Acta Biochimica Indonesiana

https://doi.org/10.32889/actabioina.177

RESEARCH ARTICLE

Open Access

Oxidative stress and antioxidant status among the elderly in Jakarta

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ABSTRACT

Background: Indonesia is experiencing a demographic shift, with the elderly population comprising 9.92% (26.82 million) of the total population, 8.21% of whom reside in Jakarta. Oxidative stress is a key contributor to the development of degenerative diseases associated with aging, while antioxidant defenses can mitigate its effects.

Objective: This study aimed to assess oxidative stress levels, measured by malondialdehyde (MDA), and antioxidant status, including catalase, reduced glutathione (GSH), and vitamin C, in the elderly population of Jakarta.

Methods: A cross-sectional study was conducted with 91 elderly participants from three sub-districts in Jakarta. Erythrocyte lysate samples were analyzed to measure MDA, catalase, and GSH levels, while plasma was used to measure vitamin C. All parameters were quantified using a spectrophotometer. MDA levels and antioxidant status were categorized based on age, blood pressure, and the number of chronic diseases, with statistical analysis performed using the Kruskal-Wallis test.

Results: The median MDA level was 2.68 (1.61–5.64) nmol/mL, with the highest levels observed in participants aged 60–64 and those with three chronic diseases. The mean catalase level was 2.41 ± 0.39 U/mL, the median GSH level was 6.16 (2.02–128.07) µmol/mL, and the mean vitamin C level was 10.87 ± 4.9 µg/mL. No significant differences in antioxidant status were observed based on age, blood pressure, or the number of chronic diseases.

Conclusion: Oxidative stress is prevalent in the elderly and is particularly influenced by the presence of multiple chronic diseases. However, antioxidant status does not significantly vary with age, blood pressure, or disease burden in this population.

Keywords: antioxidant, elderly, oxidative stress

Introduction

The World Health Organization (WHO) reports that every country is witnessing an increase in both the proportion and size of the elderly population. By 2030, it is projected that one in six people worldwide will be over 60 years old, and by 2050, the global elderly population will double to 2.1 billion [1]. Similarly, Indonesia is experiencing a demographic shift. According to the Ministry of Health of the Republic of Indonesia, the country is entering an aging population phase. In 2010, the elderly population was 18 million (7.56%), and by 2019, it had surged to 25.9 million (9.7%). This number is expected to grow further, reaching approximately 48.2 million (15.77%) by 2035 [2]. In 2020, Indonesia's elderly population accounted for 9.92% of the global total, with 8.21% residing in Jakarta [3]. This demographic shift presents significant challenges, particularly in healthcare, as an aging population is often accompanied by an increase in degenerative conditions and non-communicable diseases.

Aging is closely associated with oxidative stress, a process thought to play a key role in the development of degenerative diseases. Oxidative stress occurs when there is an imbalance between

the production of reactive oxygen species (ROS) and the body's ability to neutralize them, leading to cellular damage [4]. ROS are by-products of normal cellular metabolism, but when their levels exceed the body's antioxidant defenses, oxidative stress arises, contributing to cell damage.

Oxidative stress can damage DNA and activate the DNA damage response (DDR), which leads to the expression of p53, a protein that halts cell division. A similar response occurs due to telomere shortening during cell division, triggering the overexpression of p16, which inhibits cyclin-dependent kinases (CDKs) and causes stable growth arrest in senescent cells. Although senescent cells no longer divide, they remain metabolically active, releasing inflammatory secretions known as the senescence-associated secretory phenotype (SASP), which can influence neighboring cells [5,6]. As a result, oxidative stress impairs cellular regeneration, playing a central role in the progression of degenerative diseases such as diabetes, cancer, metabolic disorders, and cardiovascular diseases [7,8].

One key marker of oxidative stress is malondialdehyde (MDA), which is released during lipid peroxidation when ROS react with polyunsaturated fatty acids (PUFAs) in cell membranes [9,10]. This process also contributes to the formation of atherosclerotic plaques, which narrow blood vessels and increase cardiac workload.

The body combats oxidative stress through its antioxidant defense system, which includes both endogenous antioxidants, such as enzymes and proteins, and exogenous antioxidants obtained from diet, such as vitamins C and E. Catalase is an essential enzymatic antioxidant that decomposes hydrogen peroxide into water and oxygen, preventing cellular damage. Deficiency in this enzyme has been linked to degenerative diseases [7,11]. Another vital endogenous antioxidant is glutathione (GSH), a tripeptide that detoxifies hydrogen peroxide and helps maintain cellular redox balance [12,13].

Exogenous antioxidants, such as vitamin C, are obtained through dietary intake. Recommended daily intake varies with age, gender, and health status [14]. For the elderly, the recommended intake is 95 mg/day for men and 70 mg/day for women. However, higher doses (at least 400 mg/day) may be necessary in individuals with age-related chronic conditions to maintain adequate plasma levels [15]. While studies on vitamin C pharmacokinetics in the elderly are limited, evidence suggests that cellular uptake of vitamin C decreases with age [15].

