Original Research

8-HYDROXYDEOXYGUANOSINE URINE AND TOTAL NITRIC OXIDE SERUM IN CHRONIC KIDNEY DISEASE

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ABSTRACT

Oxidative stress is essential to chronic kidney disease (CKD). Several markers include 8-Hydroxydeoxyguanosine (8-OHdG) and Nitric Oxide (NO). Reactive oxygen species (ROS) and Reactive Nitrogen Species (RNS) increased in CKD and had a role in renal impairment progressivity. There are some controversies regarding oxidative markers in CKD patients in several studies. This study aimed to understand oxidative markers 8-OHdG and NO and explained the correlation of both markers in hemodialysis and non-hemodialysis CKD patients. Twenty hemodialysis patients and forty-nine non-hemodialysis patients were enrolled in this cross-sectional study. Urine patients were collected to measure 8-OHdG using the enzyme-linked immunoassay (ELISA) method, and NO was measured from serum patients using the Griss Saltzman method. Based on Bivariate Pearson analysis, there was no significant correlation between 8-OHdG urine and total NO serum in the hemodialysis group (p= 0,510, p>0.05) and in the non-hemodialysis group (p= 0.801, p>0,05). In this study, DNA oxidative marker, 8-OHdG, was not correlated with NO in CKD patients.

Keywords: *Chronic kidney disease; 8-hydroxydeoxyguanosine; nitric oxide; oxidative stress; life expectancy*

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How to cite: Ahadini, P. A., Thaha, M., & Mustika, A. (2022). 8-Hydroxydeoxyguanosine Urine and Total Nitric Oxide Serum in Chronic Kidney Disease. Folia Medica Indonesiana, 58(2), 137ï 140. https://doi.org/10.20473/fmi.v58i2.31814

pISSN:2355-8393 ● eISSN: 2599-056x ● doi: 10.20473/fmi.v58i1.31814 ● Fol Med Indones. 2022;58: 137-140
● Submitted 27 Dec 2021 ● Received 25 Jan 2022 ● Accepted 7 May 2022 ● Published 5 Jun 2022

Highlights:

1. Oxidative markers 8-Hydroxydeoxyguanosine and Nitric Oxide was found high in hemodialysis and non-hemodialysis chronic kidney disease patients.
2. There is no correlation between 8-Hydroxydeoxyguanosine and Nitric Oxide in hemodialysis and non-hemodialysis chronic kidney disease patients.

INTRODUCTION

Chronic Kidney Disease (CKD) is a clinical diagnosis of decreased kidney function characterized by a progressive decrease in the Glomerulus Filtration Rate (GFR). Kidney disease, including CKD, contributes to the world's diseases with a mortality rate of 5-10 million people (Luyckx et al. 2018). The global CKD prevalence is 11 to 13%, contributing to a significant cost burden on healthcare systems worldwide (Hill et al. 2016).

Oxidative stress is the primary key that causes the pathogenesis of CKD (Duni et al. 2019). Oxidative stress indicates an imbalance between pro-oxidants and antioxidants that lead to kidney damage (Ravarotto et al. 2018). Oxidative stress shows the progression of kidney dysfunction because Reactive Oxygen Species

(ROS) and Reactive Nitrogen Species (RNS) accumulation accelerates kidney damage through cytotoxic mechanisms (Duni et al. 2017).

Nitric Oxide (NO) is considered the key directly involved in oxidative stress-mediated kidney disease (Duni et al. 2019). An increase in NO is found in CKD patients due to an increase of inducible-Nitric Oxide Synthase (i-NOS) – one of the enzymes that trigger NO formation due to inflammation (Toualbi et al. 2020). Another evidence indicated that NO production is decreased in CKD, even in the early stage, due to endothelial dysfunction (Baylis 2006). NO interacts with one of the ROS, superoxide radicals ($O_2^{\cdot -}$), and induced peroxynitrites ($ONOO^-$) formation (Duni et al. 2019).

