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Association of MnSOD Val16Ala (rs4880) Polymorphism with Staging, Grading, and Molecular Profile of Breast Cancer Patients at Prof. Dr. I.G.N.G. Ngoerah Hospital Denpasar Bali

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ABSTRACT

Background: Aging is the major risk factor for breast cancer. Aging and breast cancer are interrelated due to the accumulation of reactive oxygen species (ROS). Manganese superoxide dismutase (MnSOD) has a pivotal role to defense ROS. The MnSOD Val16Ala (rs4880) polymorphism affects the activity of MnSOD in ROS detoxification. This study aims to prove the association of MnSOD Val16Ala (rs4880) polymorphism with stage, grading, and molecular profile of breast cancer patients at Prof. Dr. I.G.N.G. Ngoerah Hospital Denpasar Bali.
Methods: This study used a cross-sectional exploratory descriptive method. It was conducted at the Integrated Biomedical Laboratory Unit of the Faculty of Medicine, Udayana University from September to November 2022. The samples used in this study were 45 biological materials in the form of DNA from breast cancer patients who were treated at the Oncology Unit of Prof. Dr. I.G.N.G. Ngoerah Hospital from 2016 to 2018 which is stored in the Biochemistry Laboratory of the Faculty of Medicine, Udayana University. The MnSOD Val16Ala (rs4880) polymorphism was examined by DNA amplification using polymerase chain reaction (PCR) method and the identification of the polymorphism by sequencing. The results obtained were analyzed using descriptive analysis with SPSS program.
Results: The sequencing results found 45 samples (100%) with homozygous wild-type T/T. The association between MnSOD Val16Ala (rs4880) polymorphism and staging, grading, and molecular profile was unable to analyze.
Conclusions: The MnSOD Val16Ala (rs4880) polymorphism was not found in breast cancer patient in Prof. Dr. I.G.N.G. Ngoerah hospital, Denpasar, Bali. Further research is needed to understand the role of MnSOD Val16Ala (rs4880) polymorphism with breast cancer risk.

Keywords: superoxide dismutase enzyme, polymorphism, breast cancer

INTRODUCTION

Aging is a process related to the accumulation of reactive oxygen species (ROS)[1]. The accumulation of ROS increases with age which can cause DNA mutation[1,2]. It is related to the development of breast cancer (BC)[3]. The risk of BC is increasing with age[4]. BC is the most common cancer in women and its incidence is increasing every year[4]. The accumulation of ROS can be overcome by the activity of manganese superoxide dismutase (MnSOD) which works in matrix mitochondria to dismutase radical superoxide to hydrogen peroxide and singlet oxygen[5].
MnSOD has two distinct roles in cancer development, as tumour suppressor gene (TSG) or oncogene[6]. The role of MnSOD as TSG is the low expression of MnSOD

increases radical superoxide levels thus causing DNA damage, oncogenic transformation, and tumorigenesis[6]. The role of MnSOD as oncogene is due to high MnSOD expression in metastatic phase of breast cancer[7]. High expression of MnSOD can cause matrix degradation and release of cytokines, as well as growth factors, thus triggering metastasis[6,7].

Genetic variations in the MnSOD gene can alter the activity of ROS detoxification[6]. The MnSOD Val16Ala (rs4880) is the most studied single nucleotide polymorphism (SNP) in MnSOD gene. This SNP is located in the second exon of MnSOD gene that subtitutes thymine (T) to cytosine (C) at nucleotide 47, resulting the changing of amino acid valine (Val, GTT) to alanine (Ala, GCT) in 16th sequence of amino acids[8]. BC patients with Ala/Ala genotype had a low survival rate[9]. Ala allele had higher MnSOD activity in cancer cells causing invasion and metastasis of tumour cells[9]. BC patients with Val/Val genotype more often found in early stage of BC, meanwhile Val/Ala genotype related to more invasive and advance stage of BC[10].