Currently, data on oxidative stress and antioxidant levels in Jakarta's elderly population are scarce. The structural-damage hypothesis suggests that oxidative stress is closely tied to aging, as accumulated ROS contributes to cellular senescence [6]. Moreover, oxidative stress hinders cellular regeneration, leading to degenerative diseases [16]. This study aims to assess oxidative stress by measuring MDA levels and evaluate antioxidant status by analyzing catalase, GSH, and vitamin C levels in Jakarta's elderly population. Catalase and GSH are critical for neutralizing hydrogen peroxide, a long-lived ROS that can generate highly reactive hydroxyl radicals [17]. Additionally, vitamin C plays a crucial role in reducing oxidative stress by neutralizing these radicals [18]. Understanding oxidative stress and antioxidant levels in the elderly may provide predictive markers for preventing degenerative diseases in this population.

Methods

This cross-sectional study was conducted in the Kebayoran Baru, Pesanggrahan, and Cilandak subdistricts of Jakarta. A total of 91 participants, both male and female, aged 60 years or older, were recruited from Integrative Healthcare Centers for the Elderly. Stored samples from previous research were utilized [17]. Participants were eligible if they provided informed consent and were willing to adhere to study procedures. Exclusion criteria included alcohol consumption or smoking within the past year, as well as the presence of fever. The study received ethical approval (Number: KET.442/UN2.F1/ETIK/PPM.00.02/2019), and all data were treated with confidentiality.

Subject caractheristic

Blood pressure was measured once using a sphygmomanometer after obtaining informed consent, following established procedures from previous research [19]. Systolic and diastolic pressures were recorded based on the first and last Korotkoff sounds using a stethoscope over the brachial artery. Participants' medical histories, including chronic conditions such as hypertension, diabetes mellitus, stroke, and coronary heart disease, were obtained through anamnesis.

Plasma preparation

Three milliliters of venous blood were collected from each participant and placed into EDTA tubes. The blood samples were centrifuged at 3,500 rpm for 10 minutes to separate the plasma, which was then stored at -20°C for subsequent vitamin C analysis.

Erythrocyte lysate preparation

Erythrocyte lysates were prepared for the analysis of MDA, catalase, and GSH. After plasma extraction, 300 µL of the remaining blood was washed three times with 900 µL of 0.9% NaCl. Following the washing process, 900 µL of distilled water was added to create the erythrocyte lysate, which was then stored at -20°C for further analysis.

MDA analysis

MDA was measured in 90 erythrocyte lysate samples. Four hundred microliters of erythrocyte lysate were mixed with 200 µL of 20% trichloroacetic acid (TCA), vortexed, and centrifuged at 5,000 rpm for 10 minutes. The supernatant was extracted, and 0.6% thiobarbituric acid (TBA) was added to reach a final volume of 1 mL. The samples were incubated in a water bath at 96-100°C for 10 minutes, then cooled, and their absorbance was measured at 532 nm using a spectrophotometer [20].

Catalase analysis

Catalase activity was measured in 91 erythrocyte lysate samples. A blank solution consisting of 1,900 µL of 27.2 mM H_2O_2 and 100 µL of solvent was prepared, and its absorbance was recorded at 210 nm every 30 seconds for 10 minutes using a spectrophotometer. The same procedure was followed for the samples, in which 1,900 µL of H_2O_2 was mixed with 100 µL of erythrocyte lysate [20].

GSH analysis

GSH was measured in 86 erythrocyte lysate samples using the Ellman method. Fifty microliters of each sample were mixed with 200 µL of 5% TCA and centrifuged at 3,000 x g for 10 minutes. The supernatant was collected and diluted with PBS buffer (pH 8.0) to a final volume of 2 mL. Subsequently, 25 µL of 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) reagent was added, and the mixture was incubated in the dark at room temperature for 1 hour. Absorbance was measured at 412 nm [20].

Vitamin C analysis

Vitamin C levels were measured in 50 plasma samples using a vitamin C assay kit (Elabscience) according to the manufacturer's instructions. Four hundred microliters of plasma were mixed with the kit reagents and incubated at 37°C for 30 minutes. After adding 0.1 mL of additional reagent, the mixture was vortexed and incubated for 10 minutes at room temperature. Absorbance was then measured at 635 nm using a spectrophotometer.

Statistical analysis

MDA and GSH levels were expressed as medians (min–max) due to their non-normal distribution, while catalase and vitamin C levels were presented as means ± standard deviation (SD). Data were categorized by age group (60–64 years, 65–74 years, 75–84 years, >85 years), blood pressure, and number of chronic diseases. Statistical analysis was performed using the Kruskal-Wallis test. All data analysis was conducted using SPSS version 28.0.

