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RESEARCH ARTICLE

ROLE OF MALONDIALDEHYDE (MDA) IN PATIENTS WITH BREAST CANCER DISEASES

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ABSTRACT

Background: Breast cancer is one of the most common cancers as well as one of the leading causes of cancer mortality in women worldwide. Cancer risk potentially continues to increase because of the many sources of exposure to carcinogenic chemical compounds. Carcinogenic compounds can contribute to free radical formation which might further interact and damage biomolecules such as lipids. Lipid peroxidation will increase malondialdehyde (MDA) levels, triggering gene mutations that leads to cancer.

Objective: The purpose of this research was to measure and compare MDA levels between breast cancer patients and control.

Methods: This research was observational research using a cross sectional comparative design of 30 breast cancer patients and 30 healthy controls. The place of this research is in Ropanasuri specialized surgery hospital and biochemical laboratory, Faculty of Medicine, Andalas University, Padang. This research was conducted from August to September 2019. The MDA was measured using spectrophotometer and independent T-test was done.

Results: The result of this research showed the mean MDA level of breast cancer patients was 3.98 ± 0.35 nmol/ml, higher than controls was 3.04 ± 0.36 nmol/ml with p value = 0.001.

Conclusion: There were significant differences in MDA levels among breast cancer patients and control in Ropanasuri specialized surgery hospital, Padang.

Keywords : Breast Cancer, Malondialdehyde

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INTRODUCTION

Breast cancer constitutes as the second leading cause of cancer deaths among women. Breast cancer development consists of several processes involving various cell types, making its prevention remains challenging in the world.[1] Cancer can be a fatal disease, making it among leading causes of death globally.[2] Breast tumors typically begin from the ductal hyperproliferation, and then develop into benign tumors or even metastatic carcinomas after constant stimulation by various carcinogenic factors. Tumor microenvironments such as the stromal influences or macrophages play vital roles in breast cancer initiation and progression. The mammary gland of rats are inducable into neoplasms only when the stroma was exposed to carcinogens, not the extracellular matrix or the epithelium. [3] Macrophages can generate a mutagenic inflammatory microenvironment, which can promote angiogenesis and enable cancer cells to escape immune rejection. [4] Different DNA methylation patterns have been observed between the normal and tumor-associated microenvironments, indicating that epigenetic modifications in the tumor microenvironment can promote the carcinogenesis.[5]

Based on the World Health Organization (WHO), cancer is the second leading cause of death worldwide. In 2018, there were 9,6 milions of death caused by cancer. Among one in six deaths caused by cancer, five were of the most common cancers including lung cancer, breast cancer, colorectal cancer, prostate cancer and skin cancer.[6] According to the data of cancer (Globocan) in 2012, from 1,7 million cases of breast cancer in women worldwide, 47% were in developed countries and 52% in developing countries.[7] Incidents of breast cancer differ, 39% were recorded in Asia, 29% in Europe, 15% in America, 8% in Africa and 1.1% in Australia. From these data it can be concluded that the Asian continent is the continent with the highest breast cancer incidence.[8]

Based on Globacan estimates, International Agency for Research on Cancer (IARC) in 2012, the incidence of cancer among the Indonesian women was 134 per 100,000 population with breast cancer has highest incidence by 40 per 100,000 followed by cervical cancer 17 per 100,000 women. The mortality rate caused by breast cancer is 16.6 deaths per 100,000 population. In 2013 in the Indonesian Ministry of Health (2015), cancer incidence increased from 12.7 million cases in 2008 to 14.2 million cases in 2012. Estimated number of breast cancer cases in West Sumatra in 2013 was 2,285 cases, making West Sumatra as one of the leading provinces with breast cancer casess.[9]

The scale of the problem about breast cancer can also be seen from the number of cases of breast cancer found in Ropanasuri Surgical Hospital in Padang, the number of breast cancer cases is still high. In 2017, there were as many as 163 women, in 2018 as many as 204 women, and from January to September 2019 as many as 148 women. Based on research conducted by Harahap on Andalas University, risk factors that cause breast cancer are genetic factors, previous cancer history, hyperplasia, ionizing radiation, age of menarche, age of menopause, age at first time, number of births, use of hormone therapy and oral contrast, and lifestyle factors including physical activity, diet, and alcohol consumption.[10]

The potential risk of cancer continues to increase because of the sources of exposure to carcinogenic chemical compounds. Carcinogens, if exposed to humans, can contribute to free radicals formation in the body. Free radicals interaction with biomolecules can trigger the formation of cancerous cells. Cancer cells experience abnormal growth and differ from normal cells due to changes in gene expression or mutagenesis which leads to an imbalance of cell proliferation and cell death. Cancer cells can attack other tissues through blood vessels and lymph vessels.[11]

