ARTÍCULO ORIGINAL

Gac Méd Caracas 2021;129(Supl 2):S373-S378
DOI: 10.47307/GMC.2021.129.s2.15

Association between bleeding volume with heme oxygenase-1 and malondialdehyde levels in patients of acute intracerebral hemorrhage

Asociación entre el volumen de sangrado con la hemo oxigenasa-1 y los niveles de malondialdehído en pacientes con hemorragia intracerebral aguda

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SUMMARY

Background: *Oxidative stress plays an important role in secondary brain damage after a stroke of intracerebral hemorrhage because it causes permanent damage to grey matter, white matter taken by brain blood barrier disorders, and brain edema with brain cells. This study aimed to determine the correlation between bleeding volume and heme oxygenase-1 (HO-1) and malondialdehyde (MDA) levels in stroke patients with acute intracerebral hemorrhage at Dr. Soetomo Hospital, Surabaya.*

Methods: *This was a cross-sectional analytic study with 34 study subjects who met the inclusion and exclusion criteria from August 2019 to November 2019. Subjects were measured for bleeding volume and blood collection for examination of HO-1 levels and MDA levels at hospital admission in stroke patients with acute intracerebral hemorrhage.*
Results: *The mean bleeding volume in this study was 15.34±10.26 mL, the mean level of HO-1 was 55.51±138.06 ng/mL, and the mean level of MDA was 1066.03±907.97 ng/mL. Statistical analysis did not obtain any significant correlation between bleeding volume and HO-1 level (p= 0.605). However, there was a significant correlation statistically between bleeding volume and MDA level (r= 0.509; p= 0.002).*
Conclusion: *There was a correlation between bleeding volume level and the level of MDA in stroke patients with acute intracerebral hemorrhage, although there was no significant correlation between bleeding volume and HO-1 levels.*

Keywords: *Intracerebral hemorrhage stroke, heme oxygenase 1, malondialdehyde, oxidative stress, lipid peroxidation*

DOI: https://doi.org/10.47307/GMC.2021.129.s2.15

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Recibido: 11 de mayo 2021
Aceptado: 22 de jun io 2021

RESUMEN

Antecedentes: *El estrés oxidativo juega un papel importante en el daño cerebral secundario después de un accidente cerebrovascular con hemorragia intracerebral porque causa daño permanente a la sustancia gris, sustancia blanca blanca tomada*

por los trastornos de la barrera hematoencefálica y edema cerebral con las células cerebrales. Este estudio tuvo como objetivo determinar la correlación entre el volumen de sangrado y los niveles de hemo oxigenasa-1 (HO-1) y malondialdehído (MDA) en pacientes con accidente cerebrovascular con hemorragia intracerebral aguda en el Hospital Dr. Soetomo, Surabaya.

Métodos: *Este fue un estudio analítico transversal con 34 sujetos que cumplieron con los criterios de inclusión y exclusión, desde agosto de 2019 hasta noviembre de 2019. Se midió el volumen de sangrado y se recolectó sangre de los sujetos para examinar los niveles de HO-1 y los niveles de MDA, al ingreso hospitalario en pacientes con accidente cerebrovascular con hemorragia intracerebral aguda.*

Resultados: *El volumen de sangrado medio en este estudio fue de 15,34 ± 10,26 mL, el nivel medio de HO-1 fue de 55,51 ± 138,06 ng/mL y el nivel medio de MDA fue de 1 066,03 ± 907,97 ng/mL. El análisis estadístico no obtuvo ninguna correlación significativa entre el volumen de sangrado y el nivel de HO-1 (p= 0,605). Sin embargo, hubo una correlación estadísticamente significativa entre el volumen de sangrado y el nivel de MDA (r= 0,509; p= 0,002).*

Conclusión: *Hubo una correlación entre el volumen de sangrado y el nivel de MDA en pacientes con ictus con hemorragia intracerebral aguda, aunque no hubo una correlación significativa entre el volumen de sangrado y los niveles de HO-1.*

Palabras clave: *Ictus hemorragia intracerebral, hemo oxigenasa-1, malondialdehído, estrés oxidativo, peroxidación lipídica.*