Results

Participant characteristics

The participants had a mean age of 70.14 years, with nearly half falling within the 65 to 74-year age group. Approximately 50% of the participants were diagnosed with hypertension, and the majority had a history of one or more chronic conditions, including hypertension, diabetes mellitus, stroke, and coronary heart disease. Only 30% of the participants were free from chronic diseases (Table 1).

Table1. Characteristics of the participants

No.	Categorization	n (%)
1.	Mean of age: 70.14 years	
	Middle age: 60 – 64 years	20 (21.9 %)
	Youngest Old: 65 – 74 years	44 (48.4 %)
	Middle Old: 75 – 84 years	25 (27.5 %)
	Oldest Old: > 85 years	2 (2.2 %)
2.	Blood pressure:	
	Normal	29 (31.9 %)
	Pre-hypertension	22 (24.2 %)
	Hypertension grade 1	29 (31.9 %)
	Hypertension grade 2	11 (12 %)
3.	The number of chronic disease history:	
	No chronic disease	29 (31.9 %)
	1 chronic disease	32 (35.2 %)
	2 chronic diseases	24 (26.4 %)
	3 chronic diseases	6 (6.5 %)

MDA level and antioxidant status

The median MDA level was 2.68 (1.61–5.64) nmol/mL. The highest MDA levels were observed in participants aged 60–64 years and those with a history of three chronic diseases, as shown in Figure 1C. The mean catalase level was 2.41 ± 0.39 U/mL, the median GSH level was 6.16 (2.02–128.07) µmol/mL, and the mean vitamin C level was 10.87 ± 4.9 µg/mL. No significant differences in antioxidant status (catalase, GSH, and vitamin C levels) were observed based on age distribution, blood pressure, or the number of chronic diseases (Figures 1-3).

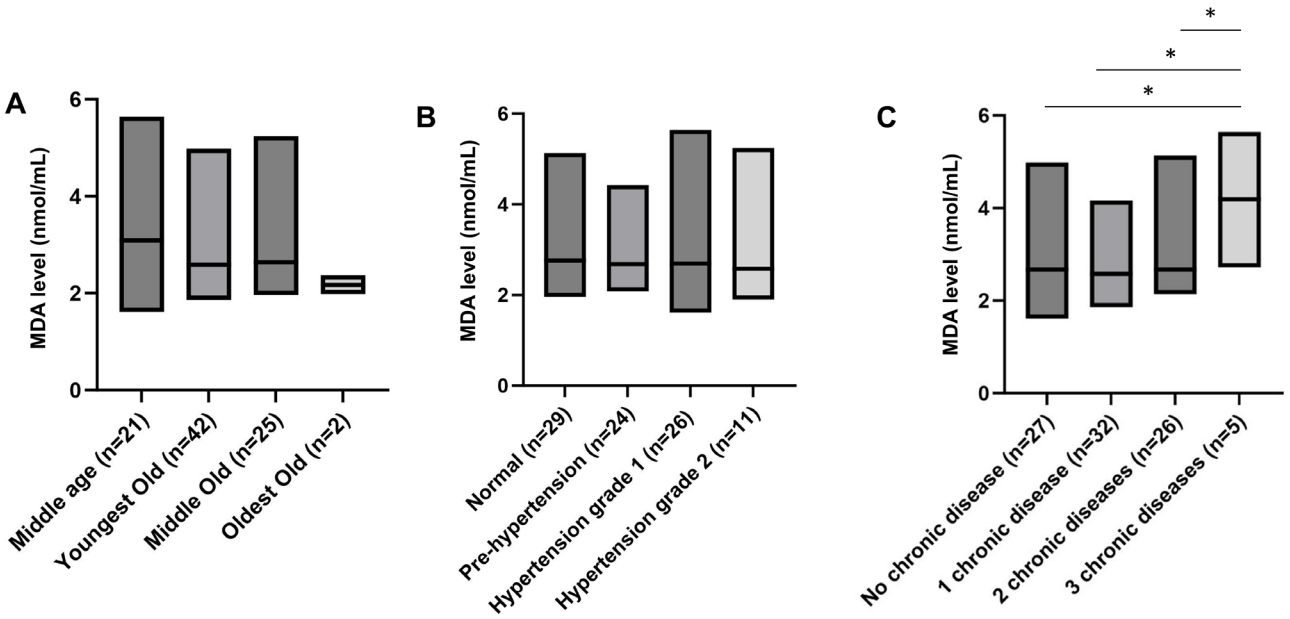

Figure 1. Malondialdehyde (MDA) levels categorized by age group, blood pressure, and number of chronic diseases. MDA levels (nmol/mL) in participants, categorized by (A) age group (middle age n=21, youngest old n=42, middle old n=25, oldest old n=2), (B) blood pressure status (normal n=29, pre-hypertension n=24, hypertension grade 1 n=26, hypertension grade 2 n=11), and (C) number of chronic diseases (no chronic disease n=27, 1 chronic disease n=32, 2 chronic disease n=26, 3 chronic diseases n=5). The box plots represent the range of MDA levels, with the thick horizontal line indicating the median for each group. *p < 0.05 using Kruskal Wallis test

