

## Oxidative Stress in the Elderly with Hypertension: A Cross-Sectional Study

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### Abstract

Hypertension is a major health problem, especially in the elderly, because it serves as a risk factor for cardio- and cerebrovascular diseases. The involvement of oxidative stress in hypertension has been shown in animal studies. However, the data about oxidative stress in humans with hypertension, especially in the elderly, are still limited.

The aim of this study is to analyze oxidative stress by measuring carbonyl and superoxide dismutase (SOD) in hypertensive elderly. It was a cross-sectional study conducted on 70 elderly subjects, 35 subjects with hypertension and 35 subjects with normotension, in Jakarta, Indonesia. Subjects were classified into the hypertensive group if their systolic blood pressure was  $\geq 130$  mmHg or their diastolic blood pressure was  $> 80$  mmHg according to American guidelines. Plasma carbonyl and SOD were measured using a spectrophotometer. Correlation analysis and Independent T-test were used for statistical analysis. Carbonyl was significantly higher, while SOD was significantly lower in hypertensive elderly compared to the control. There was a negative correlation between SOD and systolic blood pressure, as well as a weak positive correlation between carbonyl and mean arterial pressure.

In conclusion, a significant increase of carbonyl and decrease of SOD were detected in hypertensive elderly, reflecting oxidative stress.

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### Introduction

Hypertension is a type of vascular disturbance characterized by the elevation of blood pressure.<sup>1</sup> European guidelines define hypertension as blood pressure  $\geq 140/90$  mmHg;<sup>2</sup> however, American guidelines determine a lower threshold to define hypertension ( $\geq 130/80$  mmHg).<sup>3</sup> Hypertension is a dangerous pathological condition that presents a crucial cardiovascular risk, especially in elderly subjects.<sup>4</sup> In the United State of America (USA) and Europe, about 50-75% of the elderly suffer from hypertension, and it is predicted that approximately 60% of hypertension occurrence is dominated by subjects over 75 years old.<sup>5</sup> Hypertension occurs in aging individuals and

could be an important risk factor for the morbidity and mortality of cardiovascular diseases.<sup>4</sup> Moreover, hypertension in the elderly contributes to declines in cognitive function and physical capability, as well as an upsurge in the risk for fracture due to falls.<sup>4</sup> The rise of blood pressure in the elderly is occasioned by several age-associated physiological alterations, such as endothelial dysfunction, arterial rigidity, upregulation of sympathetic response, low-grade inflammation, and oxidative stress.<sup>4</sup>

Oxidative stress is an imbalance between high levels of reactive oxygen species (ROS) and low levels of antioxidant defense. It can damage macromolecules within the cells such as protein, lipids, and deoxyribonucleic acid (DNA).<sup>6</sup> The damage of these macromolecules can be detected by measuring carbonyl levels, which reflect protein damage; malondialdehyde, which reflects lipid damage; and 8-hydroxy-deoxyguanosine, which reflects DNA damage.<sup>6</sup> ROS, such as superoxide anions, are produced physiologically in the mitochondria during cell respiration due to electron leakage.<sup>7</sup> Moreover,

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many kinds of enzymes in the cells, such as the nicotinamide adenine dinucleotide phosphate oxidase, xanthine oxidase, and nitric oxide synthase also generate superoxide anion.<sup>8</sup> Accumulation of superoxide anions in the vascular system is correlated with hypertension. Superoxide anions degrade nitric oxide (NO), a vasodilator that maintains vascular relaxation. This is on top of the decreasing availability and production of NO that comes with increasing age.<sup>9</sup>

Our bodies are equipped with an endogenous antioxidant system to eliminate the accumulation of ROS, consisting of superoxide dismutase (SOD), glutathione peroxidase, catalase, and glutathione reductase. SOD catalyzes the conversion of superoxide anions to hydrogen peroxide, which is further converted into water molecules by catalase or glutathione peroxidase.<sup>7</sup> The role of endogenous antioxidant defense in hypertension has mostly been explored in animal studies, however studies in humans, especially the elderly, are still limited. Therefore, this study aims to analyze the carbonyl levels that reflect protein damage and SOD concentration as an endogenous antioxidant in the elderly with hypertension compared to a control. A correlation analysis between carbonyl, SOD, and blood pressure was performed in order to predict the role of carbonyl and SOD in the elderly with hypertension. This research is important as a basis for the consideration of antioxidant supplementation for hypertensive elderly patients in subsequent studies.

