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8- Hydroxy -deoxyguanosine (8-OHdG) urine as a biomarker of oxidative damage in early elderly hypertension

Hardjono Sumarlie
Master's Program in Biomedical Sciences Concentration in Anti Aging and Regenerative Medicine, Graduate School, Hasanuddin University, Makassar, Indonesia
*Corresponding author email: hardjonosumarlie@gmail.com

Firdaus Hamid
Departement of Microbiology, Faculty of Medicine Hasanuddin University, Makassar, Indonesia

Arif Santoso
Departement of Pulmonology, Faculty of Medicine Hasanuddin University, Makassar, Indonesia

Suryani As'ad
Departement of Clinical Nutrition, Faculty of Medicine Hasanuddin University, Makassar, Indonesia

Agussalim Bukhari
Departement of Clinical Nutrition, Faculty of Medicine Hasanuddin University, Makassar, Indonesia

Andi Alfian Zainuddin
Departement of Public Health Sciences, Faculty of Medicine Hasanuddin University, Makassar, Indonesia

***Abstract*---**According to JNC VII, patients with hypertension are patients who clinically have systolic and diastolic blood pressure ≥140 mmHg and ≥90 mmHg, respectively. The aging processes in hypertension are stiffening of the blood vessels and decreasing arterial flexibility. The underlying path mechanism is oxidative stress due to reactive oxygen species (ROS) elevation. 8-Hydroxy-Deoxyguanosine (8-OHdG) is an oxidized product of DNA repair as a kind of oxidative stress biomarker. This study aimed to analyze urinary 8-OHdG in hypertensive patients and healthy control aged 46-55 years old by involveing 80 participants. Questionnaires and medical records were

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collected to obtain relevant clinical data. The level of 8-OHdG is measured by the Enzyme-Linked Immunosorbent Assay (ELISA). The results showed that there is no difference in the average urinary level of urinary 8-OHdG of hypertensive patients (1.871 ± 0.689 ng/mL) with the healthy controls (1.743 ± 0.364 ng/mL). However, further analysis found that the level of urinary 8-OHdG in female hypertensive patients (2.367 ± 0.206 ng/mL) was significantly higher (p=0.01) compared to the male hipertensive patients (1.374 ± 0.644 ng/mL); there is no difference in level the 51-55 year age group (1.963 ± 0.643 ng/mL) and the 46-50 year age group (1.680 ± 0.768 ng/mL); hypertensive patients who were inconsistent in taking medication (2.374 ± 0.216 ng/mL) was significantly higher (p=0.001) compared to those who took medication regularly (1.535 ± 0.694 ng/mL). The level of urinary 8-OHdG can be considered as one of the examination biomarker of oxidative damage in hypertensive patients.

***Keywords*---**hypertension, aging, urinary 8-OHdG, oxidative stress.

Introduction

Hypertension is one of the main cause of premature death in the world. WHO estimatesd, that the prevalence of hypertension globally is 22% of the world's total population, but only less than a fifth of makes efforts to control their blood pressure. Southeast Asia is in the 3rd highest position with a prevalence of 25% of the total population[1]. Based on WHO's data in the Global Status Report on Non-Communicable Disease, the prevalence of hypertension in 2014 in adults aged 18 years and over was around 22%, causing 40% of deaths from heart disease and 51% of deaths from stroke. Hypertension is one of the most common non-communicable disease in Indonesia (57.6%), proved by the increasing number of hypertension visits at First Level Health Facilities every year. Data from the Makassar City Health Office, hypertension is in the 2nd place of the 10 most common diseases, where the prevalence of hypertension in 2016 reached 27.61% while the mortality rate reached 18.6%[2].

Hypertension is a common disease in the elderly, the prevalence increases with age. Hypertension is an independent predictor of cardiovascular and cerebrovascular outcomes, can lead to fatal cardiovascular disease as a silent killer. Aging is associated with changes in vascular structure and function through various pathways, including the overproduction of reactive oxygen species (ROS) which causes oxidative stress, cell aging, and inflammation. Hypertension can cause changes in vascular structure and function associated with aging, especially endothelial function[3]. One of the biomarkers of oxidative stress that is often used is 8-Hydroxy-deoxy-guanosine (8-OHdG) which is an important component of endogenous and exogenous gene damage that involves oxidative processes, leading to the generation of nucleic acid oxidation products. 8-OHdG is a noninvasive marker of oxidative stress damage to the genome, as measured in urine which is non-invasive, and is also highly stable[4].