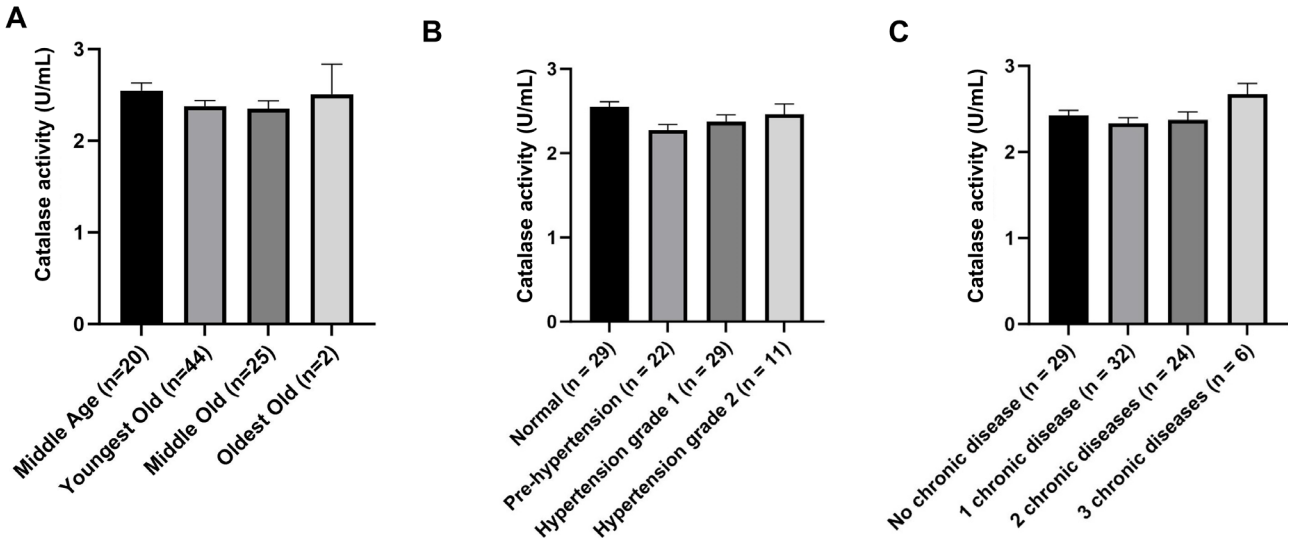

Figure 2. Catalase activity categorized by age group, blood pressure, and number of chronic diseases. The catalase activity (U/mL) in participants, categorized by (A) age group (middle age n=20, youngest old n=44, middle old n=25, oldest old n=2), (B) blood pressure status (normal n=29, pre-hypertension n=22, hypertension grade 1 n=29, hypertension grade 2 n=11), and (C) number of chronic diseases (no chronic disease n=29, 1 chronic disease n=32, 2 chronic disease n=24, 3 chronic diseases n=6). The bars represent the mean catalase activity for each group, with error bars indicating the standard error of the mean (SEM)