The difference of MnSOD activity in BC affects ROS in cancer cells which can activates signalling pathways related to cancer invasion and metastasis[7]. MnSOD Val16Ala (rs4880) gene polymorphism may determine the BC susceptibility to invade and metastasis. Thus, we consider it important to determine the frequency of the MnSOD Val16Ala (rs4880) gene polymorphisms variants, and whether there is an association between this polymorphism with stage, grade, and molecular profile of BC patients in Prof. Dr. I.G.N.G. Ngoerah Hospital, Denpasar, Bali.

METHODS

DNA samples were collected from 45 women (38-86 years old) participating in the study with clinically and histologically confirmed BC. They were treated at the Oncology Department of Prof. Dr. I.G.N.G. Ngoerah Denpasar Bali since 2016-2018. Clinical and pathological data were obtained from medical records. This research using real time PCR to identify the MnSOD Val16Ala (rs4880) gene polymorphism. The primer used in this research was forward primer : 5'- AGCCCAGCCTGCGTAGAC -3', and reverse primer : 5'-TACTTCTCCTCGGTGACG -3'. PCR were carried out in 25 µl reaction volume containing 1 µl of each forward and reverse primer, 12.5 µl of MyTaq HS Red Mix, 1 µl of DNA template, and 9.5 µl of nuclease-free water. Thermocycler (Biometra, Germany) was used to do amplification. The setting used was 5 min pre-denaturation at 94ºC; 35 cycles with denaturation for 1 min at 94ºC, annealing for 1 min at 57ºC, elongation for 1 min at 72ºC; and final elongation of 5 min at 72ºC.

The electrophoresis was conducted in 1% agarose with ethidium bromide 5 µl in 100 A, 50 Watt for 40 min. The PCR results were visualized with transilluminator with UV Rays (UV Star 312 nm, Biometra, Germany).

The PCR products were sent to Genetika Science Indonesia company to do sequencing of the DNA. The electropherogram of the DNA samples were examined using the *Snapgene*®.

The SPSS statistical software version 25 (SPSS Inc., Chicago, IL) was used to analyse the data.

RESULT

The clinical data from BC patients showed the mean age at diagnosis, age at menarche, menopause status (Table 1).

Table 1. Demographic data of samples

Variables	**N=45**
Age at diagnosis (mean±SD)	52.11 ±10.579
≥ 50 tahun [n, (%)]	24 (53.3%)
< 50 tahun [n, (%)]	21 (46.7%)
Age at menarche (mean±SD)	**13.44 ± 1.407**
≥ 13 tahun [n, (%)]	32 (71.1%)
< 13 tahun [n, (%)]	13 (28.9%)
Menopause status	
Premenopausal [n, (%)]	20 (44,4%)
Postmenopausal [n, (%)]	25 (55,6%)

The clinical characteristics of the subjects are shown in Table 2. The clinical characteristics of BC patients were in T4

(60%), N1 (48,9%), no metastasis (64,4%), advance stage (80%), grade 3 (44,4%), and Luminal B (46,7%).

Table 2. Clinical characteristics data of samples

Variables		N=45
Tumour		
	T1 [n, (%)]	3 (6,7%)
	T2 [n, (%)]	7 (15,6%)
	T3 [n, (%)]	8 (17,8%)
	T4 [n, (%)]	27 (60%)
Nodule		
	N0 [n, (%)]	13 (28,9%)
	N1 [n, (%)]	22 (48,9%)
	N2 [n, (%)]	7 (15,6%)
	N3 [n, (%)]	3 (6,7%)
Metastatic status		
	No metastasis [n, (%)]	29 (64,4%)
	Metastasis [n, (%)]	16 (35,6%)
Stage		
	Early stage [n, (%)]	9 (20%)
	Advance Stage [n, (%)]	36 (80%)
Histologic Grade		
	Grade 1 [n, (%)]	8 (17,8%)
	Grade 2 [n, (%)]	17 (37,8%)
	Grade 3 [n, (%)]	20 (44,4%)
Molecular profile		
	Luminal A [n, (%)]	9 (20%)
	Luminal B [n, (%)]	21 (46,7%)
	HER2 [n, (%)]	9 (20%)
	TNBC [n, (%)]	6 (13,3%)

The results showed the presence of single band (207 bp) of the target sequence of MnSOD Val16Ala (rs4880) gene in agarose gel as in figure (1). The sequencing of DNA samples was examined in the NCBI website to confirm the MnSOD gene with BLAST (Basic Local Alignment Search Tool).

