Sapporo Medical Journal
Volume 55, Issue 01, January, 2021

OXIDATIVE STRESS EVALUATION USING URINARY MDA AND 8-OHdG BASED ON ACEi/ARB CONSUMPTION IN CKD PATIENTS

Hana Aqilah Nur Imania[1], Mochammad Thaha[2,3]*, Nurina Hasanatuluddhiyah[4], Maftuchah Rochmanti[4], Nadira Muthi Tsania[1], Arief Bakhtiar[5]

[1.] Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia
[2.]Department of Internal Medicine, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia
[3.]Department of Internal Medicine, Universitas Airlangga Hospital, Surabaya, Indonesia
[4.] Department of Pulmonology and Respiratory Medicine, Universitas Airlangga/Dr.Soetomo Teaching Hospital, Surabaya, Indonesia
[5.] Department of Pharmacology, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia

Abstract— Based on the data from "World Kidney Day", there are at least 1 out of 10 people around the world with a certain level of morbidity that leads to Chronic Kidney Disease (CKD). Many studies have shown that oxidative stress contributes to the progression of CKD. This study aims to compare malondialdehyde (MDA) and 8-dihydro-2-deoxyguanosine (8-OHdG) as oxidative stress markers in CKD patients who were treated with angiotensin-converting enzyme inhibitor (ACEi) or angiotensin receptor blocker (ARB) and who were not treated with both therapies. In this study, fifty-four hospitalized CKD patients were recruited randomly from three different hospitals in Surabaya, Indonesia. This study's subjects were grouped based on their consumption of ACEi/ARB. Oxidative stress markers, MDA and 8-OHdG, were calculated using the Mann-Whitney test with a p-value<0.05. Urinary MDA in the patients receiving ACEi/ARB was lower than those who did not receive (3.75 (2.24-9.92) vs 5.16 (2.61-19.41); p=0.002). Urinary 8-OHdG in the patients receiving ACEi/ARB was lower than those who did not receive any (4.11 (0.36-20.26) vs 6.61 (1.76-31.35); p=0.028). There were positive correlations between urinary MDA and urinary creatinine (R=0,547; p=0,000) and between urinary 8-OHdG and urinary creatinine (R=0,630; p=0,000). These results implicate that ACEi/ARB administration in CKD patients is beneficial for reducing urinary oxidative stress markers.

Keywords— chronic kidney disease, MDA, 8-OHdG, ACEi, ARB

1. Introduction

Chronic Kidney Disease (CKD) is a structural and functional kidney abnormality that occurs more than 3 months with various health implications [1]. In 2017, 697.5 million CKD cases (all stages) were reported, with a global prevalence of 9.1%, and 1.2 million people died from CKD [2]. Based on the progressive reduction in Glomerular Filtration Rate (GFR), CKD is then classified from stage 1 with normal GFR (>90 mL/min/1.73 m^2) and might be accompanied by other symptoms like proteinuria to stage 5 when end-stage renal disease (ESRD) has occurred. Thus, medical treatment such as dialysis or organ transplant is needed for ESRD [3]. In the course of the disease, one of the basic mechanisms responsible for CKD is the presence of increased oxidant agents such as Reactive Oxygen Species (ROS). An excessive oxidant is dangerous for cells because it may damage either the body's defenses or the delivery process of other physiological signals [4]. The ROS enhancement that disrupts the balance between oxidant and antioxidant compounds leads to a state called oxidative stress [5].

Increased levels of oxidative stress also have a significant role in the Renin-Angiotensin-Aldosterone System (RAAS). Meanwhile, Renin also plays a role in Angiotensin II (AngII) formation as well as NADPH oxidase (NOx) regulation [6]. NOx is also essential in producing various oxidant compounds so that oxidative stress and RAAS overlap each other, which can exacerbate CKD progression. Based on many previous studies, oxidative stress levels in CKD patients always increase, especially in diabetes and hypercholesterolemia patients [7]. Some oxidative stress markers that are frequently used by clinicians and researchers are malondialdehyde (MDA) as a by-product of lipid peroxidase or 8-Hydroxy-2'-deoxyguanosine (8-OHdG), a part of an oxidized DNA nucleotide. Those oxidative stress markers may worsen renal dysfunction and increase not only the progression of heart function but also endothelial tissue failure [8,9,17]. Therefore, CKD patients' treatment is the drugs that act as RAAS inhibiting agents [10].