INTRODUCTION

Stroke is the second leading cause of death in the world and the highest cause of disability in the world, with intracerebral hemorrhage strokes having an incidence of 10-20 cases per 100 000 population and an estimated 10-15 of all strokes worldwide each year (1,2). Indonesian stroke registry data in 2014 reported that the number of hemorrhage strokes was 32.9 % of all strokes in Indonesia. The highest mortality recorded in hemorrhage strokes is equal to 20.3 % after 48 hours and 18.3 % less than 48 hours, greater than the mortality rate in infarct stroke. 52 % of patients died within the first month and only 20 % lived independently within 6 months (3).

In an intracerebral hemorrhage stroke, it will form a hematoma that can damage the anatomical structure of the brain, causing a neurological deficit and the effect of the urgency of space that can cause an increase in intracranial pressure. Hematoma volume >30 mL is associated with increased mortality due to intracerebral hemorrhage stroke, with mortality rates that increase to >90 % in hematoma volumes >60 mL (4). Hematoma expansion and perihematomal edema result in secondary brain injury and worsen clinical outcomes (4,5).

The interactions between cytotoxicity, excitotoxicity, oxidative stress, and lysis product inflammation from red blood cells and plasma components are the cause of secondary brain injury after intracerebral hemorrhage stroke (6). Lysis of red blood cells in the hematoma will release hemoglobin which is converted by the enzyme heme oxygenase-1 (HO-1) into neurotoxic components, heme, and iron which play a role in secondary brain injury through the induction of oxidative stress by HO-1 activation and iron-related free radicals through the Fenton reaction (7). The early activation of microglia, the release of proinflammatory mediators, and the influx of peripheral leukocytes will trigger neuroinflammation after intracerebral hemorrhage stroke that releases large amounts of reactive oxygen species and lipid peroxidation (5,7). Lipid hydroperoxide is the main product of the lipid peroxidation process. The structure of lipid hydroperoxide is very unstable and can easily turn into malondialdehyde (MDA), 4-hydroxy-2-nonenal (4-HNE), and several other forms of aldehyde. MDA is the main secondary product in the lipid peroxidation process because it is more mutagenic than other aldehydes (8). This compound was first used in 1950 as a sign of damage to food. Currently, MDA is more often used in biomedical research as a marker of oxidative stress, especially in various clinical conditions related to the lipid peroxidation process. The chemically stable nature of MDA makes it more often used as a marker of oxidative stress compared to 4-HNE (9).

Research on intracerebral hemorrhage stroke and its relation to oxidative stress has not been conducted in Indonesia and has never been performed in Dr. Soetomo Hospital, Surabaya. This study aims to determine the volume of bleeding with levels of HO-1 and MDA as a

marker of oxidative stress in stroke patients with acute intracerebral hemorrhage.

METHODS

Subjects

This was a cross-sectional observational analytic study aiming to determine the correlation between bleeding volume with HO-1 and MDA levels. The study was conducted at Dr. Soetomo Hospital, Surabaya, Indonesia, from August to November 2019. The study population was intracerebral hemorrhage stroke patients who came for treatment at Dr. Soetomo Hospital, Surabaya. The study sample was all hemorrhage stroke patients who came for treatment and met the criteria for sample acceptance. Criteria for acceptance of the sample were stroke patients with the first acute intracerebral hemorrhage who had clinical examinations and head CT scans without contrasting the onset of stroke between 24-72 hours, aged ≥20 years, were willing to participate in the study, and signed informed consent. Criteria for rejection of the sample were stroke patients with acute intracerebral hemorrhage accompanied by intraventricular hemorrhage or subarachnoid hemorrhage, having an infection upon hospital admission, suffering from diabetes mellitus, acute coronary heart disease, Parkinson's disease, and a history of malignancy. The sampling technique was conducted by consecutive sampling by taking research subjects who meet the criteria for receiving samples sequentially until the desired sample size was met. The total sample of the study was 34 patients.

Methods

Bleeding volume was measured from digital CT images with the ABC/2 formula described by Broderick (A: the largest bleeding diameter by CT, B: Diameter 90 to A, and C: estimated number of CT slices with bleeding multiplied by slice thickness).