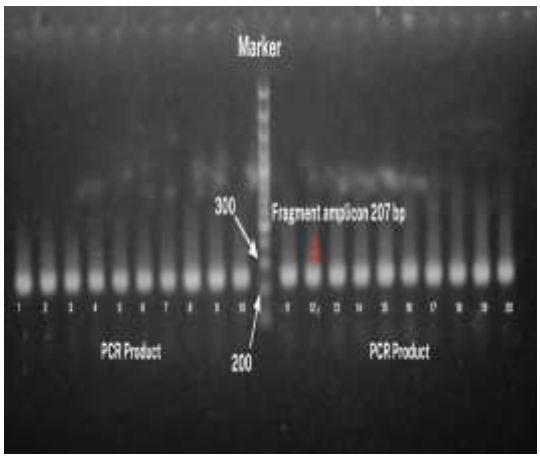

Figure 1. Agarose gel electrophoresis showed patterns of MnSOD Val16Ala (rs4880)

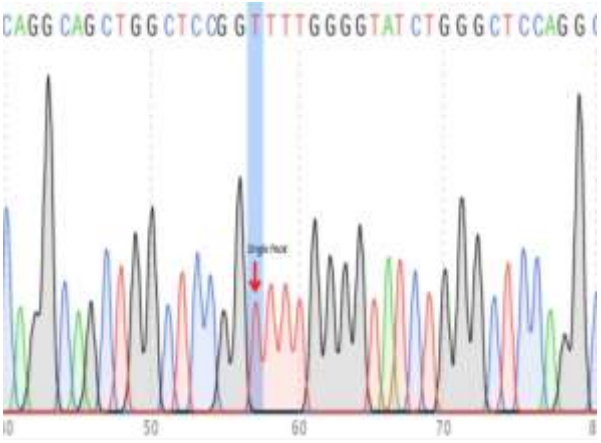

Figure 2. The electropherogram from DNA sequencing with Forward Primer showing Homozygote T/T Genotype

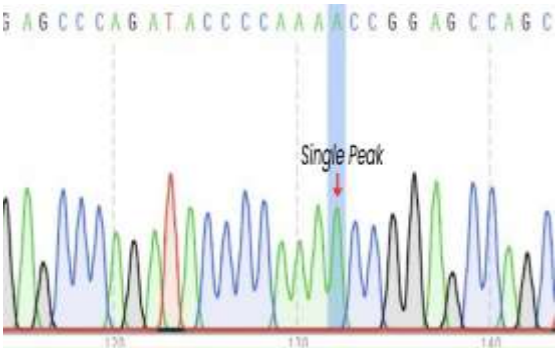

Figure 3. The electropherogram from DNA sequencing with Reverse Primer showing Homozygore A/A Genotype

All of the samples (45 samples) were homozygote wild type T/T genotype. The bivariate analysis of MnSOD Val16Ala (rs4880) gene polymorphism with stage, grade, and molecular profile was not able to conduct.

Table 3. Genotype distribution of rs4880 polymorphism

Hasil *sequencing* gen MnSOD		N=45
	TT [n, (%)]	45 (100%)
	CT [n, (%)]	0 (0%)
	TT [n, (%)]	0 (0%)

DISCUSSION

In the present study, we describe a brief clinical characteristics of the samples. BC is considered as a major health problem in women around 50 years of age[4,11,12]. The role of aging can be seen in this study, majority of the samples is more than 50 years of age and in postmenopausal status. Aging is related to the accumulation of genetic and cellular defects which increasing the carcinogenic potential and carcinogenesis[4]. The age of menarche in this study was ≥13 years of age. This finding

was inconsistent with the study conducted in the United States which found BC was more likely to occur in women with early menarche, under 12 years old[13]. This difference of this findings may be caused by error recording of the medical record[14]. Recording of menarche age in self-reported middle age patients was found to be inaccurate[15]. Patients' education and social status also affects the accuracy of menarche age medical records[15].