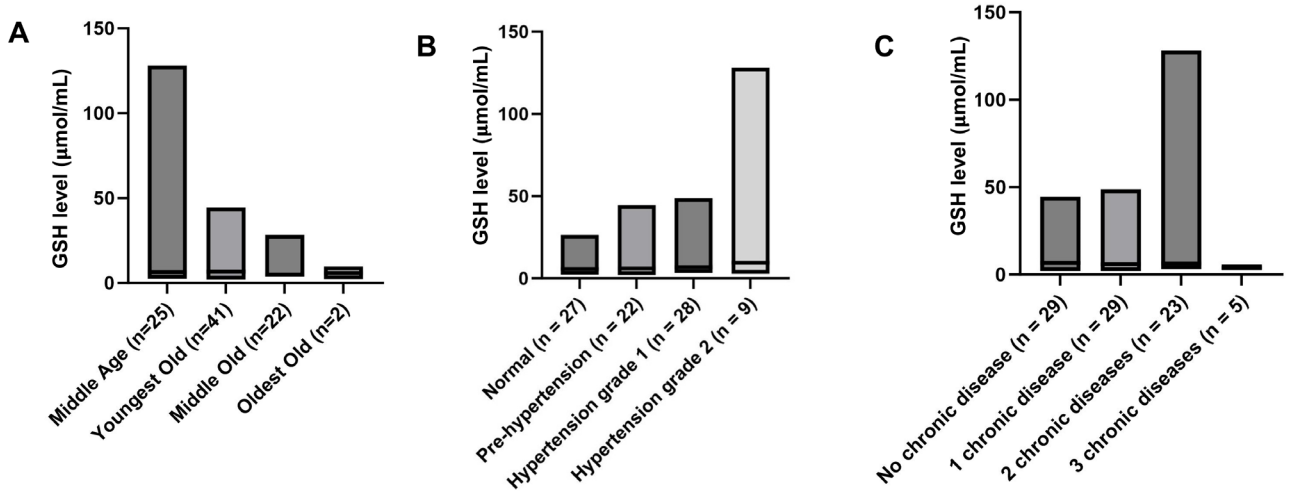

Figure 3. GSH levels categorized by age group, blood pressure, and number of chronic diseases. GSH levels (µmol/mL) in participants, categorized by (A) age group (middle age n=21, youngest old n=41, middle old n=22, oldest old n=2), (B) blood pressure status (normal n=27, pre-hypertension n=22, hypertension grade 1 n=28, hypertension grade 2 n=9), and (C) number of chronic diseases (no chronic disease n=29, 1 chronic disease n=29, 2 chronic disease n=23, 3 chronic diseases n=5). The box plots represent the range of MDA levels, with the thick horizontal line indicating the median for each group

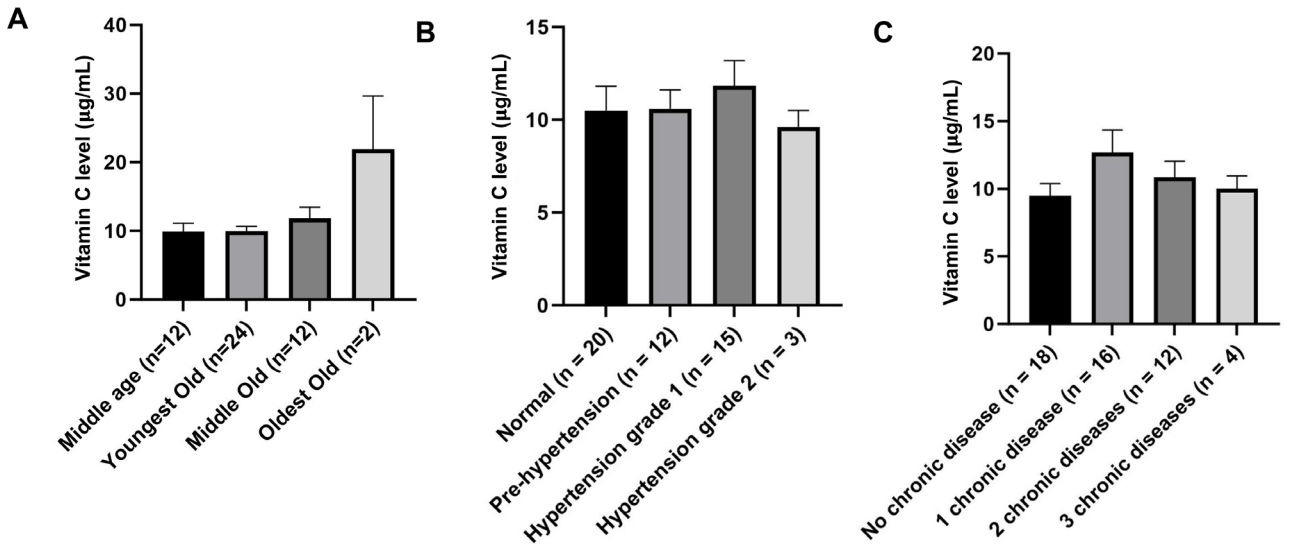

Figure 4. Vitamin C levels categorized by age group, blood pressure, and number of chronic diseases. Vitamin C level (µg/mL) in participants, categorized by (A) age group (middle age n=12, youngest old n=24, middle old n=12, oldest old n=2), (B) blood pressure status (normal n=20, pre-hypertension n=12, hypertension grade 1 n=15, hypertension grade 2 n=3), and (C) number of chronic diseases (no chronic disease n=18, 1 chronic disease n=16, 2 chronic disease n=12, 3 chronic diseases n=4). The bars represent the mean catalase activity for each group, with error bars indicating the standard error of the mean (SEM)

Discussion

This study found that the median MDA level among elderly participants was 2.68 (1.61–5.64) nmol/mL, consistent with previous studies reporting similar levels in individuals aged 60–70 years, with a median of 2.070 nmol/mL [21]. Interestingly, the oldest age group (>85 years) exhibited a slightly lower median MDA level (2.17 nmol/mL), although no significant differences were observed across other age groups. In contrast, Akter et al. [22] reported that MDA concentrations tend to increase with age, a finding supported by Muralidharan et al. [23], who observed a significant rise in serum MDA levels between individuals younger than 30 years (0.963 µM/L ± 0.0484) and those aged 31–50 years (1.945 µM/L ± 0.0816). These findings suggest that lipid peroxidation and oxidative stress may become more pronounced with advancing age.