Blood samples were centrifuged at 4°C at a speed of 3 000 rpm for 10 minutes; and after the serum was separated, it was stored at -80°C until analysis. HO-1 and MDA levels were measured by the Enzym-linked immunosorbent assay (ELISA) technique.

Data analysis

Data obtained from the data collection sheet were analyzed. Data analysis was performed using the Statistical Package for Social Science (SPSS) version 21.0 program with a statistical significance of $p<0.05$. The correlation between bleeding volume with HO-1 and MDA levels in stroke patients with acute intracerebral hemorrhage was analyzed by Spearman rank correlation tests.

RESULTS

Demographic characteristics based on age obtained that the youngest age of patients with intracerebral hemorrhage stroke in this study was 40 years, and the oldest was 87 years, with an average age of study 60.74±13.10 years. Based on the sex, there were 22 (64.7 %) male subjects and 12 (35.3 %) female subjects.

Clinical characteristics of the study subjects included hypertension, smoking, bleeding volume, HO-1 levels, and MDA levels. In this study, 26 subjects (76.5 %) had hypertension, and 8 subjects (23.5 %) did not suffer from hypertension. Patients with a history of smoking were 8 subjects (23.5 %), and the non-smokers were 26 subjects (76.5 %). In this study, the minimum value of bleeding volume was 0.5 mL, and the maximum value of bleeding volume was 37.40 mL, with the mean value of bleeding volume being 15.34±10.26 mL. At the level of HO-1, the minimum value was 0.08 ng/ml, and the maximum value was 816.00 ng/ml, with the mean value of HO-1 being 55.51±138.06 ng/ml. The level of MDA obtained a minimum value of 202.50 ng/mL and the maximum value of 3173 ng/mL, with an average value of MDA levels of 1066.03±907.97 ng/mL. The characteristics of study subjects, including demographic and clinical data, are summarized in Table 1.

The mean value of HO-1 levels in study

Table 1

Characteristics of study subjects

Variable	n	Percentage (%)	Mean±SD
Age (years)			60.74±13.10
Sex			
Male	22	64.7	
Female	12	35.3	
Hypertension			
Yes	26	76.5	
No	8	23.5	
Smoking			
Yes	8	23.5	
No	26	76.5	
Bleeding volume (cc)			15.34±10.26
HO-1 levels (ng/mL)			55.51±138.06
MDA levels (ng/mL)			1066.03±907.97

HO-1: Heme oxygenase-1; MDA: Malondialdehyde

subjects who smoked was 23.31±16.37 ng/mL, while the mean value of HO-1 levels in non-smoking study subjects was 65.42±157.01 ng/mL. Based on statistical analysis, there was no difference in the levels of HO-1 in the smoking and non-smoking groups with p= 0.569. The mean value of MDA levels in smoking subjects was 1283.78±952.89 ng/ml, while the mean value of MDA in non-smoking research subjects was 999.03±902.30 ng/mL. Based on statistical analysis, there were no differences in the levels of MDA in the smoking and non-smoking groups (p= 0.503).

The results of the correlation analysis between age and HO-1 levels found a statistically significant correlation between age and HO-1 levels with p= 0.043. The results of the correlation analysis between age and MDA levels found no statistically significant correlation between age with MDA heme levels with a value of p= 0.613.

In this study, the normality test was first performed on the variable bleeding volume with levels of HO-1 and MDA with the Kolmogorov-Smirnov test. From the analysis, it was found that the bleeding volume variable has a normal data distribution, but the variable HO-1 and MDA levels had an abnormal data distribution. Hence, the next analysis was performed using the spearman rank correlation. The results of this study did not find a statistically significant correlation between the volume of bleeding with HO-1 levels with a value of p= 0.605, but there was a positive correlation between the volume of bleeding with levels of MDA and statistically significant correlation with values of p= 0.002 and r= 0.509.