All of the participants in this study were homozygous wild-type T/T. The frequency of Val16 and Ala16 in western population is considered equal[16]. However, in Asian population the Val16 variant is found to be dominant with more than 85% in population[16]. This finding supports the statement that the Val variant in MnSOD Val16Ala (rs4880) polymorphism has β-sheet configuration that causing some of it retained in the mitochondrial inner membrane[17]. Thus the Val variant was found to form 30-40% of MnSOD protein which is less active than the Ala variant[17]. The MnSOD Val/Val genotype had lower MnSOD activity which is harmful to breast epithelial cells[18]. The mechanism of cancer initiation was mediated by the accumulation of ROS which cause oxidative damage[19]. The MnSOD works in the main location where ROS production occurs[6]. The decrease in the activity of MnSOD triggers the accumulation of ROS at the cellular level[19]. The accumulation of it causes oxidation and leads to cancer[10].

The majority of the patients were in an advance stage (80%). These findings were consistent with the study conducted in Jogjakarta, Indonesia which found the majority of the BC patients were in an advance stage[20]. However, this finding is not consistent with other studies conducted in China and Jordan which found Val/Val genotype was tend to be in early stage of BC (stage I – II)[10,21]. In the developing country, women with breast cancer often come to seek medical advice in advance stage of BC due to several factors such as level of knowledge, socio-economic problems, and stigma against BC[4,22].

This study found the majority of participants (64.4%) had no metastases BC. There result is consistent with the study conducted in the United States and Norway populations which found the Val variant did not cause metastases compared to Ala variant[9]. The Ala variant triggers higher MnSOD activity in cancer cells causing tumour cells becomes more susceptible to invasion and metastases than Val variant[6,9]. The increasing activity of MnSOD in cancer cells without the increasing activity of catalase leads to the accumulation of hydrogen peroxide (H_2O_2)[6]. It causes upregulation of MMP-1, VEGF, and MMP-9 which has contribution in invasion and metastases of tumour cells[6].

Most patients in this study were found in histological grade 3 (44.4%). These results are similar with the study conducted in West Java, Indonesia which found the most common histological grade was grade 3[23]. Grade 3 BC has poor differentiation, rapid, uncontrolled growth, and undifferentiated[24]. The most common molecular profile in this study was luminal B (46.7%). This result is consistent with the similar study conducted in East Kalimantan which found most common molecular profile of the BC patients was luminal B[25]. The result of histological grade and molecular profile in this study were consistent with the statement states molecular profile luminal B often found with higher histological grade[4].

In this study, the association between the MnSOD Val16Ala (rs4880) polymorphism with stage, grade, and molecular profile of BC patients could not be assessed. However, this result is consistent with meta analyses study which showed there was no significant association between MnSOD Val16Ala (rs4880) polymorphism with BC or the survival rate of BC patients [26].

This study has limitations. This study did not compare the MnSOD Val16Ala (rs4880) polymorphism with controls (healthy participants) and with BC patients. Thus, the association between the MnSOD Val16Ala

(rs4880) polymorphism with risk of BC in Prof. Dr. I.G.N.G. Ngoerah Denpasar Bali is unknown.

CONCLUSION

The MnSOD Val16Ala (rs4880) polymorphism was not found in BC patient in Prof. Dr. I.G.N.G. Ngoerah hospital, Denpasar, Bali. The association of MnSOD Val16Ala (rs4880) polymorphism with stage, grade, and molecular profile of BC could not be assessed. Further research with case control method is needed to understand the role of MnSOD Val16Ala (rs4880) polymorphism with breast cancer risk.