Carcinogenic compounds can contribute to the formation of reactive oxygen species (ROS) in the body. This ROS can interact with biomolecules such as DNA, lipids and proteins. ROS attack reaction on lipids can cause damage to lipids in the cellular membrane, forming lipid peroxidation which yield to Malondialdehyde (MDA). The low molecular weight malondialdehyde can be produced from the free radical attack on polyunsaturated fatty acids. The lipid peroxidation constitutes the oxidative conversions of polyunsaturated fatty acids to MDA, the main sensitive parameter of lipid peroxidation.[12] Malondialdehyde is a sign of oxidative stress, especially in various clinical conditions related to the process of lipid peroxidation.[13] Malondialdehyde can be formed when hydroxyl free radicals such as ROS react with fatty acid components of cell membranes so that a chain reaction is known as fat peroxidation. The fat peroxidation will break the chain of fatty acids into toxic compounds and cause damage to cell membranes.[14]

The mechanism of MDA formation through lipid peroxidation begins with the removal of hydrogen atoms (H) from long-chain unsaturated lipid molecules by hydroxyl radical groups (OH), making radical lipids. Then these lipid radicals react with oxygen atoms (O_2) to form peroxyl radicals, which subsequently results in MDA (with more than three unsaturated bonds).[15]

Malondialdehyde is a natural product from lipid peroxidation capable of DNA interaction to form different adducts, including Malondialdehyde-1-deoxyguanosine (M1dG). Malondialdehyde-1-deoxyguanosine is mutagenic and triggers carcinogenesis.[16] Increased ROS levels in cancer cells are often regarded as adverse factors that cause genetic instability. In cancer cells there is an abnormal increase in ROS with high oxidative stress which makes the cancer cells to be more susceptible to further oxidative stress.[17] Based on the background above, the researcher was interested to measure and compare MDA levels between breast cancer patients and control.

MATERIAL AND METHODS

This research had been approved by the Ethics Commission of The Faculty of Medicine, Universitas Andalas (No.381/KEP/FK/2019). We conducted an observational study using cross-sectional comparative design to measure and compare MDA levels between breast cancer patients and healthy controls. This research was conducted at the Hospital for Specialized Surgery Ropanasuri Padang with subjects of 30 breast cancer patients who have not received chemotherapy and radiation and 30 healthy people as controls. Criteria for both groups were: aged <50 years old or premenopausal and did not have comorbidities (hypertension, diabetes, and obesity). Research subjects were interviewed and blood drawn (3 ml) and then taken to the biochemical laboratory of

the Andalas University Medical School Padang for analysis. MDA level was measured using Thiobarbituric acid and results were read using a spectrophotometer.

To measure MDA levels, the blood samples were let stand for 30 minutes in room temperature and centrifuged (2000 rpm in 15 minutes) to get serum, then prepare the tube by the following procedure: aquades, standard, add 2.5 ml of 5% TCA at each tube, mix using a vortex mixer then centrifuge for 15 minutes, at a speed of 3000 RPM, after which it is taken using a 1 ml pipet, put in a tube according to the label and add 1 ml each of Na Thiobarbituric Acid and mix it using a vortex mixer then heat it in boiling water bath for 30 minutes then cool and finally read the absorbance with a spectrophotometer at λ 550 nm.

The data were processed using the Statistical Package for Social Science (SPSS). Statistical tests utilized were based on the distribution of the obtained data. If the data were normally distributed the independent T-test, but if the data were not normally distributed after log10 transformation of data, Mann-Whitney test will be utilized.

RESULTS

There were 30 breast cancer patients aged 39.83 ± 6.58 and 30 control aged 34.80 ± 7.40 (Table 1). The normality test was carried out using the Shapiro Wilk test (Table 2) and it was found that the MDA level in breast cancer patients and healthy controls were normally distributed with p> 0.05, then continued with the independent T-test. MDA levels were determined in 30 patients with breast cancer and 30 healthy controls, which is considered as a control group. Based on statistical test (Table 3) there was a significant difference in MDA levels in breast cancer patients and healthy controls (p = 0.001). MDA serum levels were higher in breast cancer sufferers compared to control.

Table 1. The age of the breast cancer patient and healthy controls.