DISCUSSION

Our finding showed that there was a significant positive correlation between the bleeding volume with MDA levels. The results of this study are consistent with an earlier study examining the levels of oxidative stress using MDA and Total Antioxidant Capacity (TAC) in 24 bleeding stroke patients and 24 ischemic stroke patients confirmed by CT head scan associated with the level of consciousness and National Institutes of Health Stroke Scale (NIHSS) in infarction strokes, as well as the location and volume of the hematoma in bleeding strokes. In hemorrhage stroke patients, a strong positive association has been established between MDA levels and bleeding volume, and a negative correlation between TAC levels and bleeding volume (10). This finding is consistent with several reports. MDA levels increased in patients with both bleeding and acute ischemic strokes compared to controls in healthy people (11). This is different from a previous study that compared the levels of Total Oxidant Status (TOS), Total Antioxidant Status (TAS), and MDA in intracerebral hemorrhage stroke patients with healthy controls. The TOS, TAS, and MDA levels in intracerebral hemorrhage stroke were substantially higher than in the control group. Nevertheless, the Glasgow Coma Scale (GCS) total value and the volume of hematoma were not correlated between TOS, TAS, and MDA (12).

Several factors affecting levels of HO-1 and MDA include age and smoking activity. In this study, the results of the analysis of the correlation between smoking and HO-1 levels were not statistically significant. These results are different from studies in experimental animals which reported that the expression of HO-1 in the carotid arteries of rats that smoked was much higher than in normal rats. HO-1 levels can protect vascular cells from oxidative stress induced

by cigarette smoke by reducing endogenous ROS (13). Similarly, the results of the analysis for the correlation of smoking with MDA levels were not statistically significant. This result is different from another study which stated that smoking significantly increased levels of MDA with a value of p= 0.001 (14). This difference in results can be caused because in this study most of the study subjects did not smoke (76.5 %).

The results of the analysis of age variables with HO-1 levels were statistically significant. This is in accordance with a research in 2003 stating that there is a significant positive correlation between age and HO-1 levels with r= 0.894 and p= <0.01 (15). The results of the analysis of age variables with MDA levels were not statistically significant with a p-value of 0.613. Thus, age was not a confounding factor for MDA levels although these results differed from other studies which stated that age >50 years had MDA levels higher than age ≤49 years (16).

The results of the analysis of the correlation between bleeding volume with HO-1 levels were not statistically significant. The results of this study differ from studies in 2015 stating that levels of HO-1 increase in stroke patients with intracerebral hemorrhage compared with healthy people (p= <0.001) (17). Extracellular heme is derived from hemoglobin after bleeding or released from damaged cells induces the expression of HO-1 which metabolizes heme to carbon monoxide, iron, and biliverdin. Immunohistochemical studies showed that the HO-1 protein was highly detected in the peri-ICH region, especially in microglia/macrophages and endothelial cells after ICH (18).

This study has advantages. This is the first study conducted in Indonesia, especially in Surabaya, about the correlation between bleeding volume and levels of HO-1 and MDA in stroke patients with acute intracerebral hemorrhage in Dr. Soetomo Hospital Surabaya. The limitation of this study is the small number of study subjects because this study was only conducted in one hospital and many confounding factors, such as age and smoking activity that can bias the results of the study.

CONCLUSION

There was a significant correlation between the level of bleeding volume with the level of MDA in stroke patients with acute intracerebral hemorrhage in Dr. Soetomo Hospital, Surabaya, although there was no significant correlation between bleeding volume and HO-1 levels.