Our finding that participants with three chronic diseases had the highest MDA levels (4.19 nmol/mL) is consistent with the well-documented relationship between chronic diseases and oxidative stress. Studies have shown that conditions such as diabetes, hypertension, and coronary heart disease significantly contribute to lipid peroxidation, as evidenced by elevated MDA levels. Sunita et al. [24] reported significantly higher MDA levels in individuals with type 2 diabetes mellitus (2.400 nmol/L) compared to non-diabetic controls (1.690 nmol/L). Similarly, Sreenivasulu et al. [25] found elevated MDA levels in patients with chronic renal failure (4.26 ± 1.04 µmol/L) compared to healthy controls (1.29 ± 0.2 µmol/L). Elevated MDA concentrations have also been observed in patients with coronary heart disease, particularly among smokers [26]. Additionally, studies by Armas-Padilla et al. [27] and Verma et al. [28] found that hypertensive patients had both higher MDA levels and reduced serum antioxidant capacity, indicating a possible link between oxidative stress and hypertension. Hasan et al. [29] also observed that MDA levels increase with age and the duration of illness in hypertensive patients, further supporting the association between chronic diseases and oxidative stress. These results underscore the importance of managing chronic diseases to reduce oxidative stress and prevent further cellular damage in the elderly population.

The body's antioxidant defense system, including endogenous antioxidants such as catalase and GSH, and exogenous antioxidants like vitamin C, is crucial for counteracting oxidative stress. Catalase,

an enzymatic antioxidant, plays an essential role in decomposing hydrogen peroxide into water, thereby preventing oxidative damage. Studies have shown higher hydrogen peroxide levels in hypertensive individuals compared to normotensive controls, indicating a link between hydrogen peroxide accumulation and hypertension [30]. However, in our study, no significant differences in catalase activity were observed based on blood pressure. This may be due to confounding factors such as gender and body weight. For example, men generally have higher blood pressure than women of the same age, though postmenopausal women tend to have elevated blood pressure compared to men, likely due to hormonal changes [31]. Additionally, excess body weight is a well-known risk factor for hypertension [32].

Besides catalase, other antioxidants like glutathione peroxidase also play critical roles in detoxifying hydrogen peroxide. Glutathione peroxidase uses GSH as a co-substrate and may be more effective than catalase under physiological conditions [33,34]. GSH is vital for maintaining redox balance and cellular function [35]. Although our study did not find significant differences in GSH levels based on age, blood pressure, or the number of chronic diseases, other studies have reported different outcomes. Rybka et al. [36], for example, found higher GSH levels in elderly hypertensive patients, possibly due to the antioxidant effects of antihypertensive medications. The absence of information on antihypertensive drug use in our study may have influenced the results. Other factors, such as chemical exposure, degenerative diseases, or apoptosis, could also impact GSH levels [37].

Vitamin C, a potent exogenous antioxidant, neutralizes free radicals by donating electrons. Research shows that elderly individuals often have lower plasma ascorbate levels due to decreased intake and the presence of chronic diseases, although aging itself does not significantly affect vitamin C levels [39]. Ascorbic acid, a water-soluble antioxidant in human plasma, plays a protective role in cardiovascular diseases and hypertension [40]. While our study did not find significant differences in vitamin C levels based on blood pressure, participants with grade 2 hypertension had the lowest vitamin C levels. Li Ran et al. [40] observed a negative correlation between blood pressure and vitamin C levels, suggesting that individuals with hypertension often have lower ascorbic acid concentrations. Moreover, Guan et al. [41] demonstrated that vitamin C supplementation can significantly reduce blood pressure in patients with essential hypertension, highlighting the potential role of vitamin C in managing blood pressure.

This study has some limitations. Participants were recruited from only three subdistricts in Jakarta, and lifestyle factors, nutritional status, medication use, and the specific types of chronic diseases were not accounted for. Chronic disease data were based on self-reporting, which is subject to recall bias. These limitations may have contributed to the lack of significant differences in the antioxidant parameters observed in our study.

Conclusion

Oxidative stress is prevalent in the elderly and is influenced by age and the presence of multiple chronic diseases. However, age and the number of chronic conditions do not appear to significantly affect antioxidant status. Further research is warranted, including multivariate analyses that consider confounding factors influencing oxidative stress and antioxidant status in the elderly population.

Acknowledgment

None.

Funding

Not applicable.

Author contributions

NSH: conceptualization, data analysis, review and finalized the manuscript. DQA, JVA, PCN, RTT: data collection, data analysis, writing manuscript.

Declaration of interest

The authors declare no conflict of interest.

