DIPONEGORO MEDICAL JOURNAL
(Jurnal Kedokteran Diponegoro)
Online : http://ejournal3.undip.ac.id/index.php/medico
E-ISSN : 2540-8844
Volume 10, Number 2, March 2021

DIFFERENCE IN PROFILES OF OXIDATIVE STRESS MARKER (MDA) IN STEMI AND NSTEMI

Kaninta Nuga Sekunda[1], Ariosta[2*], Charles Limantoro[3], Andreas Arie Setiawan[3]
[1]Undergraduate Program, Faculty of Medicine, Diponegoro University, Semarang, Indonesia
[2]Department of Clinical Pathology, Faculty of Medicine, Diponegoro University, Semarang, Indonesia
[3]Department of Internal Medicine, Faculty of Medicine, Diponegoro University, Semarang, Indonesia
[*]Corresponding author: Email: ariosta.lecturer@gmail.com

ABSTRACT

Bacground: Acute Myocardial Infarction (AMI) results in necrosis of the myocardium due to blockage in the coronary artery. AMI is classified into two categories, STEMI and NSTEMI. AMI is a multifactorial condition closely related to the increase in production of reactive oxygen species (ROS). As the end product of lipid peroxidase, Malondialdehyde (MDA) is often used as a biomarker for oxidative stress. **Objective:** To prove the difference between profiles of oxidative stress marker (MDA) in STEMI and NSTEMI. **Methods:** An observational analytic study with a cross-sectional study approach done in Dr. Kariadi Central Public Hospital and Diponegoro National Hospital, Semarang, Central Java, between April and September 2020. Subjects were diagnosed by the presence of chest pains and an increase in CKMB and troponin levels. The study was conducted on 47 subjects consisting of 20 STEMI samples and 27 NSTEMI samples. Serum MDA was examined using the TBARS method. Data were analyzed by a computer program. **Results:** Mean serum MDA in STEMI and NSTEMI was 0,22 ± 0,12 μmol/L and 0,82 ± 0,92 μmol/L respectively. From the statistical analysis, results showed that the difference in serum MDA concentrations between STEMI and NSTEMI were significant (p = 0,007). **Conclusion:** Malondialdehyde concentration in NSTEMI was significantly higher than in STEMI. Further studies are needed to confirm which type of MDA is more accurate, to understand the effect of lipid profile towards STEMI and NSTEMI, and to put patient's medical history into consideration.

Key Words: Acute Myocardial Infarction, Reactive Oxygen Species, Oxidative Stress, Malondialdehyde

INTRODUCTION

Globally, cardiovascular disease (CVD) is the number one cause of death. According to the World Health Organization (WHO), an estimated 7.4 million deaths were caused by coronary heart disease in 2015.[1] Based on the Sample Registration System (SRS) survey in Indonesia in 2014, coronary heart disease (CHD) is the second highest cause of death in all ages, which is 12.9%.[2] It is estimated that 23.6 million people will die from CVD by 2030. Indonesia's Basic Health Research (Riskesdas) data for 2018 shows that the highest prevalence for CVD in Indonesia is CHD at 1.5% .[3]

Atherosclerosis is a significant component of most cardiovascular diseases, including myocardial infarction. Acute myocardial infarction (AMI) is the occurrence of myocardial tissue necrosis caused by decreased oxygen supply in the blood due to critical blockage of the coronary arteries by embolus or thrombus.[4] Acute myocardial infarction is classified based on the presence or absence of ST segment elevation on the ECG. The presence of ST segment elevation is categorized as ST-elevation myocardial infarction (STEMI), whereas if it is absent it is categorized as non-ST-elevation myocardial infarction (NSTEMI).[5] Ischemia is more severe in STEMI as there is total blockage compared to NSTEMI.