REFERENCES

1. Broderick J, Connolly S, Feldmann E, Hanley D, Kase C, Krieger D, et al. Guidelines for the management of spontaneous intracerebral hemorrhage in adults: 2007 update: A guideline from the American Heart Association/American Stroke Association Stroke Council, High Blood Pressure Research Council, and the Quality of Care and Ou. Circulation. 2007;116(16):e391-413.
2. Ikram MA, Wieberdink RG, Koudstaal PJ. International epidemiology of intracerebral hemorrhage. Curr Atheroscler Rep [Internet]. 2012;14(4):300-306. Available from: https://doi.org/10.1007/s11883-012-0252-1
3. Yudiarto F, Machfoed M, Darwin A, Ong A, Karyana M, Siswanto -. Indonesia Stroke Registry (S12.003). Neurology [Internet]. 2014;82(10 Suppl):S12.003. Available from: http://n.neurology.org/content/82/10_Supplement/S12.003.abstract
4. Elliott J, Smith M. The acute management of intracerebral hemorrhage: a clinical review. Anesth Analg. 2010 May;110(5):1419–27.
5. Duan X, Wen Z, Shen H, Shen M, Chen G. Intracerebral hemorrhage, oxidative stress, and antioxidant therapy. Oxid Med Cell Longev [Internet]. 2016;2016:1203285. Available from: http://europepmc.org/abstract/MED/27190572
6. Hu X, Tao C, Gan Q, Zheng J, Li H, You C. Oxidative stress in intracerebral hemorrhage: Sources, mechanisms, and therapeutic targets. In: Santos R, editor. Oxid Med Cell Longev [Internet]. 2016.p.3215391. Available from: https://doi.org/10.1155/2016/3215391
7. Mracsko E, Veltkamp R. Neuroinflammation after intracerebral hemorrhage [Internet]. Frontiers in Cellular Neuroscience. 2014;8:388. Available from: https://www.frontiersin.org/article/10.3389/fncel.2014.00388
8. Ayala A, Muñoz MF, Argüelles S. Lipid Peroxidation: Production, Metabolism, and Signaling Mechanisms of Malondialdehyde and 4-Hydroxy-2-Nonenal. In: Ramana K V, editor. Oxid Med Cell Longev

[Internet]. 2014.p.360438. Available from: https://doi.org/10.1155/2014/360438

9. Grotto D, Maria LS, Valentini J, Paniz C, Schmitt G, Garcia SC, et al. Importance of the lipid peroxidation biomarkers and methodological aspects FOR malondialdehyde quantification. Química Nova. scielo; 2009;32:169-174.
10. Shoeibi A, Razmi N, Ghabeli Juibary A, Hashemy I. The evaluation and comparison of oxidative stress in hemorrhagic and ischemic stroke. Casp J Neurol Sci. 2017;3:206-213.
11. Beg M, Ahmad S, Gandhi S, Akhtar N, Ahmad Z. A study of serum malondialdehyde levels in patients of cerebrovascular accident. J Indian Acad Clin Med. 2005;6(3):229-231.
12. Çevik MU, Acar A, Yücel Y, Varol S, Akıl E, Arıkanoğlu A, et al. Investigation of Total Oxidants/Antioxidants in Patients with Intracerebral Haemorrhage. Turkish J Neurol [Internet]. 2013;19(1):1–4. Available from: https://dx.doi.org/10.4274/Tnd.77698
13. Yang G, Li Y, Wu W, Liu B, Ni L, Wang Z, et al. Anti-oxidant effect of heme oxygenase-1 on cigarette smoke-induced vascular injury. Mol Med Rep [Internet]. 2015;12(2):2481-2486. Available from: https://doi.org/10.3892/mmr.2015.3722
14. Shah AA, Khand F, Khand TU. Effect of smoking on serum xanthine oxidase, malondialdehyde, ascorbic acid and α-tocopherol levels in healthy male subjects. Pakistan J Med Sci. 2015;31(1):146-149.
15. Hirose W, Ikematsu K, Tsuda R. Age-associated increases in heme oxygenase-1 and ferritin immunoreactivity in the autopsied brain. Leg Med (Tokyo). 2003;5(Suppl 1):S360-S366.
16. Suresh D, Kumaran S, Annam V, Veena H. Age-related changes in malondialdehyde: Total antioxidant capacity ratio - A novel marker of oxidative stress. Int J Pharma Bio Sci. 2010;1.
17. Li X, Li C, Hou L, He M, Song G, Ren S, et al. Higher level of serum heme oxygenase-1 in patients with intracerebral hemorrhage. Int Surg [Internet]. 2015;100(7-8):1220-1224. Available from: https://doi.org/10.9738/INTSURG-D-14-00086.1
18. Wang J, Doré S. Heme oxygenase-1 exacerbates early brain injury after intracerebral hemorrhage. Brain. 2007;130(Pt 6):1643-1652.