Profile of malondialdehyde (MDA) and catalase specific activity in plasma of elderly woman

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Abstrak

Latar belakang: *Malondialdehida (MDA) merupakan petanda stres oksidatif yang merupakan produk akhir dari reaksi berantai proksidasi lipid. Untuk mencegah stres oksidatif, tubuh mensintesis katalase, suatu enzim antioksidan endogen yang mengkatalisis hidrogen peroksida (H_2O_2) menjadi air dan oksigen. Sampai saat ini kadar MDA dan katalase pada populasi usia lanjut (usila) masih memberikan hasil yang bervariasi dan kadar tersebut pada kelompok usia yang berbeda dalam populasi usila belum pernah dilaporkan. Dengan demikian, penelitian ini bertujuan untuk menganalisis profil kadar MDA dan aktivitas spesifik katalase pada plasma populasi usila berdasarkan peningkatan usia.*

Metode: *Penelitian ini menggunakan 60 subjek wanita usila sehat yang tinggal di Jakarta. Subjek dibagi dalam 2 kelompok berdasarkan kategori usia, kelompok yang lebih muda (60 – 70 tahun) dan kelompok yang lebih tua (> 70 tahun). Kadar MDA dan aktivitas spesifik katalase dianalisis pada plasma dengan spektrofotometer.*

Hasil: *Kadar MDA pada kelompok yang lebih muda (60 – 70 tahun) sedikit lebih tinggi dibandingkan kelompok yang lebih tua (> 70 tahun) namun tidak bermakna secara statistik. Selain itu, akitivitas spesifik katalase pada kelompok yang lebih muda lebih rendah bermakna dibandingkan dengan kelompok yang lebih tua.*

Kesimpulan: *Tidak ada perbedaan bermakna kadar MDA plasma pada populasi usila. Namun, aktivitas spesifik katalase meningkat bermakna seiring dengan pertambahan usia.* ***(Health Science Journal of Indonesia 2019;10(2):132-6)***

Kata kunci: *Malondialdehida, katalase, wanita usila*

Abstract

Background: Malondialdehyde (MDA) is a marker of oxidative stress as an end product from the chain reaction of lipid peroxidation. In order to prevent oxidative stress, our body synthesizes catalase, an endogenous antioxidant enzyme that catalyzes hydrogen peroxide (H_2O_2) into water and oxygen. Until now, the level of MDA and catalase in aging population were still varied and those level at different age in elderly population has not been yet reported. Therefore, the purpose of this study was to analyse the profile of MDA level and catalase specific activity in plasma of elderly women based on increasing age.

Methods: This research used 60 healthy elderly women as the subjects living in Jakarta. The subjects were divided into 2 groups based on age category, the younger group (60 – 70 years old) and the older group (>70 years old). MDA and specific activity of catalase were analyzed in plasma using spectrophotometer.

Results: MDA level in the younger group (60-70 years old) was slightly higher than MDA levels in the older group (>70 years old) but it was not significant. Moreover, specific activity of catalase in the younger group was significantly lower than the older group.

Conclusions: There was no difference in MDA level of elderly woman between younger and older group. However, catalase specific activity significantly increased with increasing age. ***(Health Science Journal of Indonesia 2019;10(2):132-6)***