The pathogenesis of atherosclerosis has a high correlation with reactive oxygen species (ROS) .[6] Reactive oxygen species are a group of small reactive molecules that play an important role in the homeostasis of the vascular cells. However, uncontrolled production of ROS has implications for vascular injury. Endogenous anti-oxidants serve as checkpoints for avoiding unwanted consequences of ROS. This imbalance in the oxidant / anti-oxidant mechanism causes a condition known as oxidative stress. [7]

The level of oxidative stress can be measured by calculating the concentration of Malondialdehyde (MDA). MDA is a secondary product of lipid peroxidation, a chain phenomenon that produces various active compounds that damage cells.[8] Oxidant compounds such as free radicals or non-radical species attack lipids that contain carbon-carbon double bonds, especially

DIPONEGORO MEDICAL JOURNAL
(Jurnal Kedokteran Diponegoro)
Online : http://ejournal3.undip.ac.id/index.php/medico
E-ISSN : 2540-8844
Volume 10, Number 2, March 2021

unsaturated fatty acids (PUFA).[9] The process of forming MDA can be enzymatic or non-enzymatic. MDA is widely used as a biomarker for assessing oxidative stress.

Determination of serum MDA levels can be done in several ways, one of which is the thiobarbituric acid-reactive substance (TBARS) test because MDA is included in the TBARS category. This method is based on the reaction between the MDA complex and the Thiobarbituric Acid Assay (TBA) in an acidic atmosphere to form a pink MDA-TBA complex. The MDA-TBA complex formed is then measured for its intensity using a spectrophotometer. The method was developed by adding HPLC and gas chromatography mass spectrometry (GC-MS) to increase its specificity and sensitivity.

MDA biomonitoring has been used in in-vivo and in-vitro research as a major biomarker of various disease patterns including hypertension, diabetes, atherosclerosis, heart failure and cancer.[8] It is clear that MDA has a role in the pathogenesis of atherosclerosis. However, there is still a clear knowledge gap about MDA as a marker for AMI in particular. Therefore, this study aims to identify differences in the markers of oxidative stress (MDA) in STEMI and NSTEMI.

METHOD

This study is an analytical observational study with a cross sectional approach, which aims to determine differences in MDA levels as a marker of oxidative stress in STEMI and NSTEMI patients. The subjects of this research are acute myocardial infarction (AMI) patients of RSUP. Dr. Kariadi and the Diponegoro National Hospital (RSND), Semarang in 2020 in the April-September period.

Subjects consisted 47 acute myocardial infarction patients divided into 2 groups, namely 20 STEMI patients and 27 NSTEMI patients who were selected through cluster sampling by means of consecutive sampling that met the inclusion and exclusion criteria. Subjects in the study were patients suffering from acute myocardial infarction (STEMI and NSTEMI) who were excluded if the patient regularly took antioxidant supplements, patients regularly took cholesterol or triglyceride drugs, patients with cancer or malignancy, and patients who had infections.

The independent variable of this study was acute myocardial infarction (STEMI and NSTEMI). The dependent variable of this study is the level of MDA. MDA levels in IMA patient samples were measured using a spectrophotometer with the thiobarbituric acid-reactive substance (TBARS) method. The sample used in this study was blood serum taken from AMI patients after signing an informed consent. MDA levels were measured at the Diponegoro National Hospital (RSND) laboratory.

The collected data was processed with a computer program. All research results were presented in the form of means and their standard deviation. The Saphiro-Wilk test was used to test normality of the data. The differences between groups were compared using the Mann Whitney test. In this study, ethical clearance was obtained from the Health Research Ethics Commission of the Faculty of Medicine (KEPK, Diponegoro University, Semarang with No. 110 / EC / KEPK / FK-UNDIP / VI / 2020.

RESULTS

The research was conducted at RSUP Dr. Kariadi and RSND from April to September 2020. A total of 47 respondents participated in the study, 19 patients from RSUP Dr. Kariadi and 28 patients from Diponegoro National Hospital. There were 20 STEMI patients and 27 NSTEMI patients.

Subject Descriptive Statistics

The distribution of subject characteristics in the two groups is presented in Table 1. Of the 47 subjects, there were 30 males and 17 females. The mean age of the subjects was older in the NSTEMI group than in the STEMI group, with age of 59.96 ± 11.16 years and 56.35 ± 7.44 years, respectively, with the youngest age being 35 years and the oldest being 87 years.