The Angiotensin-Converting Enzyme Inhibitor (ACEi) or Angiotensin Receptor Blocker (ARB) was recommended as a therapeutic option in CKD patients. They lower blood pressure, reduce proteinuria, slow the progression of kidney disease, and likely reduce CVD risk by mechanisms in addition to lowering blood pressure [11]. ACEi, such as Captopril, works to inhibit various enzymes that convert Angiotensin I (inactive decapeptide) to Angiotensin II (active vasoconstrictor) while ARB competes with Angiotensin II at its receptor site, the AT1 receptor [12]. In vivo, AngII inhibitor which works to inhibit AT1 receptors, for example, Losartan, reduces levels of oxidative stress by suppressing the number of oxidant compounds such as -O2 or vascular H2O2 through AngII receptors [13].

However, exact data describing the effect of ACEi/ARB antihypertensive drug administration in CKD patients on urinary MDA and urinary 8-OHdG in relation to urinary creatinine have not been massively discussed. Therefore, this study was conducted to determine the comparison of levels of oxidative stress markers and their correlation with renal function indicators between CKD patients who consumed and did not consume ACEi/ARB.

2. Methods

2.1 Study design and participants

This study was an observational study with a cross-sectional design. This research was approved by Universitas Airlangga Hospital Ethics Committee (Approval Number: 116/KEH/2019). With a minimum of 18 CKD patients based on previously described calculation, the subjects of this study's subjects were patients diagnosed with CKD and have been hospitalized in three different hospitals in Surabaya, Indonesia. All the subjects agreed and signed the informed consent. Fifty-four CKD subjects of both sexes were recruited with the inclusion criteria of people with minimum stage 1 CKD and above 21 years old.

The subjects were divided based on ACEi/ARB consumption: Group A who did not consume ACEi or ARB (n=25) and Group B who consumed ACEi or ARB (n=29). Most of the patients were also receiving other antihypertensive agents such as β_1-blocker (BB), calcium-channel blocker (CCB), statin, diuretics, and antioxidant agents. Data on age, sex, blood pressure, body mass index (BMI), comorbidities, smoking and hemodialysis status, pharmacological therapy consumed, urinary MDA and urinary 8-OHdG, urinary creatinine were collected.

2.2 Laboratory examinations

The samples for laboratory tests were collected from urine. The MDA and 8-OHdG marker were measured from urine and processed by enzyme-linked immunosorbent assay (ELISA) based kits. Urinary creatinine level was respectively measured with Hitachi 7600 using a turbidimetric immunoassay and an enzymatic method.

2.3 Statistical Analysis

Analyses were supported by SPSS Statistics version 25. All quantitative data were shown separately by each group and performed to normality test using Saphiro-Wilk method. The difference of both oxidative stress markers, MDA and 8-OHdG, between Group A and B were calculated using Mann-Whitney test. All comparison tests utilized in this study used two-tailed 95% Confidence Interval. A significant difference was defined if the p-value<0.05. A Spearman test was used for the correlation between variables.

3. Result

3.1 Characteristic of subjects

Fifty-four patients were observed in this study. Twenty-four subjects (44%) were on stage 5; 12 subjects were on stage 4 (22%); stage 3 and stage 2 had 11 (20%) and 4 (7%) subjects respectively; and 3 subjects were on stage 1 (5%). The baseline characteristics of the study participants were shown in **Table 1**. Forty-seven subjects had hypertension, 42 had type 2 diabetes, and 8 had coronary heart disease. Some patients might also receive other pharmacological therapy, namely β-blocker (BB), calcium channel blocker (CCB), statin, diuretics, and antioxidants as many as 24, 41, 17, 5, and 28 subjects.