## Materials and methods

This is a cross-sectional study using 70 elderly subjects (35 subjects with hypertension and 35 subjects with normotension) in three different sub-districts in Jakarta, Indonesia. Subjects were recruited for a consecutive sampling in several integrative healthcare centers from July to September 2019. The inclusion criteria were men or women aged 60 years old and over. They had to sign informed consent and be willing to follow all procedures. The exclusion criteria were a history of smoking or drinking alcohol in the last year, severe pain in the lower extremities, or fever. This study was approved by the ethics committee of the Faculty of Medicine at Universitas Indonesia (Number:

KET-442/UN2.F1/ETIK/PPM.002.02/2019).

### Blood pressure measurement

Blood pressure assessment was performed with patients in the seated position. Before measurement, subjects were given a five-minute, quiet resting period. Systolic blood pressure and diastolic blood pressure were defined by Korotkoff phases I and V, respectively. The subjects were classified in the hypertensive group if their systolic blood pressure  $\geq 130$  mmHg or diastolic blood pressure  $> 80$  mmHg.<sup>3</sup>

### Body mass index calculation

Body mass index (BMI) data were obtained from weight and height measurements. Due to limitations in the elderly, we used knee height (the distance from the foot to the knee) measurement to obtain body height. Body weight measurements were taken using a SECA 803 digital body weight scale, and the knee height measurements were taken using a knee height caliper. The results of the knee height measurements were converted to body height using the following formula:<sup>10</sup>

$$\text{Men's Height (cm)} = (1.924 \times \text{knee height [cm]}) + 69.38$$

$$\text{Women's Height (cm)} = (2.225 \times \text{knee height [cm]}) + 50.25$$

### Analysis of random blood glucose test

We obtained random blood glucose levels with a rapid glucose assessment using blood glucose test strips.

### Analysis of carbonyl level

The carbonyl level was analyzed from the subjects' plasma using a colorimetric assay based on a 2,4-dinitrophenylhydrazine reaction. The sample absorbance was read at 360 nm using a spectrophotometer.<sup>11</sup>

### SOD measurement

The activity of SOD enzymes was analyzed from subjects' hemolysate using a RanSOD kit (Randox®) according to manufacturer's guidelines. This kit utilizes xanthine and xanthine oxidase to produce superoxide radicals, which form a red formazane color after reacting with the 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (INT) reagent. The SOD activity is measured based on the degree of this reaction inhibition. One unit of SOD is defined as 50% inhibition of a red color product.

### Statistical analysis

We categorized the results into two groups, the hypertensive and the control group.

Independent T-test and Mann Whitney U test were used to determine the difference in mean between the two groups. Pearson and spearman correlation tests were used to analyze the correlation between blood pressure with oxidative stress. Statistical analysis was performed using the SPSS program. P values < 0.05 were defined as significant.

## Results

The characteristics of the subjects are shown in Table 1. There were more female elderly subjects than male in both the control and hypertensive groups. Blood pressure, pulse, and mean arterial pressure (MAP) were significantly higher in the elderly with hypertension compared to the control. However, there was not a significant difference in BMI and blood glucose between the groups (Table 1).