Keywords: Malondialdehyde, Catalase, elderly woman

Aging is a multidimensional process, in which the mechanism of destruction and repair in the body or the system occurs alternately at different speeds and times.[1] The aging process is difficult to understand because it is also difficult to distinguish between the normal aging process and the process due to an illness.[1] One factor as the contributor in aging process is oxidative stress which occurs due to excessive production of reactive oxygen species (ROS) exceed antioxidant capacity.[2] ROS is produced regularly from cellular respiration in mitochondria, phagocytosis process and hydroxylation of drug in liver. Moreover, environment pollution and radiation contamination exposed in the body act as external source of ROS.[3] Excessive ROS can irreversibly damage cellular components and cause cell death through the intrinsic apoptosis pathway in the mitochondria and triggers mitochondrial DNA damage.[4] Increased apoptosis is associated with cell reshuffle and telomere shortening at the ends of the DNA which limits the amount of cell mitosis. The increase in the number of telomeres lost due to imbalance in ROS production is one of the factors in the aging process.[5] ROS is very reactive and destructs various biomolecule around it such as protein, deoxyribose nucleic acid (DNA) and lipid especially polyunsaturated fatty acids (PUFAs).[6] Oxidation of PUFA form malondialdehyde (MDA), 4-hydroxy-2-nonenal (HNE) and other products such as *F2-isoprostanes*. MDA, HNE and *F2-isoprostanes* are oxidative stress biomarker which is widely used to detect lipid peroxidation.[6] MDA is highly reactive compound, easily penetrate into the tissues and able to form covalent bond with protein and nucleic acid allowing modification its structure and function.[7] The process caused loss of cell membrane integrity which can subsequently lead to disruption of cell function and ultimately cause dysfunction of individual organs.[7]

In order to overcome oxidative stress, the body synthesizes an endogenous antioxidant such as catalase.[8] Catalase is an antioxidant enzyme found in almost all living organisms that catalyze the decomposition of hydrogen peroxide (H_2O_2) into water and oxygen.[8] Hydrogen peroxide (H_2O_2) is produced during cellular respiration in all living cells.[3] H_2O_2 is dangerous and must be disposed of as soon as possible. The cells containing small amount of catalase are very susceptible to be oxidized by H_2O_2. Therefore catalase plays an important role in the cell's defense mechanism against the oxidation attack of H_2O_2.[8] Until now, the researches about MDA and catalase level in aging population especially in Indonesia is still limited. MDA level in 41 elderly subjects age 60 – 90 years old at Social Rehabilitation *Unit Pucang Gading Semarang* was 12.69 ± 1.373 nmol/mL.[9] However, no report about catalase level in those population. In India, Akila et al[10] found that MDA level in 13 subjects of elderly age 60- 75 years old was 3.96 ± 43.58 nmol/mL, while catalase level was 48.03 ± 24.002 Unit per gram hemoglobin. They found that MDA level increased while catalase decreased in elderly compared to 15 subjects of normal young age 20 - 32 years old. Most of research compare oxidative stress between elderly and adult subjects, however there is no research which elaborates oxidative stress level at different age in elderly population. Therefore, the purpose of this study was to analyse the profile of plasma malondialdehyde (MDA) level and specific activity of catalase in elderly women based on increasing age. The elderly women used in this research because the number of elderly women in Indonesia (9,53%) are greater than the number of elderly men (8,54%) according to Data and Information Center (2017), Ministry of Health, Republic of Indonesia.

METHODS

It was a cross sectional research using 60 subjects of healthy elderly women aged 60 years and over who live in Kali Anyar, Tambora, West Jakarta.This location was selected because it is a slum and populous urban area thus the exposure to free radical was probably high. The women were chosen as the research subjects due to life span of women is longer than men, therefore this study might be able to provide an overview of oxidative stress profile in longer life span population who live in slum urban area, The subjects were divided into 2 groups based on age category, 30 subjects of the younger group (60 – 70 years old) and 30 subjects of the older group (>70 years old). The inclusion criteria in this study were women aged 60 years and over who were willing to be the subject. While, the exclusion criteria were the subjects with total immobility, acute phases of diseases such as respiratory infections (such as pneumonia), acute arthritis, stroke, coronary heart disease, hypertensive emergencies/ urgency as well as acute exacerbation of chronic obstructive pulmonary disease (COPD). Whole blood was taken from each subject and then centrifuged at 3000 rpm for 15 minutes to obtain plasma for MDA and catalase assay. All procedures have been approved by the Ethical Committee of Faculty of Medicine, Universitas Indonesia number 0910/UN2.F1/ETIK/2018.

MDA level

MDA assay was analyzed in plasma using spectrophotometer by thiobarbituric acid method.[11] Two hundred microliters of Trichloroacetic acid (TCA) were added to the sample and centrifuged at 5000 rpm for 10 minutes, pellet discarded and 0,4 mL of thiobarbituric acid (TBA) reagent was added. The solution was incubated in a boiling water bath for 10 min to produce pink color. After cooling at room temperature, samples were read at 532 nm using a spectrophotometer.