Characteristic	Breast cancer mean±SD	Healthy controls mean±SD
Age	39.83 ± 6.58	34.80 ± 7.40

Table 2. Normality test results for MDA levels

	Groups	Shapiro-Wilk	
		N	*P value*
Levels of MDA	Breast cancer	30	0.128
	Healthy controls	30	0.106

Table 3. MDA comparison between breast cancer patient and healthy control

Groups	N	Levels of MDA (nmol/ml) Mean±SD	*P value*
Breast cancer	30	3.98 ± 0.35	0.001
Healthy controls	30	3.04 ± 0.36	

DISCUSSION

According to the research find out that the age of the respondent average in the breast cancer fertile women age and premenopause are 39.83 + 6.58 years and healthy controls subject 34.80 + 7.40 years. Among various factors known to be the initiators of breast cancer, age is a trigger

factor for breast cancer. The incidence of breast cancer according to research conducted by Pane et al (2014) states that the age range of breast cancer sufferers aged 27-81 years and the average age of 48 years, there is a tendency for cases to be diagnosed at an earlier age due to the lifestyle of the respondents. The research conducted by Thangjam et al (2014) also found that out of 507 cases of breast cancer, 160 cases (31.56%) were under 40 years old and 347 (68.44%) were over 40 years.[18]

Similar research was also conducted by Rahmatya & Khambri among 46 people, the highest age of breast cancer sufferers in the Surgery Section of RSUP Dr. M. Djamil Padang in 2012 was found to be in the age range of 40-49 years with an average age of 46.87 years. The youngest was 32 years old while the oldest was 67 years old. These results were also consistent with research conducted by Azamris in Padang in 2006 which stated that the peak age of breast cancer sufferers at RSUP Dr. M. Djamil Padang among the ages of 40-50 years (34.3%) with an average age of 46.7 years.[19]

The Statistical test results showed that the mean MDA level in breast cancer patients was 3.98 + 0.35 nmol/ml while the average MDA level in healthy controls was 3.04 + 0.36 nmol/ml. The result of independent T-test obtained p-value = 0.001, therefore there was significant difference in MDA levels between breast cancer patients and healthy controls. Increased MDA levels in breast cancer sufferers might be due to induction of breast cancer cells to increase ROS that can induce oxidative stress followed by molecular damage and including lipid peroxidation.[20]

ROS level elevation, redox balance alteration, and redox signaling deregulation are common hallmarks of cancer progression and treatment resistance. ROS generation is mainly contributed by mitochondria during oxidative phosphorylation. Elevated ROS levels detected in cancer cells might due to several aspects, such as high metabolic activity, cellular signaling, peroxisomal activity, mitochondrial dysfunction, oncogene activation, and increased enzymatic activity of oxidases, cyclooxygenases, lipoxygenases, and thymidine phosphorylases. Intracellular homeostasis is maintained by developing an immense antioxidant system including catalase, superoxide dismutase, and glutathione peroxidase. Besides these enzymes, important antioxidant glutathione and transcription factor Nrf2 also contribute to balancing oxidative stress. ROS-mediated signaling pathways activate pro-oncogenic signaling which promotes cancer progression, angiogenesis, and survival. Additionaly, to maintain ROS homeostasis and evade cancer cell death, cancer cells increase antioxidant capacity level.[21]

One of the most produced lipid peroxidation aldehydes is MDA. It can react with proteins and DNA causing gene mutations that will trigger the formation of cancer cells besides increasing MDA levels as a marker of cancer cell development.[22] Increased MDA in breast cancer patients is associated with excessive ROS production and deficiency of antioxidant defenses. Excessive ROS production is triggered by exposure to chemical, biological and physical carcinogenic substances. A significant increase in MDA in cancer along with a decrease in antioxidants indicates the higher levels of oxidative stress and lower levels of antioxidant defenses. This event plays an important role in tumor development and the pathogenesis that

results from gene mutations caused by increased levels of MDA.[20]

A similar research conducted by Sahu et al showed the increase in MDA levels in breast cancer patients with an average of 5.8 + 3.2 nmol/ml and a control group of 1.9 + 0.28 nmol/ml with p-value = 0.01 ($p < 0.05$) so that there were statistically significant differences between the breast cancer group and the control group. MDA is a product of lipid peroxidation caused by an increase in ROS in the body, which can lead to the development of breast cancer cells.[23]

CONCLUSION

There was a significant difference in MDA levels in breast cancer patients and healthy controls. MDA level measurement is expected to be used as an indicator of breast cancer in women who are often exposed to carcinogenic substances as an effort to detect and prevent breast cancer in women.

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REFERENCES

1. Sun Y, et al. Risk factors and preventions of breast cancer. Internasional Journal of Biological Sciences. 2017; 13(11): 1387–1397.