Variable	Control Group	Hypertensive Group	P
Age (Years)	70.83 ± 5.61 <sup>IT</sup>	68.94 ± 6.06 <sup>IT</sup>	0.18
Gender			
- Male (n)	11	8	
- Female (n)	24	27	
BMI (kg/m <sup>2</sup> )	23.65 ± 4.36 <sup>IT</sup>	23.51 ± 2.94 <sup>IT</sup>	0.87
Blood Pressure			
- Systolic	110 (100 – 120) <sup>S</sup>	130 (100 – 170) <sup>S</sup>	<0.001 <sup>MT*</sup>
- Diastolic	70 (60 – 80) <sup>S</sup>	80 (60 – 110) <sup>S</sup>	0.006 <sup>MT*</sup>
Pulse Pressure (PP)	72 (68 – 82)	80 (71 – 88)	<0.001 <sup>MT*</sup>
MAP	86.29 ± 6.04 <sup>IT</sup>	97.71 ± 6.04 <sup>IT</sup>	<0.001 <sup>IT*</sup>
Blood Glucose	117 (78 – 204) <sup>S</sup>	112 (79 – 306) <sup>S</sup>	0.75
Comorbidities			
- Diabetes Mellitus	17	29	
- Coronary heart disease	5	14	

<sup>IT</sup>: Mean ± standard deviation (SD)

<sup>S</sup>: Median (minimum-maximum)

IT: Independent T-test

MT: Mann Whitney U test

Table 1. Characteristics of subjects.

Variables	Control Group (Mean ± SD)	Hypertensive Group (Mean ± SD)	T-value	P
Carbonyl (nmol/mL)	4.79 ± 1.49 <sup>IT</sup>	6.15 ± 1.95 <sup>IT</sup>	-3.26	0.002 <sup>IT*</sup>
SOD (U/mL)	165.19 ± 57.06	112.81 ± 49.92	4.09	0.001 <sup>IT*</sup>

IT: Independent T-Test, \*: p < 0.05

Table 2. Carbonyl level and SOD.

Both groups had the comorbidities of diabetes mellitus and coronary heart disease, which were more prevalent in the hypertensive group. We found that carbonyl level was significantly higher in the hypertensive group compared to the control group, while SOD activity as an endogenous antioxidant enzyme was lower in the hypertensive group compared to

the control (Table 2). A correlation analysis was conducted to predict the role of carbonyl and SOD in blood pressure, pulse pressure, and MAP. There was a weak positive correlation between carbonyl and MAP (Table 3), as well as a weak negative correlation between SOD and systolic blood pressure.

Variable	SBP (mmHg)		DPB (mmHg)		PP		MAP (mmHg)	
	r	p-value	r	p-value	r	p-value	r	p-value
Carbonyl	0.19 <sup>SP</sup>	0.13	0.23 <sup>SP</sup>	0.054	0.14 <sup>SP</sup>	0.24	0.25 <sup>PR</sup>	0.036*
SOD	-0.31 <sup>SP</sup>	0.008*	-0.02 <sup>SP</sup>	0.85	-0.19 <sup>SP</sup>	0.11	-0.17 <sup>PR</sup>	0.17

Table 3. Correlation of blood pressure with oxidative stress marker.

<sup>SP</sup>: Spearman correlation test, <sup>PR</sup>: Pearson correlation test,

r : Correlation coefficient, \*: p < 0.05.

## Discussion

The female elderly subjects in this research were more numerous than the men, which is suitable based on data from the Indonesia Central Bureau of Statistics, which indicates that elderly people in Indonesia constitute 8.9 % of the population in Indonesia, consists of 52.52% females and 47.48% males.<sup>12</sup> Chinnakali et al.<sup>13</sup> found that the prevalence of hypertension in elderly females (40.8%) was slightly higher compared to males (39.2%). It was also reported that the prevalence of hypertension was slightly higher in post-menopausal females compare to males.<sup>14</sup> The increase of systolic and diastolic blood pressure in the hypertensive group caused the elevation of pulse pressure (PP) and MAP. PP and MAP are used as predictors for cardio- and cerebrovascular diseases.<sup>15</sup> A study by Zheng et al.<sup>16</sup> of uncontrolled hypertension demonstrated that MAP can predict ischemic stroke in patients aged ≥ 65 years old.