3.2 Kidney function profile of subject

The subject's urinary creatinine was categorized based on the reference value and shown as the median (95% LL-UL) in **Table 2**. The urinary creatinine of Group B 63.09 (27.6-179.54) was lower than Group A 81.42 (37.07-252.01), but it is not statistically significant.

3.3 Comparison of Urinary MDA, Urinary 8-OHdG, and Urinary Creatinine

There was a statistically significant reduction of the oxidative stress markers among CKD patients who consume ACEi/ARB (**Table 3**). Urinary MDA in Group A (5.16 (2.61-19.41)) was higher than Group B (3.75 (2.24-9.92)) with a p-value of 0,002. Urinary 8-OHdG in Group A (6.61 (1.76-31.35)) was also higher than Group B 4.11 (0.36-20.26)) with p-value of 0,028.

3.4 Correlation of Urinary MDA, Urinary 8-OHdG, and Urinary Creatinine

The data showed positive correlations between urinary MDA with urinary creatinine (R=0,547; p=0,000) and urinary 8-OHdG with urinary creatinine (R=0,630; p=0,000) as listed in **Table 4**.

4. Discussion

Based on the data, participants are dominated by male patients. Differences in sex hormones between both genders may explain the slower progression of CKD in men [14]. Both in the ACEi/ARB and non-

ACEi/ARB groups, most of the subjects were over 45 years. This condition was in line with many previous studies and epidemiological reports. It is the consequence of the reduction of glomerular filtration rate (GFR), around 8 mL/min/1.73 m² since the age of 40 years [15]. Patients treated with ACEi or ARB should also be monitored for hypotension, decreased GFR, and hyperkalemia. Also, the number of patients with systolic blood pressure ≥ 130 mmHg who received ACEi/ARB therapy (56%) was more than the non-ACEi/ARB group (31%). This result may prove that there is an overlapping relationship between CKD and hypertension as found in the previous study [16].

Cardiovascular disease and various associated risk factors are related to the progression of CKD. An animal experiment in angiotensin-induced mice model showed that hypertension increased ROS production. The escalation of oxidant compounds will further aggravate kidney dysfunction, marked by the increase of albuminuria. This condition leads to worsening CKD degrees [16]. The high systolic and diastolic pressure in CKD patients was also associated with the inflammatory process [17]. A lot of studies about anti-inflammation drugs in CKD patients, such as statin, is conducted to prove its role in overcoming the inflammation condition in CKD [6].

ROS levels enhancement disrupt the balance between oxidant and antioxidant compounds. Imbalanced oxidative levels can make the kidneys more vulnerable, which occurs in both the renal cortex and medulla, with various effects ranging from changes in renal blood flow to retention of sodium or other inflammatory substances. Furthermore, intracellular ROS formation is mediated by NOx. Whereas outside the cell, this process is triggered by the presence of AngII which binds to the AT1 receptor. Hence, AngII inhibitor agents such as ACEi/ARB can also suppress the production of various oxidant compounds.

Several studies have been conducted to assess the effectiveness of urinary MDA and urinary 8-OHdG as promising potential markers. *In vivo,* urinary MDA will increase along with lipid peroxidation caused by vitamin E deficiency and exposure to iron nitrilotriacetate [20]. Urinary MDA has been used as a marker in obesity plus visceral and has been very useful as a non-invasive test for oxidative stress [23]. In 2015, Zargari et al. also reported that MDA levels were increased in patients who had received hemodialysis therapy [21]. Although the level of MDA can be measured from both serum and tissue, a serum sample for measuring biomarkers can stress the patient due to the blood sampling process. Currently, the use of alternative biofluids in clinical practice is being tested because they offer an easier way for people to not get stress or trauma, such as those related to saliva and urine which tend to be cheaper, easier to obtain, quicker, and not affected by injury or infection of the blood vessels [27]. In addition, some argue that urine is a better marker of oxidative stress than plasma because of its lower content of metal-containing organic and inorganic compounds [24]. Because of the lower levels of the ROS promoter as well, urine is less susceptible to increased oxidative stress markers created when taking and storing samples [22].