Received: July 28, 2024
Revised: September 17, 2024
Accepted: September 18, 2024
Published online: October 16, 2024

References

1. World Health Organization. Ageing and health [Internet]. Geneva: World Health Organization. 2021 [cited 2022 Feb 24]. Available from: https://www.who.int/news-room/fact-sheets/detail/ageing-and-health
2. Kementrian Kesehatan RI. Indonesia masuki periode aging population -sehat negeriku [Internet]. 2019 [cited 2021 Oct 26]. Available from:https://sehatnegeriku.kemkes.go.id/baca/rilismedia/20190704/4530734/indonesia-masuki-periode-aging-population/
3. Badan Pusat Statistik. Statistik penduduk lanjut usia 2020. Jakarta: Badan Pusat Statistik; 2020.
4. Pizzino G, Irrera N, Cucinotta M, Pallio G, Mannino F, Arcoraci V, et al. Oxidative stress: harms and benefits for human health. Oxid Med Cell Longev. 2017:463-93. https://doi.org/10.1155/2017/8416763
5. Pole A, Dimri M, Dimri GP. Oxidative stress, cellular senescence and ageing. AIMS Mol Sci. 2016;3(3):300-24. https://doi.org/10.3934/molsci.2016.3.300
6. Kumari R, Jat P. Mechanisms of cellular senescence: cell cycle arrest and senescence associated secretory phenotype. Front Cell Dev Biol. 202;9. https://doi.org/10.3389/fcell.2021.645593
7. Yoshikawa T, Naito Y. What Is Oxidative Stress?. JMAJ. 2002;45(7).
8. Liguori I, Russo G, Curcio F, Bulli G, Aran L, Della-Morte D, et al. Oxidative stress, aging, and diseases. Clin Interv Aging. 2018;13:757-72. https://doi.org/10.2147/CIA.S158513
9. Ayala A, Muñoz MF, Argüelles S. Lipid peroxidation: Production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. Oxid Med Cell Longev. 2014;2014. https://doi.org/10.1155/2014/360438
10. Yang X, Li Y, Li Y, Ren X, Zhang X, Hu D, et al. Oxidative stress-mediated atherosclerosis: mechanisms and therapies. Front Physiol. 2017;8(AUG). https://doi.org/10.3389/fphys.2017.00600
11. Nandi A, Yan L, Jana CK, Das N. Role of catalase in oxidative stress- and age-associated degenerative diseases. Oxid. Med. Cell. Longev. 2019;11:2019. https://doi.org/10.1155/2019/9613090
12. Lubos E, Loscalzo J, Handy DE. Gluthathione peroxidase-1 in health and disease: from molecular mechanisms to therapeutic opportunities. Antioxid Redox Signal. 2011;15(1): 1957-1997. https://doi.org/10.1089/ars.2010.3586
13. Forman HJ, Zhang H, Rinna A. Gluthathione: overview of its protective roles, measurement, and biosynthesis. Mol Aspects Med. 2009; 30(1-2): 1-12 https://doi.org/10.1016/j.mam.2008.08.006
14. Padayatty SJ, Levine M. Vitamin C physiology: the known and the unknown and Goldilocks. Curr Hypertens Rev. 2016;22(6):462-93. https://doi.org/10.1111/odi.12446
15. University OS. Micronutrients for older adults [Internet]. 2022 [cited 2022 Jul 29]. Available from: https://lpi.oregonstate.edu/mic/life-stages/olderadults#
16. di Micco R, Krizhanovsky V, Baker D, d'Adda di Fagagna F. Cellular senescence in ageing: from mechanisms to therapeutic opportunities. Nat Rev Mol Cell Biol. 2021;22(2):75-95. https://doi.org/10.1038/s41580-020-00314-w
17. Winterbourn C.C. Toxicity of iron and hydrogen peroxide: The Fenton reaction. Toxicol. Lett. 1995;82-83:969-974. https://doi.org/10.1016/0378-4274(95)03532-X
18. Padayatty SJ, Levine M. Vitamin C physiology: the known and the unknown and Goldilocks. Curr Hypertens Rev. 2016;22(6):462-93. https://doi.org/10.1111/odi.12446
19. Nurul KA, Erfi P, Silvia HN. Correlation between age, body mass index, and blood selenium level with glutathione peroxidase activity among elderly in south jakarta. Int J Hum Heal Sci. 2020;04(02):3-7. http://dx.doi.org/10.31344/ijhhs.v4i2.181
20. Hardiany NS, Karman AP, Calista ASP, Anindyanari BG, Rahardjo DE, Novira PR, et al. The Effect of Fasting on Oxidative Stress in the Vital Organs of New Zealand White Rabbit. Rep Biochem Mol Biol. 2022;11(2):190-199. https://doi.org/10.32889/actabioina.112
21. Hardiany NS, Sucitra S, Paramita R. Profile of malondialdehyde (MDA) and catalase specific activity in plasma of elderly woman. Health Sci J Indones. 2020;10(2):132-6. https://doi.org/10.22435/hsji.v12i2.2239
22. Akter A, Mamun SA, Rizwan A, Talukder IJ, Mamataj S. Malondialdehyde level as oxidative stress in geriatric population in Bangladesh. M Abdur Rahim Medical College Journal. 2022;15(2):198-201.
23. Muralidharan N, Bhat T, Kumari SN. A study on effect of ageing on the levels of total antioxidant and lipid peroxidation. IJCMR. 2017;4(12):8-10.
24. Sunita R, Sahidan S, Hidayat R. Evaluation of malondialdehyde in type 2 diabetes mellitus patients as oxidative stress markers in Bengkulu population. Bio Sc Med. 2020;4(3):45-54. https://doi.org/10.32539/bsm.v4i3.146
25. Sreenivasulu U, Prasad BRS, Durga T. Study of serum malondialdehyde levels in chronic renal failure patients: a hospital based study in govt. general hospital, Anantapuramu, Andhra Pradesh. Int J Clin Biochem Res. 2020;7(1): 138-41. https://doi.org/10.18231/j.ijcbr.2020.028