Oxidants that interact with cellular nucleic acids can cause cell death due to the inactivation of mitochondrial enzymes and directly cause DNA damage, transcription factors, and enzymes for DNA repair (Putri & Thaha 2014). Hydroxyl (OH-) targets the DNA of cells and mitochondria, causing them to contain free radicals and forming several oxidation products (Sung et al. 2013). Damage to DNA due to the accumulation of oxidants causes the emergence of oxidative stress markers, namely 8-OHdG (Putri & Thaha 2014). 8-Hydroxydeoxyguanosine is a product of DNA damage caused by oxidative stress produced by specific enzymatic cleavage following 8-hydroxylation of guanine bases (Ghanayem et al. 2017). Levels of 8-OHdG can be known through intact urine specimens without going through other metabolic stages (Sung et al. 2013).

Theoretically, there was a correlation between 8-OHdG and NO in CKD, caused by oxidative stress (Alsagaff et al. 2020). Peroxynitrite formed due to the reaction between NO and O_2 increases RNS cytotoxic. These compounds can cause oxidative DNA damage, leading to the appearance of 8-OHdG. Thus, this study aimed to prove whether there was a correlation between 8-OHdG, DNA damage marker, and NO in CKD.

MATERIALS AND METHODS

A cross-sectional study was conducted on 20 hemodialysis patients and 49 non-hemodialysis patients at Universitas Airlangga Hospital, Royal Hospital, and Premiere Hospital in Surabaya, Indonesia, and conducted in April 2017-July 2017. The Ethical Committee of Universitas Airlangga Hospital had approved this study under decree No. 187/KEP/2021. The inclusion criteria for the participants were patients over 21 years old with CKD undergoing routine treatment for more than three months at three hospitals (non-hemodialysis group); patients over 21 years old with CKD undergoing chronic hemodialysis for more than three months at these hospitals (hemodialysis group). Data were collected through blood and urine sampling. Blood serum was measured for NO, and urine sampling for 8-OHdG.

According to the manual instruction, 8-OHdG was measured in urine using the enzyme-linked immunoassay method with Bioxytech 8-OHdG-EIA (Cat. No. 21026, Oxis Health, USA). The patients were measured by Microplate Reader 680 with 96 healthy plate format (Biorad, USA). The concentration of urinary 8-OHdG was then determined by comparing the patients' optical density (OD) to the standard curve, then corrected by renal filtration rate using creatinine, then reported in units of ng/mg creatinine. Total nitric oxide was measured using the sample's nitrite and nitrate measurement approach using the colorimetric principle with the Griess reaction method. The reagent used was the Total Nitric Oxide Parameter kit (R&D Systems, USA) with catalog number KGE001. The tool used was Microplate Reader 680 with 96 healthy plate formats (Biorad, United States), and a 10 kDa ultrafilter used was Vivaspin 500 (Sartorius, Germany). Total NO serum was reported in units of umol/L.

Data were presented as mean±SD. Bivariate Pearson tests were performed to assess the correlation between variables. $P < 0.05$ following two-tailed analysis was considered to indicate statistical significance. Statistical analyses were performed using SPSS version 26 (IBM Corporation, Armonk, New York, USA).

RESULTS

The characteristics of the patients are shown in Table 1. The age range of the study patients was 21-80 years. In both groups, most patients were between the age of 51-60. The study sample was divided into two groups: patients who did continuous hemodialysis and those who did not. Most patients in the continuous hemodialysis group were 12 males (17.39%) and eight females (11.59%). The total number of patients for the group undergoing continuous hemodialysis was 20 (29.99%). The group that did not undergo hemodialysis consisted of 27 males (39.13%) and 22 females (31.89%). The total number of patients for the group that did not undergo hemodialysis was 49 (71.01%). Thus, male patients outnumbered females in both groups.

Table 1. Patient's characteristic

Characteristic	Hemodialysis Group	Non-hemodialysis group
Age (years)	56.7±11.41	58.6±5.96
Male/female n (%)		
Male	12 (17,39%)	27 (39,13%)
Female	8 (11,59%)	22 (31,89%)
Total of sample n (%)	20 (29,99%)	49 (71,01%)
Total all patients n (%)	69 (100%)	

The mean level ± SD of 8-OHdG urine in the hemodialysis group was 4.46±3.24 ng/mg creatinine, while the mean level in the non-hemodialysis group was 10.55±6.07 ng/mg creatinine. The normality Unpaired T-Test showed that the data of the two variables in this group were not normally distributed (Sig. <0.005). The data on the non-hemodialysis group were the mean level ± SD of 8-OHdG urine was 72.65±36.79 umol/L, while the mean level of urinary Total NO serum from the patients was 61.81±61.80 umol/L. The normality unpaired t-test showed that the data of the two variables in this group were typically distributed or homogenous (Sig. >0.005). Thus, NO was found to increase, and 8-OHdG was high in both groups. Those proved the occurrence of oxidative stress in CKD patients.