Declaration by Authors

Ethical Approval: This research has an ethical clearance number: 2315/UN14.2.2.VII.14/LT/2022 from the ethics commissions of Udayana University.

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Conflict of Interest: The authors declare no conflict of interest.

REFERENCES

1. López-Otín C, Blasco MA, Partridge L, Serrano M, Kroemer G. The hallmarks of aging. Vol. 153, Cell. Elsevier B.V.; 2013. p. 1194.
2. Pangkahila W. Anti Aging Medicine Tetap Muda dan Sehat. Penerbit Buku Kompas. 2019;9–21.
3. Cui H, Kong Y, Zhang H. Oxidative Stress, Mitochondrial Dysfunction, and Aging. J Signal Transduct. 2012 Oct 2;2012:1–13.
4. Łukasiewicz S, Czeczelewski M, Forma A, Baj J, Sitarz R, Stanisławek A. Breast Cancer—Epidemiology, Risk Factors, Classification, Prognostic Markers, and Current Treatment Strategies - An Updated Review. Cancers (Basel) [Internet]. 2021 Aug 25;13(17):4287. Available from: https://www.mdpi.com/2072-6694/13/17/4287
5. Kumari S, Badana AK, G MM, G S, Malla R. Reactive Oxygen Species: A Key Constituent in Cancer Survival. Biomark Insights [Internet]. 2018 Jan 1;13:117727191875539. Available from: http://journals.sagepub.com/doi/10.1177/1177271918755391
6. Alateyah N, Gupta I, Rusyniak RS, Ouhtit A. SOD2, a Potential Transcriptional Target Underpinning CD44-Promoted Breast Cancer Progression. Molecules [Internet]. 2022 Jan 26;27(3):811. Available from: https://www.mdpi.com/1420-3049/27/3/811
7. Becuwe P, Ennen M, Klotz R, Barbieux C, Grandemange S. Manganese superoxide dismutase in breast cancer: From molecular mechanisms of gene regulation to biological and clinical significance. Free Radic Biol Med [Internet]. 2014 Dec;77:139–51. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0891584914004171
8. Liu G, Sun G, Wang Y, Wang D, Hu W, Zhang J. Association between manganese superoxide dismutase gene polymorphism and breast cancer risk: A meta-analysis of 17,842 subjects. Mol Med Rep. 2012 Oct;6(4):797–804.
9. Glynn SA, Boersma BJ, Howe TM, Edvardsen H, Geisler SB, Goodman JE, et al. A mitochondrial target sequence polymorphism in manganese superoxide dismutase predicts inferior survival in breast cancer patients treated with cyclophosphamide. Clinical Cancer Research. 2009 Jun 15;15(12):4165–73.
10. Atoum M, Abdel-Fattah M, Nimer N, Abdel-Rahman S, Abdeldayem SA. Association of Alanine-Valine Manganese Superoxide Dismutase Gene Polymorphism and Microheterogeneity Manganese Superoxide Dismutase Activity in Breast Cancer and Benign Breast Tissue. J Breast Cancer [Internet]. 2012 Jun;15(2):157. Available from: https://ejbc.kr/DOIx.php?id=10.4048/jbc.2012.15.2.157
11. Amandito R, Viryawan C, Santoso F, Gautami W, Soni Panigoro S. The Characteristics of Breast Cancer Patients in "Dharmais" Hospital National Cancer Center Jakarta Based on Occupational and Environmental Status. Vol. 7, ARTIKEL PENELITIAN Indonesian Journal of Cancer. 2013.
12. Putra IGIP, Saputra H, Maker LPII, Sriwidyani NP. Karakteristik Klinikopatologi Penderita Karsinoma Payudara di RSUP Sanglah Tahun 2013-2016. Jurnal Medika Udayana [Internet].