DIPONEGORO MEDICAL JOURNAL
(Jurnal Kedokteran Diponegoro)
Online : http://ejournal3.undip.ac.id/index.php/medico
E-ISSN : 2540-8844
Volume 10, Number 2, March 2021

Table 1. Characteristic Distribution of STEMI & NSTEMI Subjects

Variable	Mean ± SD	Median (Min – Max)
Age (years)		
- STEMI	56,35 ± 7,44	56 (41-67)
- NSTEMI	59,96 ± 11,16	57 (35-87)
Sex		
- Male n= 30 (63,8%)		
- Female n= 17 (36,2%)		

Table 2. MDA Serum Levels for STEMI and NSTEMI Groups

Variable	Mean ± SD	Median (Min – Max)	Differential Analysis (p)
MDA (µmol/L)			
- STEMI	0,22 ± 0,12	0,20 (0,04-0,62)	0,00
- NSTEMI	0,82 ± 0,92	0,33 (0,04-2,84)	0,00

Normality and Difference Test

The data obtained from the examination of MDA levels is a numerical ratio data. MDA levels were measured by an electro photometer. Descriptive analysis of MDA levels can be seen in Table 2.

MDA levels in STEMI patients had an average value of 0.22 ± 0.12 µmol/L with a median value of 0.20 µmol/L, a minimum value of 0.04 µmol/L and a maximum value of 0.62 µmol/L. MDA levels in NSTEMI patients had a mean value of 0.82 ± 0.92 µmol/L with a median value of 0.33, a minimum value of 0.04 µmol/L and a maximum value of 2.84 µmol/L. The results of the normality test using Saphiro Wilk showed $p < 0.05$, which indicates that the data distribution was not normal. Data transformation has been carried out but the data distribution is still not normal.

DISCUSSION

Characteristic of research subjects

This study included 47 AMI patients who were willing to take part in the study and met the inclusion and exclusion criteria. The gender of the research subjects consisted of 30 men (63.8%) and 17 women (36.2%). The percentage of male patients in this study was greater than that of women. This is in accordance with the research of Deborah R. Zucker et al, which received more male subjects with AMI than women.[10] Roshan Raut (2017) stated that men suffer from AMI more than women because of risk factors associated with CAD are more common in men.[11] Of the various risk factors, smoking and dyslipidemia play the biggest role. These two risk factors are more common in men. Meanwhile, women have protective factors such as the endogenous hormone estrogen (17-β-estradiol which is biologically active) which has been shown to protect the heart from damage.[12 13 14]

The median age of the study subjects in the STEMI group was 56 years, with a mean age of 56.35 ± 7.44 years. Meanwhile, the median age of the research subjects in the NSTEMI group was 57 years with a mean age of 59.96 ± 11.16 years. This is in accordance with the research of Boyer, et al (2018) which found a significant increase in the relative proportion of AMI patients between the ages of 45 and 65 years.[15] Advanced age has been found to be the most common risk factor among men and women although there is a delay of up to 7-10 years for women to develop AMI compared to men.[16]

Differences in MDA levels in STEMI and NSTEMI patients

The results of this study indicated that MDA levels in the NSTEMI group were higher than in the STEMI group. Malondialdehyde (MDA) is an end product of the lipid peroxidation chain reaction which is often used as a marker of oxidative stress.[17]

DIPONEGORO MEDICAL JOURNAL
(Jurnal Kedokteran Diponegoro)
Online : http://ejournal3.undip.ac.id/index.php/medico
E-ISSN : 2540-8844
Volume 10, Number 2, March 2021

Apart from the unconjugated form (free MDA), MDA can also be found in the conjugated form. In the measurement of oxidative stress, conjugated MDA is used as an index to indicate recent damage[18], whereas unconjugated MDA is a marker of prolonged injury[19]. The sum amount of unconjugated (free MDA) and conjugated MDA is often referred to as total MDA.