In the previous study, the content of 8-OHdG based on nuclear and mitochondrial deoxyribonucleotrotein in hypertensive subjects was significantly higher than the normotensive controls. Moreover, urine 8-OHdG reflects the oxidation status of hypertensive subjects. and these markers can be used to monitor changes in oxidative stress in these patients[5]. In vivo monitoring of oxidative stress is easier with the ability to use non-invasively obtained samples, such as urine (Evans et al, 2010). In this study, we applied the ELISA procedure for the human urine sample to see if the sample could be used to estimate the physiological age of a person aged 46-55 years. [5].

Research Methods

This study used cross sectional design with ethical approval protocol number UH2009053. The study was conducted by taking samples of morning urine of hypertension patients at the Mangasa and Kalukubodoa Health Centers, and healthy controls. Determination of the criteria for the patient sample is based on the medical record of outpatients at the Public Health Center and by screening of pressure checks. The urine sample was then brought by the researcher to the Research Unit in the HUMRC laboratory/Hasanuddin University Teaching Hospital to examine the level of 8-Hydroxy-deoxyguanosine (8-OHdG) using the Enzyme-Inked Immunosorbent Assay (ELISA) method. The study population was hypertensive patients and healthy controls in the early elderly. The study sample was a reachable population that met the research criteria (inclusion criteria) with hypertension in the early elderly 45-55 years, no history of somatic or psychiatric disorders registered in the medical record, no history of medication, smoking or alcohol consumption for 2 weeks, and respondents agree to sign the Informed Consent. The sample was determined by purposive sampling technique in total of 80 respondents, 40 samples for the hypertension group and 40 samples for healthy controls. Data analysis was carried out according to the objectives and measuring scale and then analyzed with computer using Statistical Product and Service Solution (SPSS).

Result

This study was conducted on 40 hypertensive patients and 40 healthy controls who met the inclusion and exclusion criteria.

Table 1
Responden Characteristics

Parameter	Hipertensive n=40	Healthy Control n=40
[a]Gender		
Male	20 (50.0)	20 (50.0)
Female	20 (50.0)	20 (50.0)
[a]Age		
46-50 Year	13 (32.5)	28 (70.0)
51-55 Year	27 (67.5)	12 (30.0)

[a]Period of illness		
1 Year	18 (45)	-
2 Year	5 (12.5)	-
3 Year	17 (42.5)	-

n=The amount of sample
[a]: Parameters for categorical data (n %)

Table 1. The total number of subjects in this study were 40 people with hypertension, consisting of 20 men (50.0%) and 20 women (50.0%). Likewise, there were 40 healthy control subjects in this study, consisting of 20 men (50.0%) and 20 women (50.0%). Of the 40 subjects with hypertension, 13 people (32.5%) aged 46-50 years and 27 (67.5%) aged 51-55 years old were subjects; and from 40 healthy control subjects, 28 subjects (70.0%) aged 46-50 years and 12 (30%). And based on the period of the illness of hypertensive patients, there were 18 people with hypertension who were sick for 1 year (45%), 5 people who were sick for 2 years (12.5%), and 17 people who were sick for three years (45.5%)

Table 2
The Differences in the Expression of Urine 8-OHdG Level Based on Gender in Hipertension

20	1.374± 0.644	0.001
20	2.367± 0.206	

*p: Uji Independent Sampel Test

Based on the Independent sample test, it showed that there was a significant difference in urine 8-OHdG level between hypertensive men and women ($p<0.05$). The average urine 8-OHdG level in women with hypertension was higher at 2,367 ± 0.206 ng/mL, compared to men with hypertension, which was 1.374 ± 0.644 ng/mL.