26. Khan MA, Baseer A. Increased malondialdehyde levels in coronary heart disease. J Pak Med Assoc. 2000;50(8):261-4.
27. Armas-Padilla MC, Armas-Hernández MJ, Sosa-Canache B, Cammarata R, Pacheco B, Guerrero J, et al. Nitric oxide and malondialdehyde in human hypertension. Am J Ther. 2007;14(2):172-6. https://doi.org/10.1097/01.pap.0000249914.75895.48
28. Verma MK, Jaiswal A, Sharma P, Kumar P, Singh AN. Oxidative stress and biomarker of TNF-α, MDA, and FRAP in hypertension. J Med Life. 2019;12(3):253-9. https://doi.org/10.25122/jml-2019-0031
29. Hasan AA, Sayyah SG. Oxidative stress marker malondialdehyde and glutathione antioxidant in hypertensive patient. Eur J Biomed Res. 2023;2(1):31-6. https://doi.org/10.24018/ejbiomed.2023.2.1.47
30. Lacy F, Kailasam MT, O'Conner DT, Schmid-Schönbein GW, Parmer RJ. Plasma hydrogen peroxide production in human essential hypertension: role of heredity, gender, and ethnicity. Hypertension. 2000; 36: 878-884. https://doi.org/10.1161/01.HYP.36.5.878
31. Reckelhoff JF. Gender differences in the regulation of blood pressure. Hypertension. 2001; 37: 1199-1208. https://doi.org/10.1161/01.HYP.37.5.1199
32. Pikilidou MI, Scuteri A, Morrell C, Lakatta EG. The burden of obesity on blood pressure is reduced in older persons: the Sardinia study. Obesity (Silver Spring). 2013 Jan; 21(1): E10-E13. https://doi.org/10.1002/oby.20010
33. Griendling KK, Camargo LL, Rios FJ, Alves-Lopes R, Montezano AC, Touyz RM. Oxidative stress and hypertension. Circulation Research. 2021; 128: 993-1020. https://doi.org/10.1161/CIRCRESAHA.121.318063
34. Lubos E, Loscalzo J, Handy DE. Glutathione peroxidase-1 in health and disease: from molecular mechanisms to therapeutic opportunities. Antioxid Redox Signal. 2011;15(7): 1957-1997. https://doi.org/10.1089/ars.2010.3586
35. Kalinovic S, Stamm P, Oelze M, Daub S, Kroller-Schon S, Kvandova M, et al. Comparison of three methods for in vivo quantification of glutathione in tissues of hypertensive rats. Free Radical Research. 2021;55(11-12): 1048-61. https://doi.org/10.1080/10715762.2021.2016735
36. Rybka J, Kupczyk D, Kedziora-Kornatowska K, Motyl J, Czuczejko J, Szewczyk-Golec K, et al. Glutathione-related antioxidant defense system in elderly patients treated for hypertension. Cardiovasc Toxicol. 2011;11(1):1-9. https://doi.org/10.1007/s12012-010-9096-5
37. Schulz JB, Lindenau J, Seyfried J, Dichgans J. Glutathione, oxidative stress and neurodegeneration. Eur. J. Biochem. 2000;267:4904-4911. https://doi.org/10.1046/j.1432-1327.2000.01595.x
38. M Abdullah, RT Jamil FA. Vitamin C (ascorbic acid). Vol. 3, Encyclopedia of Toxicology. 2022. 962-963 p. https://doi.org/10.1016/B978-0-12-386454-3.01250-1
39. Travica N, Ried K, Hudson I, Sali A, Scholey A, Pipingas A. Gender differences in plasma vitamin C concentrations and cognitive function: A pilot cross-Sectional study in healthy adults. Curr Dev Nutr.2020;4(4):nzaa038. https://doi.org/10.1093/cdn/nzaa038
40. Ran L, Zhao W, Tan X, Wang H, Mizuno K, Takagi K, et al. Association between serum vitamin C and the blood pressure: a systematic review and meta-analysis of observational studies. Cardiovasc Ther. 2020;2020:4940673. https://doi.org/10.1155/2020/4940673
41. Guan Y, Dai P, Wang H. Effects of vitamin C supplementation on essential hypertension A systematic review and meta-analysis. Medicine. 2020;99(8):e19274. https://doi.org/10.1097/MD.0000000000019274