Annals of Medical Research
DOI: 10.5455/annalsmedres.2018.12.276 2019;26(2):270-4

Original Article

Oxidant and antioxidant mechanisms in chronic kidney disease

Ahmet Karatas[1], Ahmet Bayrak[2], Tulin Bayrak[2], Ebru Canakci[3]

[1]Ordu University Faculty of Medicine, Department of Internal Medicine, Nephrology Division, Ordu, Turkey
[2]Ordu University Faculty of Medicine, Department of Biochemistry, Ordu, Turkey
[3]Ordu University Faculty of Medicine, Department of Anesthesiology and Reanimation, Ordu, Turkey

Abstract

Aim: The objective of this study was to evaluate the correlation of inflammation, oxidant and antioxidant biomarkers with the stages of CKD.

It is well known that inflammation has an important role in CKD. While PON-1 and PAF-AH are biomarkers with antioxidant characteristics, MDA is an oxidant biomarker.

Material and Methods: The participants were divided into 3 groups. Control (n=37) group, non-hemodialysis chronic kidney disease (non-HD CKD) group and hemodialysis group (n=40) . One hudred twenty-one participants were included in this cross-sectional and observational study. Serum PON-1, PAF-AH, MDA levels were measured.

Results: There was a significant difference between the groups regarding the median MDRD values (p<0.001). The median MDRD value in the control, non-HD CKD and dialysis groups was 93. 36 and 7 respectively. There was also a significant difference between the groups regarding the median PON-1 value (p<0.001). The median PON-1 value in the control, predialysis and dialysis groups was 67, 63.1 and 62 respectively. There was also no significant difference between the groups regarding the median PAF-AH value (p=0.469). The median PAF-AH value in the control, predialysis and dialysis groups was 115.7, 116.95 and 117.4 respectively. There was also a significant difference between the groups regarding the median MDA value (p<0.001).

Conclusion: We concluded that PON-1 and MDA might be considered as useful biomarkers in CKD patients. The correlation between PAF-AH and CKD, larger subject sizes are needed. We believe that our study will be a starting point for larger studies focused on CKD severity and antioxidant/oxidant biomarkers.

Keywords: Chronic Kidney Disease; Platelet-Activating Factor-Acetylhydrolase; Paraoxonase-1; Malondialdehyde.

INTRODUCTION

Chronic kidney disease (CKD) is a widespread public health problem, which may have several adverse consequences like renal failure, cardiovascular disease, and premature death (1). CKD is defined as a structural or functional disorder of kidneys that persist longer than 3 months (2). It is also well known that inflammation plays an important role in CKD (3). In the late '90s, it was demonstrated that inflammation, which emerges as a result of monocyte and interleukin-1 release, played an important role in the pathogenesis and progression of CKD. IL-1, the main cytokine of the inflammation was the starting point related to the major complications and increased mortality in patients with chronic dialysis program (4). Atherosclerosis, which emerges in CKD and oxidative stress, inflammation, hypertension, dyslipidemia, vascular calcification and endothelial dysfunction participate in the pathogenesis of cardiovascular disease (5-7).

Platelet-activating factor (PAF-AH) is a pro-inflammatory phospholipid mediator, found in several physiopathological conditions and plays an important role in several syndromes or diseases like kidney diseases (8). Animal and human studies showed that PAF-AH could be an important component in kidney damage and demonstrated the efficacy of its products and activities in CKD (9). Plasma malondialdehyde (MDA) concentration is one of the most common biomarkers for the lipid peroxidation level (10).

The antioxidant defense system preserves the homeostasis of the reactive oxygen species (ROS) in cells.