2019 [cited 2022 Aug 11];8(11). Available from: https://ojs.unud.ac.id/index.php/eum/article/view/55021/32627
13. Goldberg M, D'Aloisio AA, O'Brien KM, Zhao S, Sandler DP. Pubertal timing and breast cancer risk in the Sister Study cohort. Breast Cancer Research. 2020 Dec 1;22(1).
14. Moradzadeh R, Mohammad ;, Mansournia A, Ghiasvand R, Baghfalaki T, Haidar Nadrian ;, et al. Impact of Age at Menarche on Breast Cancer: The Assessment of Recall Bias [Internet]. Vol. 22, Arch Iran Med. 2019. Available from: www.aimjournal.ir
15. Cooper R, Blell M, Hardy R, Black S, Pollard TM, Wadsworth MEJ, et al. Validity of age at menarche self-reported in adulthood. J Epidemiol Community Health (1978) [Internet]. 2006 Nov 1;60(11):993–7. Available from: https://jech.bmj.com/lookup/doi/10.1136/jech.2005.043182
16. Kim A. Modulation of MnSOD in Cancer: Epidemiological and Experimental Evidences. Toxicol Res. 2010;26(2):83–93.
17. Sutton A, Imbert A, Igoudjil A, Ronique Descatoire V, Cazanave S, Pessayre D, et al. The manganese superoxide dismutase Ala16Val dimorphism modulates both mitochondrial import and mRNA stability. Vol. 15, Pharmacogenetics and Genomics. 2005.
18. Bica CG, de Moura Da Silva LL, Toscani NV, da Cruz IBM, Sá G, Graudenz MS, et al. MnSOD gene polymorphism association with steroid-dependent cancer. Pathology and Oncology Research. 2009 Mar;15(1):19–24.
19. Klaunig JE, Wang Z. Oxidative stress in carcinogenesis. Curr Opin Toxicol. 2018 Feb 1;7:116–21.
20. Sinaga ES, Ahmad RA, Shivalli S, Hutajulu SH. Age at diagnosis predicted survival outcome of female patients with breast cancer at a tertiary hospital in Yogyakarta, Indonesia. Pan African Medical Journal. 2018;31.
21. Li L, Zheng L, Lin M, Li P. Association between manganese superoxide dismutase gene Val16Ala polymorphism and breast cancer in Hainan province. Cancer Research and Clinic. 2020 Apr 1;32(4):280–4.
22. Indra, Manginstar C, Islam AA, Sampepajung D, Hamdani W, Bukhari A, et al. The relationship between NFKB, HER2, ER expression and anthracycline -based neoadjuvan chemotherapy response in local advanced stadium breast cancer: A cohort study in Eastern Indonesia. Annals of Medicine and Surgery. 2021 Mar 1;63.
23. Fajar IM, Heriady Y, Aji HW. Karakteristik Usia, Gambaran Klinis dan Histopatologi Pasien Kanker Payudara di RSUD Al-Ihsan Provinsi Jawa Barat Periode Januari 2018 - Oktober 2020. Jurnal Riset Kedokteran. 2021 Dec 31;1(2):85–91.
24. WHO Classification of Tumours Editorial Board, International Agency for Research on Cancer. Breast tumours [Internet]. 5th ed. Vol. 2. Lyon (France): International Agency for Research on Cancer; 2019 [cited 2022 Aug 12]. Available from: https://publications.iarc.fr/581.
25. Paramita S, Raharjo EN, Niasari M, Azizah F, Hanifah NA. Luminal B is the most common intrinsic molecular subtypes of invasive ductal breast carcinoma patients in East Kalimantan, Indonesia. Asian Pacific Journal of Cancer Prevention. 2019;20(8):2247–52.
26. Wang P, Zhu Y, Xi S, Li S, Zhang Y. Association between MnSOD Val16Ala Polymorphism and Cancer Risk: Evidence from 33,098 Cases and 37,831 Controls. Dis Markers [Internet]. 2018 Sep 2;2018:1–16. Available from: https://www.hindawi.com/journals/dm/2018/3061974/

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