Relationship between Plasma Neuregulin-1 and MDA Levels with Severity of CAD

Gestina Aliska*, Muhammad Fadli, Yose Ramda Ihami, Elly Usman, Ivan Mahendra Raditya, Rahma Tsania Zhuhra, Robby Alfadi

Introduction

About one-third of the world population died because of coronary artery disease (CAD), joined with cerebrovascular diseases [1]. The level of CAD’s severity is related to some biologic factors, such as inflammatory cytokines and lipoproteins, that influence atherosclerosis and angiogenesis processes. The relationship between vascular growth factor and CAD’s severity has not been clearly identified yet. Validated biomarkers are important screening methods to help patients with heart risks and heart diseases related symptoms. These biomarkers reflect the CAD’s severity and ischemic heart incident [2].

Neuregulin-1 (NRG-1) is an angiogenic and growth factor activated by stress, including oxidative stress. NRG-1 is activated by ischemia and physical activity in animals. NRG-1 works through the ErbB receptor in controlling the cell’s survival, growth, metabolism, and angiogenesis. Oxidative stress and inflammation play a significant role in the atherosclerosis process of CAD. A previous study has shown that neuregulin-1 is a prominent angiogenic factor in diabetic cardiomyopathy, whereas the relationship between neuregulin-1 and MDA plasma level has not been investigated [3]. The malondialdehyde (MDA) was produced during oxidative stress on lipid peroxidation [2]. MDA levels and H-CRP are significantly increasing in CAD patients [4]. Previous studies show that plasma NRG-1 levels were higher in patients with stress-induced ischemia [2]. However, the underlying physiologic and pathologic factors influencing the development of NRG-1 could improve the injured cardiac performance, unattenuated pathological changes, and prolonged survival of the cells.

METHODS: We measured plasma NRG-1 in 61 nondiabetic patients within 38–82 years old range with STEMI, NSTEMI, and UAP.

RESULTS: We found their plasma NRG1, respectively, was 10.3 (1.9–38.2) ng/ml, 14.3 ± 7.2 ng/ml, and 7.05 (4.5–0.4) ng/mL. Plasma NRG 1 increased in AMI patients.

CONCLUSION: This study concludes that NRG1’s activated during cardiac cells injury, in any AMI.
study to observe NRG-1 levels in CAD patients in Indonesia, mainly focused in Minang ethnicity. This study also analyzes the relationship between NRG-1 and MDA with CAD’s severity.

Materials and Methods

The study population consisted of a retrospective of 61 nondiabetic patients who underwent coronary angiography due to either STEMI, NSTEMI, or UAP from July to November 2017 from an academic referral center. The samples were taken in 24 h after primary catheterization and without regard to the onset of a heart attack. The CAD severity was further characterized in these subgroups using the SYNTAX score, which accounts for lesion location, degree of stenosis, and the number of vessels involved [7]. The study was performed by the Committee of the Research ethics of the Faculty of Medicine, Universitas Andalas, with number 329/KEP/FK/2017. All patients were informed, and consent was obtained.

**Plasma NRG-1 measurement**

Human NRG-1 (Neuregulin 1) ELISA Kit from Elabscience® was used to assay plasma NRG-1 levels according to the manufacturer’s instructions. The measurement was held in the Biomedicine Laboratory of Faculty of Medicine, Universitas Andalas.

**Plasma MDA measurement**

Plasma MDA levels were measured using spectrophotometry in the Biochemistry Laboratory of Faculty of Medicine, Universitas Andalas.

**CAD severity**

CAD severity was measured by the SYNTAX score (SYNergy between PCI with TAXUS™ and Cardiac Surgery). SYNTAX score is an available measurement tool to make the medical decision in managing CAD’s patients mainly focused in choosing actions based on coronary anatomy. Moderate-high severity of CAD is defined if SYNTAX score >22 and low severity is defined if SYNTAX score ≤ 22 [7].