Received: 07.12.2018 **Accepted:** 02.01.2019 **Available online:** 07.01.2019
Corresponding Author: Ahmet Karatas, Ordu University Faculty of Medicine, Department of Internal Medicine, Nephrology Division, Ordu, Turkey, **E-mail:** karatas55@hotmail.com

Paraoxonase (PON) is mostly synthesized in kidney and liver and found on the surface of high-density lipoproteins (HDL). It inhibits the peroxidation of LDL and HDL in the plasma. PON-1, a paraoxonase iso-enzyme, protects LDL and circulatory cells against the oxidative damage. Thus prevents the inflammatory response in the cells of the arterial wall (11).

In conclusion, excessive ROS production or ineffective antioxidant capacity play an important role in the development and progress of the renal and cardiovascular diseases (12,13).

The objective of this study was to evaluate the correlation of inflammation, oxidant, and antioxidant biomarkers with CKD stages.

MATERIAL and METHODS

Our study was approved by the Ethics Committee for Clinical Research of the Ordu University Medical Faculty (Date: 01.11.2018; Approval Nr: 2018/223). The patient consent was obtained from all participants.

One hudred twenty-one participants were included in this cross-sectional and observational study. Fourty-four non-hemodialysis chronic kidney disease (non-HD CKD) patients, applied to the Nephrology Department of the Training and Research Hospital in the Ordu University Medical Faculty between 01 November 2018 and 03 December 2018; 37 healthy individuals, applied for a general health check-up to the outpatient department of the internal medicine, and also 40 hemodialysis patients from the routine hemodialysis program were included in the study. The demographic data of the participants (age, BMI, gender) were recorded. Hemogram was performed with Cell-Dyn Ruby device. Cobas-c 501 module was used for the analysis of routine biochemical parameters (serum BUN (mg/dl), creatinine (mg/dl), total cholesterol (mg/dl), triglyceride (mg/dl), HDL (mg/dl), LDL (mg/dl), VLDL (mg/dl), albumin (g/dl), potassium (mmol/l), calcium (mg/dl), CRP (mg/dl), iron binding capacity (mcg/dl), uric acid (mg/dl). The routine urinary examination was done with Cobas R-6500. Roche Cobas-c 501 was used for the analysis of microalbumin (ng/ml), protein (ng/ml), creatinine, Albumin/creatinine ratio (ACR) (mcg/mg) in the spot urine. HBA1c (mmol/mol) was evaluated with immunoassay method in Roche Cobas e501. The hormones (Vit D (ng/ml), ferritin (ng/ml), PTH (ng/ml)) were analyzed with Roche Cobas e601. The PAF-AH (nmol/min/ml), PON-1 (U/ml), and MDA (nmol/ml) activities were measured with Shimatsu UV 1800 spectrophotometry.

The body mass index (BMI) was calculated for all participants [BMI= weight (kg)/height $(m)^2$]. The participants were divided into 3 groups. The healthy participants had no known diseases; they applied to the outpatient department for check-up. No biochemical anomaly was detected in the laboratory examination and no hematuria and proteinuria in the urinary examination. The estimated glomerular filtration rate (e-GFR) of healthy subjects was calculated with the Modification of Diet in Renal Disease (MDRD) equation. All e-GFR values were above 60 ml/min/1.73m^2. CKD was diagnosed according to the KDIGO Clinical Practice Guideline for the Evaluation and Management of CKD. The e-GFR values of the CKD patients were calculated with the MDRD equation and were below 60' ml/min/1.73m^2. Hemodialysis patients (Stage V) were under hemodialysis treatment at least for 3 months (3 times/week).

The exclusion criteria were as follows: Active infection/inflammation, malignancy, pregnancy, and absence of informed consent.

Statistical Analysis:
All data were analyzed with IBM SPSS v23. The non-normally distributed parameters were evaluated according to the groups with the Kruskal-Wallis test. The correlation between the variables was analyzed with the Spearman's rank correlation. The results were presented with median values (min – max). The accepted limit of significance was $p<0.05$.