Table 3
The Differences in the Expression of Urine 8-OHdG level Based on Age in Hipertension

13	1.680 ± 0.768	0.220
27	1.963 ± 0.643	

*p: Uji Mann-Whitney

Table 3. Based on the Mann Whitney test showed that there was no significant difference in urine 8-OHdG level in hypertensive patients aged 46-50 years and

51-55 years. The average urine 8-OHdG level in hypertensive patients aged 51-55 years was higher at 1,963 ± 0.643ng/mL, compared to the patients aged 46-50 years, which was 1,680 ± 0.768 ng/mL.

Table 4
The Differences in the Expression of Urine 8-OHdG level Based on Gender in Healthy Control

	20	1.575 ± 0.430	0.003
	20	1.912 ± 0.169	

*p: Uji Independent Sampel Test

Table 4. Based on the Independent sample test, it showed that there is a significant difference in urine 8-OHdG level in healthy controls, male and female. The average urine 8-OHdG level in female healthy controls was higher at 1.912 ± 0.169 ng/mL, compared to 1.575 ± 0.430 ng/mL in male controls.

Table 5
The Differences in the Expression of Urine 8-OHdG level Based on Age in Healthy Control

	28	1.705 ± 0.388	0.323
	12	1.833 ± 0.298	

*p: Uji Mann-Whitney

Table 5. Based on the Mann-Whitney test, it showed that there is no significant difference in urine 8-OHdG level in healthy controls aged 46-50 years and 51-55 years. The average urine 8-OHdG level in healthy controls aged 51-55 years was higher at 1.833 ± 0.298 ng/mL, compared to those aged 46-50 years, which was 1.705 ± 0.388 ng/mL.

Table 6
The Differences in the Expression of Urine 8-OHdG level Based on Hypertension and Healthy Control Groups

	40	1.871 ± 0.689	0.053
	40	1.743 ± 0.364	

*p: Uji Mann-Whitney

Table 6. Based on the Mann-Whitney test, it showed that there was no significant difference in urine 8-OHdG level in hypertensive patients and healthy controls. The average urine 8-OHdG level in hypertensive patients was higher at 1.871 ± 0.689 ng/mL, compared to healthy controls which was 1.743 ± 0.364 ng/mL.

Discussion

Hypertension in the elderly occurs due to the aging process which is characterized by changes in the physiological system such as the decreased immune system and increased susceptibility to various diseases like cancer, diabetes mellitus (DM), cardiovascular disorders, and neurodegenerative diseases, and could also cause death, with the result that it could affect life expectancy. Increased life expectancy can provide a picture of health and is one of the factors that greatly determines the quality of human resources[(6)(7)]. Hypertension is the most common disease that occurs in the elderly. Hypertension is considered a major risk factor for the development of heart disease and various vascular diseases in elderly people due to the stiffening of the arteries resulting in blood pressure to increase[(6)].

Hypertension can be affected by irreversible factors such as gender, age, genetics; and modifiable factors such as diet, exercise habits and others. The occurrence of hypertension requires the role corresponded risk factors (*common underlying risk factor*), in other words, one risk factor alone does not necessarily cause hypertension. With age, systolic and diastolic blood pressure increases. Systolic blood pressure increases progressively until the age of 70-80 years, while diastolic blood pressure increases until the age of 50-60 years and then tends to stay or decrease slightly. The combination of the two things above is very likely to reflect the presence of stiffening of the blood vessels and a decrease in arterial compliance and result in an increase in pulse pressure with age[(6)].

In this study, urinary 8-OHdG levels in female hypertensive patients were higher at 2,367 ± 0.206 ng/mL, compared to male at 1.374 ± 0.644 ng/mL (Table 2). In this study, samples of patients with hypertension used were men and women aged 46-55 years. Hypertension is also closely related to gender. Blood pressure in man has a higher diastolic than women at all ages, so that men suffer from hypertension more. However, hypertension that occurs in both men and women can cause stroke, left ventricular enlargement and kidney dysfunction. Research conducted by Kusumawaty (2016) regarding the relationship between gender and the incidence of hypertension in patients seeking treatment at the Adult Polyclinic of Bangkinang Health Center showed that women suffer from hypertension more (51%) than men (49%). The study also found that women suffer from hypertension more (58%) than men (42%)[(8)].