**Analysis**

Categorical variables were compared by the Chi-square test, while t-test and Mann–Whitney used for the numerical variable. Statistical analysis was performed with SPSS 20.0. Data are statistically significant results with p < 0.05.

**Results**

There were 70 patients with CAD who were included in this study, 9 of them were excluded since they were diagnosed by diabetes mellitus. A total of 61 patients were measured for NRG-1 plasma and MDA. In this study, most patients were male of 40–64 years old. Table 1 shows 39 patients with high severity of CAD based on the SYNTAX score had a higher lipid profile level, and there were no significant differences to 22 patients with low severity of CAD (SYNTAX≤22). Hypertension, dyslipidemia, and smoking are the most risk factor for CAD in these patients. STEMI was the most diagnosed of all (67.2%). There were no significant differences in Table 1: Patient characteristics

| Characteristics | All patients (n,%); SYNTAX >22 (n=39); SYNTAX ≤ 22 (n=22) | p value |
|-----------------|----------------------------------------------------------|---------|
| Gender          | Male (54 (88.5%); Female (7 (11.5%))                     |         |
| Age             | <40 years old (1 (1.6%); 40–64 years old (50 (81.9%); >65 years old (10 (16.5%)) |         |
| Diagnosis       | UAP (6 (9.8%); NSTEMI (14 (23%); STEMI (41 (67.2%))      |         |
| Risk factors    | Hypertension (34 (55.7%); Family history (8 (13.1%); Dyslipidemia (33 (54.1%); Menopause (n=7) (1/7 (14.3%); Smoking (42 (68.9%)) |         |
| Lipid profiles  | Total cholesterol (176.3 ± 41.4); HDL (31.5 (14-43); LDL (123.4 ± 38.6); Triglycerides (121.4 ± 38.6) |         |
| BMI             | 23.7 ± 2.5                                               | 0.395   |

There were no significant differences to 22 patients with low severity of CAD (SYNTAX ≤ 22). Levels of MDA plasma were higher in the group with SYNTAX >22 (2.61 ± 0.62) than in the group with SYNTAX<22 (2.61 ± 0.62). The higher levels of plasma MDA were shown in UAP and NSTEMI (Table 2). In this study, shown in Figure 1, we found that mean MDA levels in high severity patients are higher than mild to moderate severity patients, but not statistically significant (3.02 ± 0.69 vs. 2.61 ± 0.62, p = 0.074).

**NRG-1 levels**

| NRG-1 levels | Median (min-max) | p value |
|--------------|------------------|---------|
| Syntax ≤ 22  | 10.97 (1.15–32.25)| 0.775   |
| Syntax >22   | 13.59 (9.31–38.2) |         |
| Diagnosis    | 13.86 (11.91–28.5) | 0.654   |
| UAP and NSTEMI | 13.86 (11.91–28.5) |         |
| STEMI        | 10.31 (9.31–38.2)  |         |

**Table 2: Plasma NRG-1 levels in ACS patients**

**Table 3: High/lower severity patients**

Table 1: Patient characteristics

| Characteristics | All patients (n,%); SYNTAX >22 (n=39); SYNTAX ≤ 22 (n=22) | p value |
|-----------------|----------------------------------------------------------|---------|
| Gender          | Male (54 (88.5%); Female (7 (11.5%))                     |         |
| Age             | <40 years old (1 (1.6%); 40–64 years old (50 (81.9%); >65 years old (10 (16.5%)) |         |
| Diagnosis       | UAP (6 (9.8%); NSTEMI (14 (23%); STEMI (41 (67.2%))      |         |
| Risk factors    | Hypertension (34 (55.7%); Family history (8 (13.1%); Dyslipidemia (33 (54.1%); Menopause (n=7) (1/7 (14.3%); Smoking (42 (68.9%)) |         |
| Lipid profiles  | Total cholesterol (176.3 ± 41.4); HDL (31.5 (14-43); LDL (123.4 ± 38.6); Triglycerides (121.4 ± 38.6) |         |
| BMI             | 23.7 ± 2.5                                               | 0.395   |
Discussion