Table 2. Correlation between 8-OHdG and Total NO serum of the study patients based on bivariate Pearson analysis

	Hemodialysis group	Non-hemodialysis group
Correlation coeff. (r)	-0.156	-0.037
Significance (p)	0.510	0.801

A Bivariate Pearson correlation test was performed to determine the correlation between urinary 8-OHdG and serum NO in CKD. Based on Table 2, there was no significant association between 8-OHdG with NO in the hemodialysis group (p=0.510, r=-0.156) and the non-hemodialysis group (p=0.801 r=-0.037). This uncorrelation means that 8-OHdG and NO do not affect each other's value.

DISCUSSION

We obtained that the level of 8-OHdG urines was high in both groups, particularly in the non-hemodialysis group. A previous study showed that in hemodialysis patients, 8-OHdG levels were higher than those without hemodialysis because circulating ROS increased by 14x acutely after a dialysis session (Duni et al. 2019). Meanwhile, there was also an increase in serum 8-OHdG levels in pre-HD CKD patients, not associated with eGFR (Dai et al. 2019). Therefore, the more significant rise in 8-OHdG may occur in the non-hemodialysis group, so it generally has a lower severity than the hemodialysis group with higher eGFR values.

Total NO serum was increased in both groups. A study showed an increase in total serum NO in CKD patients undergoing hemodialysis (Meenaski & Agarwal 2013). It was caused by the dialysis process, which stimulates cytokines to increase Nitric Oxide Synthase (NOS), the NO-forming enzyme. This increase triggers cytotoxic substances that are responsible for causing complications, including hypertension and nitrosative stress. Toualbi et al. (2020) explained that the increase in NO was in line with the severity, and the highest was found in a group undergoing hemodialysis.

In contrast, another study showed a decrease in NO production and NO bioavailability (Roumeliotis et al. 2020). It is due to an increase in endogenous inhibitors of NOS. A decrease in NO was also found in CKD patients undergoing hemodialysis because hemolysis can cause an increase in free hemoglobin, which has a high affinity for binding NO (Roumeliotis et al. 2020).

In this study, there was no correlation between 8-OHdG urine and total NO serum in CKD, whether in the hemodialysis or non-hemodialysis group. Meanwhile, a study indicated that NO has a particular role that can cause the appearance of 8-OHdG (Hsu & Tain 2021). The study explained that in CKD, there are inhibitors of the NO-forming enzymes that cause NOS to become uncoupled and then cause NO to meet $O_2^{\cdot-}$ so that peroxynitrite ($ONOO^-$) is formed.

Peroxynitrite is a product of RNS which has a cytotoxic effect and causes oxidative damage to DNA. One of the oxidative DNA markers is 8-OHdG. Thus, further research is required to identify and focus on other factors affecting the 8-OHdG and NO values so that more significant results were obtained.

Strength and limitation

This study has several limitations, including the number of patients in the group that did not undergo hemodialysis was twice as large as other groups. Based on the unpaired t-test analysis results, the data on urine 8-OHdG levels in both groups showed that the data variation was not homogeneous. The group of CKD patients was also not differentiated based on severity. Some of these things can affect the results of the analysis and the final results of this study.

CONCLUSION

In this study, there was no significant correlation between 8-OHdG and NO in patients with CKD. Even though 8-OHdG is widely known as a biomarker of DNA oxidative stress, its role in CKD progression is the factor that affects the value, so further research is required.

Acknowledgement

The authors thank the Universitas Airlangga Hospital, Royal Hospital, and Premiere Hospital in Surabaya, Indonesia.

Conflict of interest

None.

Funding disclosure

None.

Author contribution

AM conceptualied the study and managed the administration. PAA collected and analyed the data, and rote the manuscript. MT validated, revised, and finalied the manuscript

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