Specific Activity of Catalase Enzymes

One hundred microliters of the sample were added to 1,900 µl of H_2O_2 with optimal dilution. And then 100 µl of solvent was added following by homogenization with manual shaking and its absorption measured at 210 nm.[12]

Statistical analysis

Statistical analysis was performed using Statistical Package for Social Science (SPSS) software for Windows version 2.0. All data were tested for normality using the Kolmogorov-Smirnov Test. Significance test used Mann Whitney (nonparametric) for MDA level due to abnormal distribution and unpaired (parametric) t test for catalase.

RESULTS

MDA Level

The MDA level in 30 subjects of younger group (60-70 years old) was 0,039-3,826 nmol/mL, median 2,07 nmol/mL. While the MDA level in the 30 subjects of older group (> 70 years old) was 0,163-6,079 nmol/mL, median 1,93 nmol/mL (figure 1). There was no statistically different between those groups (p>0,05).

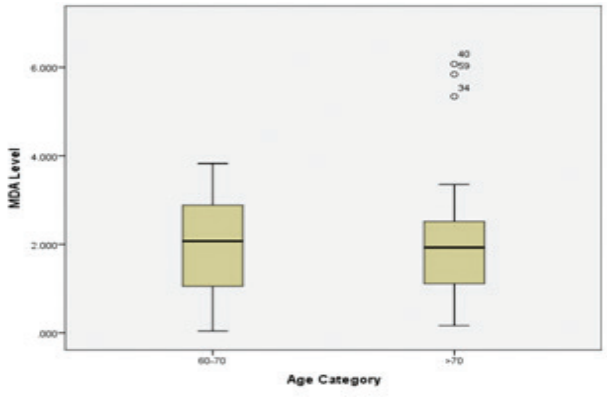

Figure 1. The plasma MDA level in the elderly 60-70 years and the elderly >70 years old. There was no significant differences (p> 0.05).

Specific Activity of Catalase Enzymes

In this study, the specific activity of the catalase enzyme in younger group (60-70 years old) was 0.047 ± 0.006 U/mg protein and 0.060 ± 0.004 U/mg in the older group (> 70 years old). From that results it meant that catalase specific activities of the older group was higher than the younger group (figure 2). That difference was stastically significant (p = 0.007).

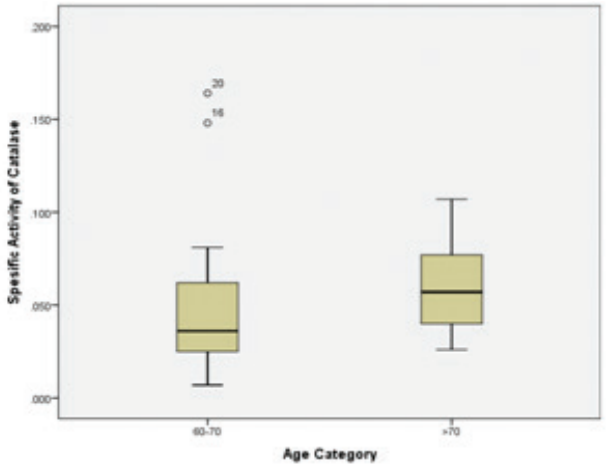

Figure 2. The specific activity of the catalase enzyme in the older group (> 70 years old) was significantly higher (p <0.05) compare to younger group (60-70 years old).

The MDA and catalase levels were also classified based on 5 years of age distance as shown in table1. MDA level tends to increased up to 69 years old. However, its level tends to decrease up to 84 years old. While in catalase, the level was gradually increased up to 84 years old. The results in this category could not be analyzed statistically since the number of samples in each category were small and not equal.