The main finding of this study was that patients with CAD plasma NRG-1 did not significantly associate with CAD severity (SYNTAX score). The results showed that patients with SYNTAX score > 22 had a higher plasma concentration of NRG-1 than SYNTAX score ≤ 22. Geisberg et al. found the negative correlation between NRG-1 levels with CAD’s severity, which lower NRG-1 levels related to higher severity. The difference is probably caused by the measurement time of NRG-1 levels [2]. This study measured NRG-1 levels 24 h after primary catheterization, not based on the onset of a heart attack. Then, also, patients who underwent coronary angiography in CAD got initial and continued treatment with cardiac medications in 24 h [11]. Antiplatelet drugs and other cardiac drugs such as aspirin and clopidogrel give an excellent vascular impact. Those lead to endothelial function improvement and may result in increasing NRG-1 expression [5], [12].

Kuramochi et al. found that releasing of NRG is induced by ischemic and reperfusion injuries. Reactive oxygen species induce activation of NRG-1 in myocardial cells [7]. NRG-1 activated during myocardial infarct and cardiomyocytes will proliferate after damage and through the regeneration helped by the intracellular and extracellular signal. NRG-1 regulates cardiogenesis and cardiac regeneration [9], [10].

Vascular endothelial growth factor (VEGF) increased in physiological and pathological state to repair endothelial function. Our study may relate to these conditions, where human endothelial progenitor cells produced together after VEGF in hypoxia and ischemic situations [13]. Thrombosis caused by atherosclerosis leads to hypoxia and released cardiac endothelial cells within hours to restore endothelial walls [14]. Recent studies on rat model myocardial ischemia show peaked of VEGF expression in 3 h after injury, and it can determine the degree of severity of the ischemic injury [15]. Intermittent hypoxia has a cardioprotective effect by regulating VEGF expression in the ischemic rat model [16], [17]. Endothelial function improvement after hypoxia by VEGF leads to an increase of NRG-1 expression [18], [19] [20]. NRG-1 also helped to strengthen the vascular and alleviate cardiac endothelial cells shown in rats’ model [21].

MDA levels increase together with CAD’s severity. CAD patients with three vessels involved have a significantly higher level of MDA than one vessel involved. However, in this study, MDA levels showed no significant increase in high severity patients. Venkata et al. reported that the MDA plasma levels were significantly higher in inpatient CAD groups compared to the control group (p<0.05) [22]. MDA level is a useful marker for the severity of CAD and reflecting the presence of easily removed plaque [23], [24]. MDA can be predictive markers of adverse cardiovascular outcomes [25]. Elevated concentration of MDA relates to severe coronary artery calcification in patients with renal disease [26]. Higher MDA levels mean worse prognosis on CAD who underwent PCI in a recent study [25]. MDA has a stable concentration in blood over 36 h in patients [28].

MDA was produced from lipid peroxidation on oxidative stress. Elevated MDA indicates free radicals production leads to atherogenesis and the development of atherosclerosis caused by coronary heart disease [29], [30]. A recent study shows that smokers’ patients got higher oxygen free radical than non-smokers patients [31]. We also found that more than half of our patients were smokers that lead them to produce more MDA. Tobacco and other risk factors play a role in coronary heart disease [31], [32].

Conclusion

Our results suggest that NRG-1 plasma levels do not correlate with CAD severity. However, There was no significant difference between both groups. Patients with SYNTAX score > 22 had a higher plasma concentration of NRG-1 than SYNTAX score ≤ 22. NRG-1 can increase the myocardial angiogenesis, probably through the direct effects of NRG-1 and through the increased expression of VEGF. There was no significant MDA plasma level in patients with moderate high and low severity of CAD. Increased
oxidative stress, such as MDA, was observed in CAD cases and potentially represented a pathogenic factor for atherosclerosis.

Acknowledgments

We thank all the medical, paramedical, and administration staff in Universitas Andalas for the support in this research.

