data was tested using *Mann-Whitney* test and found that there is no significant difference between the diastolic blood pressure, one time blood sugar level, fasting blood sugar level, and 2-hour postprandial blood sugar with infarct volume size category.

The correlation between Barthel index at admission and infarct volume category using the *chi-square test* found that there is no significant difference between categories.

IV. THE CORRELATION BETWEEN MALONDIALDEHYDE PLASMA LEVEL WITH THE SIZE OF INFARCT VOLUME AND BARTHEL INDEX AT ADMISSION

The result of plasma MDA level measurement, showed that plasma MDA level in stroke patients is higher than normal (reference for normal malondialdehyde plasma level for men is 0.41 to 1.15 μmol/liter, and women 0.33 to 1.22 μmol/liter). The minimum plasma MDA level is 11.61 μmol/liter and the maximum of 24.70 μmol/liter, with the average of 16.57 SD 3.74 μmol/liter). Compare to normal reference, the lowest in this study is 13.66 times higher than normal, while the highest plasma MDA level is 30.88 times higher than normal.

The size of the infarct volume was measured using a pixel thresholding technique in cm^3 and converted into mm^3, then categorized into three categories, namely; Small: < 50 mm^3, Medium: 50-200 mm^3, Large: 201-400 mm^3. The measured minimum size of infarct volume is 74.20 mm^3 and maximum 159740 mm^3 with a mean 13934.75 SD 32662.36 mm^3. Medium infarct volume size was found in 5 respondents (11.6 %) and the large size in 38 respondents (88.4 %). In this study we found a significant correlation between infarct volume size and BI at admission ($r = 0.369$, $p = 0.015$). There is no significant correlation between infarct volume size with an increase in plasma MDA level and BI at admission.

V. DISCUSSION

As showed in **TABLE I**, AIS occured more frequent in men than women and the average age of onset is in elderly over 60 years old. This result is consistent with numerous studies conducted in various parts of the world where there is an increasing incidence of stroke with increasing age, the risk of stroke increases two times each decade after 55 year old. For gender, the incidence of stroke is more frequent in males than females at age less than 60 years, and became the same relative to age of more than 60 years [20].

Among the risk factors, (hypertension, diabetes mellitus, heart disease, smoking and dyslipidemia), hypertension is the most common risk factor found in 31 (72.1 %) respondents. Hypertension is indeed a vascular risk factor found in most stroke patients, either as a single risk factor or together with other risk factors [20],[21]. Epidemiological studies in Toronto concluded that hypertension raises the risk of stroke 3 times compared to other risk factors, and it will increase to 9 times when combined with diabetes mellitus and hypercholesterolemia [21].

In regards to the onset of less than 72 hours, plasma MDA level is higher than normal level. This is consistent with study conducted by Beg, et al. that the plasma MDA level of AIS patients within 48 hours of stroke onset is higher than normal and significantly different than control [22]. Plasma MDA level was higher than normal due to AIS obtained an increase of calcium ions intracell which will trigger the arachidonic acid metabolism that accumulate during ischemia of the brain by the enzyme cyclooxygenase and lipooxygenase that occurs 6-48 hours of AIS onset and will produce superoxide [22].

Risk factors that can affect the release of free radicals in this study (plasma MDA) include the age [21], hypertension [23]-[24], smoking, diabetes mellitus [23] and dyslipidemia [21] may affect the release of free radicals. In this study, the increase infarct volume is not influenced by risk factors (age, hypertension, smoking, diabetes mellitus, and dyslipidemia), but it can be influenced by the level of other antioxidants (SOD, GPx, and CAT) [23]-[24]. The correlation of elevated plasma MDA level with the size of AIS infarct volume is obtained to be a very weak correlation, or there is no significant correlation

between elevated plasma MDA level with the size of the infarct volume. Previous research confounds a significant correlation between serum MDA level with the vast size of infarct lesion (cm^2) [20], this difference is due to differences in methods, sampling and the measurement of infarct lesion. This study is a cross-sectional design, whereas previous studies use case control-p methods. Sampling we did is by taking the plasma MDA level, whereas sampling of previous studies is by taking the serum MDA level, and infarct measurements on this research is by measuring the infarct volume (cm^3) whereas previous studies is by measuring the area of infarction (cm^2), the previous study also did not analyze the increase in MDA level with stroke infarct volume size.

Obtainment of no significant correlation between plasma MDA level and infarct volume size can be caused by some other influences that contributes to the size of infarct volume such as the levels of Ca, IL-1, IL-6, TNF-α, MMP, that we did not measure in this study, where each of them are interrelated through the ischemic cascade mechanism. Role of Ca level increase is to initiate the outbreak of free radicals and can start the inflammatory process after ischemia [3].

Ischemic damage to the blood brain barrier, through oxidative stress pathway is an initial stimulus for blood brain barrier injury, and can trigger the release of proteases such matrix metallo proteinase (MMP) by neurons, endothelial, and glia cells. This enzyme induces damage to the blood brain barrier. Within 24-72 hours after infarction, the second phase of blood brain barrier injury, greater tissue damage and marked leukocyte infiltration through the release of matrix metalloprotein-9 (MMP-9) from neutrophil transmigration into ischemic brain occurs. Impaired blood brain barrier allows the leakage of blood components into brain parenchyma. Extravasation results in vasogenic edema. This vasogenic edema occurs because hypodensity due to the loss of integrity of endothelial cell tight junctions. Attenuation decrease of HU brain parenchyma in AIS is equal to edema level [13].

Correlation between plasma MDA level and Barthel index (BI) at admission of AIS is obtained to be a very weak correlation where there is no significant correlation between plasma MDA level and BI at admission with onset less than 72 hours. According to previous studies conducted by Siswonoto (2008), plasma MDA level did not significantly affect the output value of activity daily living in patient with AIS. In this study, ADL is assessed using the *National Institutes of Health Stroke Scale* (NIHSS). Although in previous studies, the assessment of ADL using NIHSS can illustrate that the ADL assessment in several ways, in this research, we used BI tendencies and tend to not have a significant correlation with plasma MDA level.

There is a significant correlation (r 0.077 and $p < 0.05$) between the size of the infarct volume and BI at admission of AIS onset less than 72 hours. In this study, consistent with previous studies conducted by Damopolii (2007), the value of BI score is affected by the infarct lesion volume and infarct location. There is a significant correlation between BI score and lesion volume, the greater lesions volume in patients with AIS, the smaller value of BI (more severe degree of dependence). In AIS patients, BI is correlated strongly with CT-scan if there is a larger lesion volume. If the lesion is as small as 50 cc or less, it is a new lesion. BI is lower if lesion occurs in MCA territory compared to if lesion occurs in ACA territory. A blockage occurring in ACA territory area will make an extensive infarction due to lack of collateral on that area, but BI looks lower (moderate and severe degree of dependence) on territorial MCA and ACA+MCA.

This study has several limitations such as no examination of factors that can affect infarct volume: the levels of Ca, IL-1, IL-6, TNF-α, MMP-9, so we cannot analyze the influence of them to infarct volume size. Factors that could affect plasma MDA level include SOD, GPx and CAT, which we also did not do the examination on so we cannot analyze their effect on infarct volume size. The onset of ischemic stroke is incidence to the time of plasma MDA level, so we can find out the increasing fluctuations MDA level in patients with AIS.

VI. Conclusion

There was no significant correlation between elevated plasma MDA level with the infarct volume size and BI at admission in patients with AIS. There is a significant correlation between infarct volume size and BI at admission of less than 72 hours.

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