The examination of MDA levels in this study was carried out by measuring the levels of free MDA. So far, free MDA is more commonly used than total MDA as a biomarker of oxidative stress.[20] This is because the free MDA analysis method is easier to perform. An additional acid or base hydrolysis step is required to obtain the total MDA number.[21] However, various proteins and nucleic acids can pick up free MDA, so measuring free MDA does not accurately reflect the amount of MDA generated from lipid peroxidation. On the other hand, dietary intake may contribute to conjugated MDA.[22] The results of a study by Cui et al, 23 found that serum free MDA and total MDA levels did not have significant differences as biomarkers of oxidative stress. Therefore, it is not clear which of the two forms of MDA is more accurate to be biomarkers of oxidative stress induced by lipid peroxidation.

Malondialdehyde as a marker for oxidative stress does not accurately describe the severity between STEMI and NSTEMI. This is consistent with a study conducted by Lavall et al, where the profiles of oxidative stress in the STEMI and NSTEMI groups were not much different, and the difference between the two groups was not significant when only looking at the degree of occlusion in the arteries.[24] The study suggests that total occlusion in STEMI and partial occlusion in NSTEMI did not affect MDA levels. However, both conditions stem from the same pathophysiological process.

The pathogenesis of AMI is a multifactorial process that has many risk factors. Of the various risk factors, the one that has the greatest impact on MDA levels is dyslipidemia.[25]

Many studies have found that serum MDA is higher in subjects with hyperlipidemia. A study found that the MDA concentration in the hyperlipidemic group was 2,48-fold higher.[26] This suggests an increase in oxidative stress with progressive hyperlipidemia. It is known that the hyperlipidemic state is associated with changes in the physical properties of the cell membrane[27], which may facilitate the release of free radicals from the mitochondrial electron transport chain or the activation of NADPH oxidase[28]. Increased lipid peroxidation is thought to be a consequence of oxidative stress which occurs when the dynamic balance between prooxidant and antioxidant mechanism is disturbed.[29]

Several hyperlipidemic drugs have been shown to have beneficial effects not only for maintaining lipid profiles, but also for reducing oxidative stress.[30] The statin class of drugs has been shown to act as anti-atherogenic which can improve endothelial function, inhibit vascular inflammation, and stabilize atherosclerotic plaque. Non-steroidal anti-inflammatory drugs (NSAIDs) can also affect MDA concentration. It has been proven from a study that NSAIDs have a significant antioxidant role by scavenging radicals.[31]

The limitation of this study is that only the serum MDA concentration was examined, hence it does not describe the true state of oxidative stress. It is known that there are several factors that can affect the concentration of MDA. Information regarding medical history, risk factors, lipid profile, and medication history could affect MDA concentration was not available. These factors that were not considered caused a decrease in the accuracy of the results.

CONCLUSION AND SUGGESTIONS

Conclusion

There is a significant difference in MDA concentrations in STEMI and NSTEMI.

Suggestions

1. Further research on the correlation of free MDA and total MDA needs to be done to find out which is a more accurate form of MDA to portray MDA concentration.
2. Unconjugated MDA, conjugated MDA, and total MDA need to be examined according to history of disease.
3. Lipid profile should be examined in order to consider its effect on MDA in STEMI and NSTEMI patients.
4. History of treatment in the STEMI and NSTEMI patients needs to put into consideration.

DIPONEGORO MEDICAL JOURNAL
(Jurnal Kedokteran Diponegoro)
Online : http://ejournal3.undip.ac.id/index.php/medico
E-ISSN : 2540-8844
Volume 10, Number 2, March 2021

5. Subsequent research regarding the effect of drugs such as statins and NSAIDs on MDA levels need to be done.
6. Further research on the pathophysiology of STEMI and NSTEMI to determine the causes of the differences between the two conditions clinically.