In elderly age, there is also an aging process that characterized by the decrease of estrogen hormone along with increasing age in women of old age (menopause) resulting in urine 8-OHdG level in women will be higher[(8)]. The incidence of hypertension in women increases in middle age and elderly. During menopause, the estrogen hormone continues to decline. Hormonal changes affecting women to experience increased sensitivity to salt and weight gain. Both of these have the potential to trigger higher blood pressure[(9)]. Based on Saputri's research (2017) at

the Penumping Health Center, Surakarta, in the postmenopausal women group, the proportion of hypertension disease was found to be related to postmenopausal age[10].

In this study, urine 8-OHdG level in hypertensive patients aged 51-55 years were higher at 1,963 ± 0.643 ng/mL, compared to hypertensive patients aged 46-50 years, namely 1,680 ± 0.768 ng/mL (Table 3). Also in this study, the sum of hypertensive subjects aged 51-55 years is more than the subjects aged 46-50 years. Hypertension is one of the common diseases in the elderly. Hypertension causes fatal cardiovascular disease[3]. The risk of hypertension will increase along with age. Blood pressure generally will increase by the age of 60 years. After the age of 45 years, the arterial walls would undergo thickening, causing the blood vessels to narrow and become stiff as a result of the buildup collagen in the muscle layer. This condition causes an increase in blood pressure due to the forced pumped blood at every beat to pass through the narrow blood vessels (11). Therefore the occurrence of hypertension is strongly influenced by the aging process. Both hypertension and aging are independent predictors of cardiovascular and cerebrovascular outcomes[3].

The level of urinary 8-OHdG in healthy female controls were higher at 1.912 ± 0.169 ng/mL, compared to male healthy controls at 1.575 ± 0.430 ng/mL (Table 4). In this study, the healthy control samples were men and women aged 46-55 years. The level of urine 8-OHdG in female healthy controls were higher, one of which could be the result of hormonal factors such as estrogen. Women aged 45-55 years will enter the premenopause period. Women who have not experienced menopause are protected by estrogen hormone. Estrogen plays a very important role in the immune process in premenopausal women. Premenopausal women will start to lose by degrees the estrogen hormone which has been protecting blood vessels from damage. This condition causes the estrogen hormone to change in quantity according to the age of the woman, which generally begins to occur at the age of 45-55 years[8].

In this study, urine 8-OHdG levels in hypertensive patients were higher at 1.871 ± 0.689 ng/mL, compared to healthy controls at 1.743 ± 0.364 ng/mL (Table 6). The risk of hypertension will increase with age or is strongly influenced by the aging process. Thus both hypertension and aging are independent predictors of cardiovascular and cerebrovascular outcomes. The aging process is characterized by the decrease of organ function, which is associated with changes in vascular structure and function through various pathways, including oxidative stress, cell aging, and inflammation. The aging process causes an increase in free radicals or ROS[8]. ROS can trigger aging at the cellular level, due to the cessation of replication due to damage to the cell structure, resulting in oxidative stress. Oxidative stress occurs due to the production of free radicals that exceed antioxidant levels, resulting in an imbalance[12][13][14]. ROS can trigger inflammation in the renal glomeruli which could affect the blood pressure regulation and trigger endothelial dysfunction[15][16][3].

Increased blood pressure caused by oxidative stress, could increase the activity of NADPH (Nicotinamide Adenine Dinucleotide Phosphate) oxidate. NADPH oxidate increases the production of ROS which could react with NO causing the decrease

in NO bioavailability resulting in the impairement of vasodilation[14]. The increasing age will affect the stiffness of the blood vessel walls and could cause the blood vessels to become stiff and the decrease in arterial compliance, thereby increasing blood pressure[17][6]. The incidence of hypertension is expected to continue to increase considering the higher life expectancy[14].

Conclusion

The level of urinary 8-OHdG produced by the body can be used as one of the considerations for biomarker examination to assess the oxidative stress damage in hypertensive patients because urinary 8-OHdG is the end product of ROS which will increase along with age.

Suggestion

This research needs to be developed by using more variables, spesifically the factors that affect the level of urinary 8-OHdG to confirm the results of this research. This study can be carried out for further research to determine the relationship between urine 8-OHdG levels and antioxidant levels in patients with hypertension.

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