References

1. Xiao J, Li B, Zheng Z, Wang M, Peng J, Li Y, et al. Therapeutic effects of neuregulin-1 gene transduction in rats with myocardial infarction. Coron Artery Dis. 2012;23(7):460-8. https://doi.org/10.1097/mca.0b013e32835877da PMid:22968213

2. Geisberg CA, Wang G, Safa RN, Smith HM, Anderson B, Peng XY, et al. Circulating neuregulin-1 levels vary according to the angiographic severity of coronary artery disease and ischemia. Coron Artery Dis. 2011;22(6):577-82. https://doi.org/10.1097/mca.0b013e328334d334 PMid:22027878

3. Huang M, Zheng J, Chen Z, You C, Huang Q. The relationship between circulating neuregulin-1 coronary collateral circulation in patients with coronary artery disease. Int Heart J. 2020;61(1):115-20. https://doi.org/10.1536/ihj.19-277 PMid:31956140

4. Khaki-Khatibi F, Yaghoubi AR, Rahbani NM. Study of antioxidant enzymes, lipid peroxidation, lipid profile and immunologic factor in coronary artery disease in East Azarbijan. Int J Med Biomed Biotechnol. 2020;2019;2(1):57-60. https://doi.org/10.3892/ijmb.2019.4309 PMid:27799944

10. Rupert CE, Coulombe KL. The roles of neuregulin-1 in cardiac development, homeostasis, and disease. Biomark Insights. 2015;10(Suppl 1):1-9. https://doi.org/10.4137/bmi.s20061 PMid:25922571

11. Dunn SP, Kazmi H. Antithrombotic therapies in acute coronary syndrome. In: CCSAP Cardiology Critical Care. Lenexa, KS: American College of Clinical Pharmacy; 2017. p. 1-34.

12. Widmer RJ, Lerman A. Endothelial dysfunction and cardiovascular disease. Glob Cardiol Sci Pract. 2014;2014(3):291-308. PMid:25780786

13. Yoder MC. Human endothelial progenitor cells. Cold Spring Harb Perspect Med. 2011;2(7):a006692. https://doi.org/10.1101/cshperspect.a006692 PMid:22762017

14. Van Belle E, Bauters C, Asahara T, Isner JM. Endothelial regrowth after arterial injury: From vascular repair to therapeutics. Cardiovasc Res. 1998;36(1):54-68. https://doi.org/10.1016/a0008-6363(97)00326-x PMid:9683907

15. Mao RM, Du ZB, Gao WM, Mi L, Zhu BL. Time-dependent expression of vascular endothelial growth factor after acute myocardial ischemia in rats. Fa Yi Xue Za Zhi. 2012;28:179-84. PMid:22812217

16. Wang Z, Si LY. Hypoxia-inducible factor-1α and vascular endothelial growth factor in the cardioprotective effects of intermittent hypoxia in rats. Ups J Med Sci. 2013;118(2):65-74. https://doi.org/10.3109/03009734.2013.766914 PMid:23441597

17. Orlandi A, Bennett M. Progenitor cell-derived smooth muscle cells in vascular disease. Biochem Pharmacol. 2010;79:1706-13. https://doi.org/10.1016/j.bcp.2010.01.027 PMid:20117099

18. Niu J, Han X, Qi H, Yin J, Zhang Z. Correlation between vascular endothelial growth factor and long-term prognosis in patients with acute myocardial infarction. Exp Ther Med. 2016;12(1):475-9. https://doi.org/10.3892/etm.2016.3286 PMid:27347081

19. Wu C, Gui C, Li L, Peng Y, Tang Z, Wei J. Expression and secretion of neuregulin-1 in cardiac microvascular endothelial cells treated with angiogenic factors. Exp Ther Med. 2018;15(4):3577-81. https://doi.org/10.3892/etm.2018.5811 PMid:29545886