High levels of ROS are also thought to induce the oxidation of DNA and RNA, through the oxidation of nucleosides (especially the guanine part). The nucleoside oxidants are excreted in the urine so that it can be interpreted that urine acts as an indicator of cumulative oxidative stress [29]. Biomarkers associated with ROS-induced DNA damage in CKD patients include 8-OHdG and 8-oxodeoxyguanosine (8-oxo-dG). These two biomarkers are essential for measuring endogenous oxidative damage to DNA [18-19].

A study by Agarwal, that many other researchers have cited, examined the effect of add-on ARB, Losartan (50 mg/day for 1 month), on oxidative stress and proinflammatory state of the kidney in patients with CKD and found that urinary MDA was significantly decreased from 4.75 ± 3.23 to 3.39 ± 2.17 mol/g creatinine [28]. In contrast, plasma MDA or oxidized proteins did not change in response to additional AngII

blockade. Another study using Telmisartan (ARB) also reported a significantly lowered urinary malondialdehyde excretion [39]. Urinary MDA to creatinine ratio was also reduced by Telmisartan at week 18 ($p<0.01$ vs. placebo). Furthermore, it is said that urinary measurements of markers of oxidative stress appear to be more sensitive than plasma measurements in patients with CKD [28].

Administration of ACEi significantly decreased urinary 8-OHdG levels [38,40]. Dincer et al. reported that compared to patients taking CCB, the urinary 8-OHdG level was significantly lower in the patients taking an ACE inhibitor. Meanwhile, the serum level of 8-OHdG was not significantly different in the treatment groups [40]. Thus, according to their data, urinary measurement as a marker of oxidative damage appears to be more sensitive than serum measurement in patients with CKD [38]. In the ARB group, there was also a significant reduction in urinary excretions of 8-OHdG (-17.5 ± 6.9). The researchers argue that the level of urinary oxidative stress markers reflects the intrarenal level of oxidative stress better [41].

Our study showed that all markers of oxidative stress decreased in the patients who consumed ACEi/ARB. The MDA and 8-OHdG depletion were comparable to several other studies. For example, an *in vivo* study on 40 mice with hyperglycemia investigated whether ACEi has an antioxidant effect. As a result, ACEi has been shown to reduce H_2O_2 and suppress MDA concentrations in streptozotocin-induced mice [30]. Another study conducted on 52 ESRD patients with HD programs for at least 12 months who took Losartan, a type of ARB drug, statistically had more controlled blood pressure, decreased oxidative stress index, and lower antioxidant levels [31]. Finally, ACEi/ARB has a positive role in preventing the progression of CKD or in other words, these antihypertensive drugs are beneficial in reducing oxidative stress.

In addition, urinary creatinine was also reported for observing the relationship between the oxidative stress markers and the indicator of laboratory renal function. A study showed that changes in MDA levels in pediatric patients with ESRD on HD or continuous peritoneal dialysis (CPD) paralleled plasma creatinine, with a mean MDA/creatinine ratio of 34.4 before HD and 26.5 after HD [32]. To reduce the effect of urinary excretion rate as well as minimize differences in urine density between participants, urinary MDA and 8-OHdG concentrations were adjusted for urinary creatinine [33-34]. That report is supported by the explanation that creatinine can be a good urine dilution factor and it is affected by renal tubular processes [35]. Therefore, this study observed the relationship of urinary creatinine toward levels of markers of oxidative stress as a representation of a renal function in CKD patients.

Eventually, several conditions were different in this study compared to previous similar studies. It was the use of urine to evaluate oxidative stress levels when the majority of other studies used plasma or serum. However, the choice of this method is important because kidney function directly affects the ability to filter and excrete solutes such as oxidative stress markers [36]. Selection of urine samples to measure oxidative stress markers and observe kidney function can be considered to support the diagnosis and clinical observation of patients with CKD. Assessment with urine tends to be less invasive so it is easier to do to assess improvement after pharmacological therapy intervention, either with antihypertensive agents such as ACEi/ARB or HD [37].