REFERENCES

1. Fajardo S, García-Galvan RF, Barranco V, Galvan JC, Batlle SF. Epidemiology of Myocardial Infarction. *Intech* [Internet]. 2016 [cited 2020 Feb 4];1(1):13.
2. Kemenkes RI. Penyakit Jantung Penyebab Kematian Tertinggi. *Kemen Kes Rep Indo [Internet]*. 2017 [cited 2020 Feb 4]. Available from: kemkes.go.id
3. Kementerian Kesehatan RI Badan Penelitian dan Pengembangan. Hasil Utama Riset Kesehatan Dasar. *Kemen Kes Rep Indo [Internet]*. 2018 [cited 2020 Feb 4] :1-100. Available from: depkes.go.id
4. Kingma JG. Myocardial Infarction: An Overview of STEMI and NSTEMI Physiopathology and Treatment. *World J Cardiovasc Dis [Internet]*. 2018 [cited 2020 Feb 4];8(11):498-517.
5. Donaldson C, Dauerman HL. Acute Myocardial Infarction. *Crit Care Secrets Fifth Ed [Internet]*. 2012 [cited 2020 Feb 11]:17(4)192-196.
6. Kattoor AJ, Pothineni NVK, Palagiri D, Mehta JL. Oxidative Stress in Atherosclerosis. *Curr Athero Rep* [Internet]. 2017 [cited 2020 Feb 5]; 19(11):42.
7. Stark J. Oxidative stress and atherosclerosis. *Escardio [Internet]*. 2015 [cited 2020 Feb 5];156(28):1115-1119.
8. Khoubnasab J.M, Ansarin K, Jouyban A. Use of malondialdehyde as a biomarker for assesing oxidative stress in different disease pathologies: A review. *Iran J Public Health [Internet]*. 2015 [cited 2020 Feb 6] ;44(5):714-715.
9. Rappaport ZH. Lipid Peroxidation: Production, Metabolism, and Signaling Mechanisms of Malondialdehyde and 4-Hydroxy-2-Nonenal. *Acta Neurochir Suppl [Internet]*. 2014 [cited 2020 Feb 5];98:9-12.
10. Zucker DR, Griffith JL, Beshansky JR, Selker HP. Presentations of acute myocardial infarction in men and women. *J Gen Intern Med [Internet]*. 1997[cited 2020 Sep 29];12(2):79-87.
11. Raut R, Bahadur M, Sharma D, Rajbhandari S. Comparative Study of Risk Factors Among the Male and Female Patients with Acute Myocardial Infarction Admitted in CCU of Sahid Gangalal National Heart Centre. *Nepal Hear J [Internet]*. 2017 [cited 2020 Sep 29] ;6(1):4-7.
12. Moshki M, Zareie M, Hashemizadeh H. Sex differences in acute myocardial infarction. *N Y State J Med [Internet]*. 2015 [cited 2020 Sep 29];62(3):2336-2338.
13. Dunlay SM, Roger VL. Gender differences in the pathophysiology, clinical presentation, and outcomes of ischemic heart failure. *Curr Heart Fail Rep [Internet]*. 2012 [cited 2020 Sep 29];9(4):267-276.
14. Ilic M, Sipetic SG, Ristic B, Ilic I. Myocardial infarction and alcohol consumption: A case-control study. *PLoS One [Internet]*. 2018[cited 2020 Sep 29] ;13(6):1-2.
15. Boyer NM, Laskey WK, Cox M, Hernandez AF, Peterson ED, Bhatt EL, et al. Trends in Clinical, Demographic, and Biochemical Characteristics of Patients With Acute Myocardial Infarction From 2003 to 2008: A Report From the American Heart Association Get With The Guidelines Coronary Artery Disease Program. *J Am Heart Assoc [Internet]*. 2012 [cited 2020 Sep 30] ;1(4):1-2.
16. Maas AHEM, Appelman YEA. Gender differences in coronary heart disease. *Netherlands Hear J [Internet]*. 2010 [cited 2020 Sep 30];18(12):598-603.
17. Singh Z, Karthigesu IP, Singh P, Kaur R. Use of malondialdehyde as a biomarker for assessing oxidative stress in different disease pathologies: A review. *Iran J Public Health [Internet]*. 2014 [cited 2020 Sep 30];43(3):7-16.
18. Cighetti G, Debiasi S, Paroni R, Allevi P. Free and total malondialdehyde assessment in biological matrices by gas chromatography-mass spectrometry: What is needed for an accurate detection. *Anal Biochem [Internet]*. 1999 [cited 2020 Sep 30];266(2):222-229.
19. Mahmoodi H, Hadley M, Chang YX, Draper HH. Increased formation and degradation of