2. World Helath Organization WHO. (2016). Breast cancer:prevention and control. Available from: http://www.who.int/cancer/detection/breastcancer/en/index1.html. (Accessed 30 Maret 2019).

3. Sonnenschein C, Soto AM. Carcinogenesis explained within the context of a theory of organisms. Progress in biophysics and molecular biology. 2016;122(1): 70–76.

4. Dumars C, Ngyuen JM, Gaultier A, et al. Dysregulation of macrophage polarization is associated with the metastatic process in osteosarcoma. Oncotarget. 2016; 7(48): 78343–54.

5. Basse C, Arock M. The increasing roles of epigenetics in breast cancer: Implications for pathogenicity, biomarkers, prevention and treatment. Int J Cancer. 2015; 137 (12):2785–2794.

6. World Helath Organization WHO. (2018). Cancer. Available from: http://www.who.int/mediacenter/factsheets/fs297/en/. (Accessed 08 Juli 2019).

7. Torre AL. Surveilance and health services research. Atlanta: American cancer Society. 2016;14: 16-27.

8. Desantis CE, et al. Breast cancer statistics 2015: Convergence of Incidence rates between black and white women. CA Cancer J Clin. 2016; 66 (01): 31-42.

9. International Agency for Research on Cancer (IARC). Latest world cancer statistics global cancer burden rises to 14.1 million new cases in 2012: marked increase in breast cancers must be addressed. Lyon: International Agency Of Research on Cancer. 2012; (12).

10. Harahap WA. Cancer epidemiology & cancer risk factors.

Surgery departemen. Andalas Medical School. 2016; 12 (8):1-8.

11. Askoxylakis V, et al. Long-term survival of cancer patients compared to heart failure and stroke: A systematic review. BMC Cancer. 2010; 10 (15):1-8.

12. Gubaljević JG, et al. Serum levels of oxidative stress marker malondialdehyde in breast cancer patients in relation to pathohistological factors, estrogen receptors, menopausal status, and age. Journal of Health Sciences. 2018;8(3):154-161.

13. Ayala, A. Munoz, MF. Arguelles, S. Lipid Peroxidation: Production, metabolism and signaling mechanism of malondialdehyde and 4-Hydroxy-2-nonenal. Oxid med Cell Longev. 2010;112 (20): 1-31.

14. Asni E, et al. Pengaruh hipoksia berkelanjutan terhadap kadar malondialdehid, glutation tereduksi, dan aktivitas katalase ginjal tikus. Majalah Kedokteran Indonesia. 2009; 59 (12): 595-600.

15. Yusrika AR. Aulanni'am. Prasetyawan, S. Kadar malondialdehid (MDA) dan gambaran histologi pada ginjal tikus putih (Rattus norvegicus) pasca induksi cylosporine-A. Kimia Student Journal. 2013;1 (02) : 222-228.

16. Ma B, Villalta PW, Balbo S, Stepanov I. Analysis of a malondialdehyde-Deoxyguanosine adduct in human leukocyte DNA by liquid chromatography nanoelectrospray-high-resulation tandem mas spectrometry. Chemical Research in Texicology. 2010; 27 (10) : 1829-1836.

17. Wang J, et al. Inhibition of cancer growth in vitro and vivo by a novel ROS-modulating agent with ability to eliminate stem-like cancer cells. Offocial Journal of the Cell death Differentiation Association. 2017;8 : 1-9.

18. Thangjam S, Laishram RS, Debnath K. Breast carcinoma in young females below the age of 40 years: a hispathological perspective. South Asian Journal of Cancer. 2014; 3 (02) : 97-100.

19. Rahmatya A, Khambri D. Hubungan usia dengan gambaran klinikopatologi kanker payudara di bagian bedah RSUP Dr. M.Djamil Padang. Jurnal Kesehatan Andalas. 2015; 4 (2): 479-484.

20. Bhattacharjee, J. Jogdand, S. Shinde, RK. Goswami, S. Assessment of oxidative stress in breast cancer patients: a Hospital based study. Internasional Journal of Basic & Clinical Pharmacology. 2018;7 (5): 966-970.

21. Kumari S, Badana AK, G MM, G S, Malla R. Reactive oxygen species: A key constituent in cancer survival. Biomark Insights. 2018;13: 1177271918755391.

22. Hauck AK, Bernlohr DA. Oxidative stress and lipotoxicity. Journal of Lipid Research. 2016; 57 (31) : 1976.

23. Sahu A, Varma M, Kachhawa K. A Prognostic study of MDA, SOD and Catalase in Breast Cancer Patients. Internasional Journal of Science and Research. 2013; 4 (05) : 157-159.