The high level of carbonyl, which reflects protein damage, showed that oxidative stress occurred in the hypertensive group. Oxidative stress inhibits NO activation and diminishes its availability. NO is a powerful vasodilator that modulates the permeability of the vascular wall. Adequate NO availability is important for maintaining endothelial function. Lack of NO availability leads to endothelial dysfunction and is correlated with hypertension and atherosclerosis, which are major risk factors for cardiovascular disease.<sup>9</sup> Moreover, oxidative stress is also correlated with inflammation and stimulates the

thickening of the vascular wall due to a proliferation of vascular muscle cells and collagen accumulation.<sup>17,18</sup> Our research shows an increase of carbonyl by 1.28-fold in the hypertensive group compared to the control. The result of this study is in line with Yavuzer et al.,<sup>19</sup> who found an elevation of carbonyl in 30 elderly subjects with hypertension. Naregal et al.<sup>20</sup> also found the elevation of another oxidative stress marker (malondialdehyde, which reflects lipid damage) in elderly subjects with hypertension.

In this research, although plasma carbonyl was increased, there is no correlation between carbonyl and blood pressure. This probably indicates that carbonyl is not a main factor that influences blood pressure in the elderly. However, an animal study using rats showed that carbonyl could stimulate hypertension.<sup>21</sup> Some studies have shown an increase in ROS such as superoxide radicals, hydrogen peroxide, and lipid peroxide in hypertension.<sup>22</sup> ROS may have a direct effect on blood pressure. On the other hand, we found that there was a weak positive correlation between carbonyl and MAP. This means that the carbonyl compound might increase cardiac output and peripheral resistance.

SOD activity in the hypertensive group was significantly lower compared to the control group. It seems that the reduction of this enzyme acts as a defense to cope with oxidative stress. SOD eliminates superoxide radicals by altering them into hydrogen peroxide.<sup>7</sup> Low levels of SOD could enhance superoxide radicals, which further inhibit NO activation.<sup>23</sup> The knockdown SOD gene in mice leads to the accumulation of superoxide radicals and decreased NO levels.<sup>24</sup> Therefore, decreased SOD has an impact on the formation and progression of hypertension. Our results found that there was a negative correlation between SOD and systolic blood pressure. This means that low levels of SOD have the potential to increase systolic blood pressure. A study by Naragel et al.<sup>20</sup> showed that a decline in SOD induced elevation of systolic blood pressure in the elderly. A negative correlation between SOD and systolic and diastolic blood pressure were also detected in adult hypertensive subject (36-51 years old).<sup>25</sup> Therefore, modulation of oxidative stress and antioxidant systems could regulate blood pressure. Administration of antioxidant supplements, as well as high intake of fruits and

vegetables as a source of antioxidants, may be beneficial for hypertensive patients. However, further research is needed to evaluate blood pressure following antioxidant treatment. The limitation of this study was that both groups had comorbidities (diabetes mellitus and cardiovascular disease), which may affect oxidative stress levels. However, these comorbidities were more common in the hypertensive group, and there was no significant difference in blood glucose levels between the groups. Moreover, the history of these comorbidities was only obtained from anamnesis, which may be less accurate compared to laboratory examination finding.

## Conclusions

The significant increase of carbonyl and decrease of SOD detected in elderly subjects with hypertension reflect oxidative stress. Moreover, the elevation of carbonyl might increase MAP, and the reduction of SOD probably leads to an elevation of systolic blood pressure. Further clinical trial research is needed such as by giving antioxidant supplementation to hypertensive elderly in order to eliminate oxidative stress.

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## Declaration of Interest

The authors report no conflict of interest.

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