DIPONEGORO MEDICAL JOURNAL
(Jurnal Kedokteran Diponegoro)
Online : http://ejournal3.undip.ac.id/index.php/medico
E-ISSN : 2540-8844
Volume 10, Number 2, March 2021

malondialdehyde-modified proteins under conditions of peroxidative stress. *Lipids [Internet]*. 1995 [cited 2020 Sep 30];30(10):963-966.
20. Ho E, Karimi Galougahi K, Liu CC, Bhindi R, Figtree GA. Biological markers of oxidative stress: Applications to cardiovascular research and practice. *Redox Biol [Internet]*. 2013 [cited 2020 Sep 30];1(1):483-491.
21. Draper HH, Hadley M. Malondialdehyde determination as index of lipid Peroxidation. *Methods Enzymol [Internet]*. 2001 [cited 2020 Sep 30];186(C):421-431.
22. Draper HH, Hadley M. A review of recent studies on the metabolism of exogenous and endogenous malondialdehyde. *Xenobiotica [Internet]*. 1990 [cited 2020 Sep 30];20(9):901-907.
23. Cui X, Gong J, Han H, He L, Teng Y, Tetley T, et al. Relationship between free and total malondialdehyde, a well-established marker of oxidative stress, in various types of human biospecimens. *J Thorac Dis [Internet]*. 2018 [cited 2020 Sep 30];10(5):3088-3197.
24. Lavall MC, Bonfanti G, Ceolin RB, Schott L, Goncalves TL, Moresco RN, et al. Oxidative Profile of Patients with ST Segment Elevation Myocardial Infarction. *Clin Lab [Internet]*. 2016 [cited 2020 Sep 30];62(5):971-973.
25. Rathore V. Risk Factors of Acute Myocardial Infarction: A Review. *Eurasian J Med Investig [Internet]*. 2018 [cited 2020 Sep 30] ;2020(1):2-7.
26. Yang RL, Shi YH, Hao G, Li W, Le GW. Increasing oxidative stress with progressive hyperlipidemia in human: Relation between malondialdehyde and atherogenic index. *J Clin Biochem Nutr [Internet]*. 2008 [cited 2020 Sep 27];43(3):154-158.
27. Engelmann B, Streich S, Schönthier UM, Richter WO, Duhm J. Changes of membrane phospholipid composition of human erythrocytes in hyperlipidemias. I. Increased phosphatidylcholine and reduced sphingomyelin in patients with elevated levels of triacylglycerol-rich lipoproteins. *Biochim Biophys Acta (BBA)/Lipids Lipid Metab [Internet]*. 1992 [cited 2020 Sep 28];1165(1):32-37.
28. Ludwig PW, Hunninghake DB, Hoidal JR. Increased Leucocyte Oxidative Metabolism in Hyperlipoproteinaemia. *Lancet [Internet]*. 1982 [cited 2020 Sep 30];320(8294):348-350.
29. Kumari SS, Menon VP. Changes in levels of lipid perodixes and activities of superoxide dismutase and catalase in isoproterenol induced myocardial infarction in rats. *Indian J Exp Biol [Internet]*. 1987 [cited 2020 Sep 30] ;25(6):419-423.
30. Oesterle A, Laufs U, Liao JK. Pleiotropic Effects of Statins on the Cardiovascular System. *Circ Res [Internet]*. 2017 [cited 2020 Sep 30];120(1):229-243.
31. Končič M, Rajič Z, Petrič N, Zorc B. Antioxidant activity of NSAID hydroxamic acids. *Acta Pharm [Internet]*. 2009[cited 2020 Sep 30];59(2):235-242.