This study also had certain limitations. The overlapping cardiac and renal disease led to the administration of more than one pharmacological drug that could make it difficult to distinguish oxidant level, whether from kidney injury or exposure to chemicals and exogenous drugs. Our findings did not directly address the question regarding the contribution of ACEi/ARB to oxidative stress in the development of CKD. More clinical studies of this kind need to be done, especially regarding the injury processes that result in

increased oxidative stress levels based on the urinary biomarkers in CKD patients.

5. Conclusion

This study indicates that urinary levels of MDA and 8-OHdG as oxidative stress markers were lower in subjects with CKD patients who received ACEi/ARB than those who did not. The two markers are also related to an indicator of kidney function, namely urinary creatinine. Thus, administration of ACEi/ARB in CKD patients gives significant results on urinary MDA and 8-OHdG in urine, even the urinary creatinine levels themselves. These results mean that urinary MDA and 8-OHdG could be useful for monitoring CKD patients with ACEi/ARB treatment.

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7. Acknowledgment

We thank Professor Thaha and the team who collected the data and allowed us to use some of the data for this study.

8. Figures and Tables

Table 1. Characteristic of Study Participants

No	Characteristic	Group A (n=25)	Group B (n=29)
1	Age, mean (Years)	58 ± 7.8	58 ± 9.1
2	Sex ratio (M/F)	16/9	16/13
3	Body Mass Index ≥ 25, n (%)	14 (56)	18 (62)
4	Systolic Blood Pressure ≥ 130 mmHg, n (%)	14 (56)	9 (31)
5	Diastolic Blood Pressure ≥ 80 mmHg, n (%)	22 (88)	11 (37)
6	Hemodialysis, n (%)	14 (56)	17 (58)
7	Hypertension, n (%)	20 (80)	27 (93)
8	Type 2 Diabetes, n (%)	21 (84)	21 (72)
9	Coronary heart disease, n (%)	5 (20)	3 (10)
10	Smoking, n (%)	10 (40)	5 (17)
11	BB	8 (32)	16 (55)
12	CCB	18 (72)	23 (79)
13	Statin	7 (28)	10 (34)
14	Diuretics	3 (12)	2 (7)
15	Antioxidant	11 (44)	17 (58)

Group A (did not consume ACEi/ARB) and group B (consumed ACEi/ARB); BB= β-blocker; CCB= Calcium Channel Blocker

Table 2. Kidney function profile of the subject

Characteristic	Category	n (%)	Median (95% LL-UL)	
			Group A	Group B
Urine Creatinine	≤ 37 mg/dl women	2 (9)	81.42 (37.07-252.01)	63.09 (27.6-179.54)
	≤ 40 mg/dl men	3 (9)		
	> 37 mg/dl women	20 (91)		
	> 40 mg/dl men	29 (91)		

Group A (did not consume ACEi/ARB) and group B (consumed ACEi/ARB)

Table 3. Comparison of Urinary MDA and Urinary 8-OHdG between groups

Parameter	Median (95% LL-UL)		p
	Group A	Group B	
Urinary MDA (mmol/L)	5.16 (2.61-19.41)	3.75 (2.24-9.92)	0.002
Urinary 8-OHdG (ng/mL)	6.61 (1.76-31.35)	4.11 (0.36-20.26)	0.028

Analyzed using Mann-Whitney Test; Group A (did not consume ACEi/ARB) and group B (consumed

ACEi/ARB)

Table 4. Correlation between Urinary MDA, Urinary 8-OHdG, and Urinary Creatinine

Correlation	R	P
Urinary MDA and Urinary Creatinine	0,547**	0,000
Urinary 8-OHdG and Urinary Creatinine	0,630**	0,000

Analyzed using Spearman's Rho Test; 8-OHdG: 8-dihydro-2-deoxyguanosine
**: there is a correlation between the variables associated with a significance level of 1%