20. Zeng Z, Gui C, Nong Q, Du F, Zhu L. Serum neuregulin-1β levels are positively correlated with VEGF and angiopeptin-1 levels in patients with diabetes and unstable angina pectoris. Int J Cardiol. 2013;193677-9. https://doi.org/10.1016/j.ijcard.2013.04.088 PMid:23632614

21. Kang W, Cheng Y, Zhou F, Wang L, Zhong L, Li H, et al. Neuregulin-1 protects cardiac function in septic rats through multiple targets based on endothelial cells. Int J Mol Med. 2019;44(4):1255-66. https://doi.org/10.3892/ijmm.2019.4309 PMid:31432099

22. Tajika K, Okamatsu K, Takano M, Inami S, Yamamoto M, Murakami D, et al. Malondialdehyde-modified low-density lipoprotein is a useful marker to identify patients with vulnerable plaque. Circ J. 2012;76:2211-7. https://doi.org/10.1253/circj.cj-12-0183 PMid:22765057

23. Venkata R, Ravi K. Evaluation of correlation between oxidative stress and abnormal lipid profile in coronary artery disease. J Cardiovasc Dis Res. 2011;2(1):57-60. PMid:21716754

24. Matsuo Y, Kubo T, Okamoto Y, Ishibashi K, Komukai K,
Tanimoto T, et al. Circulating malondialdehyde-modified low-density lipoprotein levels are associated with the presence of thin-cap fibroatheromas determined by optical coherence tomography in coronary artery disease. Eur Heart J Cardiovasc Imaging. 2013;14:43-50. https://doi.org/10.1093/ehjci/jes094 PMid:23573905

25. Abolhasani A, Shahbazloo SV, Saadati HM, Mahmoodi N, Khanbabaei N. Evaluation of serum levels of inflammation, fibrinolysis and oxidative stress markers in coronary artery disease prediction: A cross-sectional study. Arq Bras Cardiol. 2019;113(4):667-74. https://doi.org/10.5935/abc.20190159 PMid:31691749

26. Jung HH, Choi DH, Lee SH. Serum malondialdehyde and coronary artery disease in hemodialysis patients. Am J Nephrol. 2004;24:537-42. https://doi.org/10.1159/000081731 PMid:15523169

27. Amioka N, Miyoshi T, Otsuka H, Yamada D, Takaishi A, Ueeda M, et al. Serum malondialdehyde-modified low-density lipoprotein levels on admission predict prognosis in patients with acute coronary syndrome undergoing percutaneous coronary intervention. J Cardiol. 2019;74:258-66. https://doi.org/10.1016/j.jcc.2019.02.012 PMid:30898480

28. Wu T, Rifai N, Roberts LJ. Stability of Measurements of biomarkers of oxidative stress in blood over 36 hours. Cancer Epidemiol Biomarkers Prev. 2004;13(8):1399-402 PMid:15289864

29. Khan MA, Baseer A. Increased malondialdehyde levels in coronary heart disease. J Pak Med Assoc. 2000;50(8):261-4. PMid:10992710

30. Cheraghi M, Ahmadvand H, Maleki A, Babaeenezhad E, Shakiba S, Hassanzadeh F. Oxidative stress status and liver markers in coronary heart disease. Rep Biochem Mol Biol. 2019;8(1):49-55. PMid:31334288

31. Bermudez V, Acosta L, Aparicio D, Finol F, Canelon R, Urdaneta A, et al. Smoking habits and cardiovascular disease. Rev Latinoam Hipertens. 2010;5:19-27.

32. Roy A, Rawal I, Jabbour S, Prabhakaran D. Tobacco and cardiovascular disease: A summary of evidence. In: Prabhakaran D, Anand S, Gaziano TA, Watkins DA, Wu Y, Mbanya JC, et al., editors. Cardiovascular, Respiratory, and Related Disorders. 3rd ed., Ch.4. Washington, DC: The International Bank for Reconstruction and Development, The World Bank; 2017. https://doi.org/10.1596/978-1-4648-0518-9_ch4