Table 1. MDA dan catalase level in women elderly based on 5 years age distance category

Age (years)	n	MDA Level Median* (min ± max)	Catalase level Mean ± SD
60-64	17	1.942 nmol/mL (0,039 ± 3,721)	0,043 ± 0,032 U/mg protein
65-69	9	2.570 nmol/mL (0,791 ± 3,826)	0,054 ± 0,047 U/mg protein
70-74	16	2.099 nmol/mL (0,215 ± 6,076)	0,055 ± 0,022 U/mg protein
75-79	13	1.680 nmol/mL (0,163 ± 3,250)	0,057 ± 0,025 U/mg protein
80-84	4	1.522 nmol/mL (1,114 ± 5,884)	0,076 ± 0,025 U/mg protein
100	1	1.929 nmol/mL	0.060 U/mg protein
	60		

Note: *Median was represented for MDA level due to abnormal distribution

DISCUSSIONS

Oxidative stress is a condition that reflects an imbalance between reactive oxygen species (ROS) and antioxidant defenses.[8] Malondialdehyde (MDA) is a marker of oxidative stress as the end result of a chain reaction of lipid peroxidation.[6] In this study, MDA levels of the elderly women in 60-70 years old group was 0,039-3,826 nmol/mL, median 2,07 nmol/mL. While MDA level in the > 70 years old group was 0,163-6,079 nmol/mL, median 1,93 nmol/mL Statistical tests showed no significant differences in the MDA levels between those groups ($p > 0.05$). The age range of research subjects was too close so that MDA levels between those groups were not significantly different. If we compared it with the MDA level in 10 young women (20 – 27 years old) that we checked (data not shown), MDA level in those elderly women was higher than young women (1,155 nmol/mL). When the subjects were grouped in 5 years distance as shown in table 1, actually MDA level tends to increase up to 69 years old and the highest MDA level was found in group 65 – 69 years old. Therefore in this age group, the women elderly probable were prone to suffer from degenerative diseases. In general, other studies compared MDA level between young and old age with a wide age range, such as Fasna et al[13] analyzed MDA levels in 150 healthy men and women aged between 20 and 90 years old. They found that plasma MDA levels increased with age, indicated rapid oxidation occurred during aging process.[13] Moreover, the increased of MDA level probably due to reduced antioxidants in the body. Muralidharan et al[14] proved that a decrease in the antioxidant level causes an increase in MDA level in the elderly population. It is known that the body continuously produces free radicals, both through normal metabolism, inflammation, malnutrition and environmental effects such as pollution, ultraviolet, cigarette smoke and others.[3] The formation of free radical compounds is the initiator of the lipid peroxidation process or MDA formation which acts as a destroyer of body tissue.[6] Therefore, as we get older, the buildup of free radicals increased in the body, resulting in oxidative stress. Currently MDA is more often used in biomedical research as the marker of oxidative stress especially in various clinical conditions related to the lipid peroxidation process. The more chemically stable properties of MDA make this compound more often used as the marker of oxidative stress.[7]

From the results of this study it was found that the specific activity of the catalase enzyme was significantly higher at the older group (> 70 years old) compared to the younger group (60-70 years old). If the subjects were groups in 5 years distance as shown in table 1, specific activity of catalase enzyme tends to increase with increasing age. It might be a protective effect of healthy elderly women in order to cope high level of oxidative damage. The function of endogenous antioxidant catalase is to suppress oxidative damage by catalyzing the change of H_2O_2 into water and oxygen.[8] High reactive free radicals could attack the cell membrane, which triggers high catalase activity in an effort to suppress the presence of oxidative stress.[6] The high specific activity of the catalase enzyme in this study seems to provide a protection thereby plasma MDA levels in this study did not increase with age. Several studies stated that free radicals are the main cause of aging.[2,15-17] Therefore, it is important to control the formation of free radicals by improving cellular antioxidant status to inhibit aging process. Until now the relationship between endogenous antioxidant activity and aging still needs to be elaborated more deeply, but it is often assumed that antioxidants serve as an anti-aging molecule.[18] All elderly subjects in this research were in healthy condition, due to catalase specific activity increased significantly in the older groups, it can be assumed that to achieve healthy aging conditions, endogenous antioxidants should be maintained at the proper level. Further study is needed to determine the value limit for endogenous antioxidant level which can be used as the standard for healthy aging status.

In conclusion, there was no difference in MDA level of elderly woman between younger (60 – 70 years old) and older group (>70 years old). However, catalase specific activity significantly increased with increasing age.

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