RESULTS

There was a significant difference between the groups regarding the median age ($p<0.001$). The median age in the control, non-HD CKD and dialysis groups was 46. 64 and 64 years respectively. However, there was no significant difference between the groups in respect to the median BMI ($p=0.129$). The median BMI value in the control, non –HD CKD group and dialysis groups was 26.6, 27.55 and 26.055 respectively. There was a significant difference between the groups regarding the median creatinine level ($p<0.001$). The median creatinine level in the control, non-HD CKD and dialysis groups was 0.8, 1.855 and 6.75 respectively. There was also a significant difference between the groups regarding the median e-GFR value ($p<0.001$). The median e-GFR value in the control, non-HD CKD and dialysis groups was 93, 36 and 7 respectively. There was also a significant difference between the groups regarding the median albumin level ($p<0.001$). The median albumin level in the control, non-HD CKD and dialysis groups was 4.5, 4.1 and 3.7 respectively. There was also a significant difference between the groups regarding the median CRP level ($p<0.001$). The median CRP level in the control, non-HD CKD and dialysis groups was 0.2, 0.455 and 1.295 respectively. There was also a significant difference between the groups regarding the median PTH level ($p<0.001$). The median PTH level in the control, non-HD CKD and dialysis groups was 35.09, 78.45 and 145.95 respectively. There was also a significant difference between the groups regarding the median ferritin level ($p<0.001$). The median ferritin level in the control, non-HD CKD and dialysis groups was 57.4, 91 and 1225.41 respectively. There was also no significant difference between the groups regarding the median vitamin D level ($p<0.382$). The median vitamin D level in the control, non-HD CKD and dialysis groups was 15, 15.9 and 14 respectively. There was also a significant difference between the groups regarding the median microalbumin/creatinine ratio ($p<0.001$). The median microalbumin/

creatinine ratio in the control, non-HD CKD and dialysis groups was 7, 117.5 and 648.75 respectively. There was also a significant difference between the groups regarding the median PON-1 value (p<0.001). The median PON-1 value in the control, non-HD CKD and dialysis groups was 67, 63.1, and 62 respectively. There was also no significant difference between the groups regarding the median PAF-AH value (p=0.469). The median PAF-AH value in the control, non-HD CKD and dialysis groups was 115, 116.95 and 117.4 respectively. There was also a significant difference between the groups regarding the median MDA value (p<0.001). The median MDA value in the control, non-HD CKD and dialysis groups was 4, 4,3 and 4.4 respectively. The descriptive statistical values of the groups are summarized in Table 1.

There was no significant difference between the groups considering the gender (p=0.314) and the related values are listed in Table 2.

There was a weak and positive correlation within the control group for e-GFR and PON-1 (r=0.378; p=0.021). However, there was no significant correlation between BMI and PON-1 in the control group. Also in the non-HD CKD and dialysis groups, there was no significant correlation between PON-1 and age, BMI and e-GFR. In the dialysis group, there was a weak negative correlation between CRP and PON-1; PON-1 levels decreased with the increase of CRP. There was a weak negative correlation between MDA and PTH in the dialysis group (r=384; p=0.014). The correlation between other variables was not significant. The correlation analysis of the groups is listed in Table 3.

Table 1. The comparison of the parameters between the groups

	Control (n=37)	non-HD CKD (n=44)	Dialysis (n=40)	p
Age (year)	46 (25-78)	64 (28-85)	64 (22-84)	<0.001
BMI(weight(kg)/height (m)^2)	26.6 (20.41 – 39.2)	27.55 (19.8 – 40.7)	26.055 (18.2 – 50.5)	0.129
Creatinine (mg/dl)	0.8 (0.56 – 0.98)	1.855 (1.13 – 4.89)	6.75 (3.18 – 12.5)	<0.001
e-GFR (ml/min/1.73m^2)	93 (65.91-121)	36 (10-61)	7 (4-12)	<0.001
Albumin (g/dl)	4.5 (3.5 – 5.18)	4.1 (3.1 – 4.7)	3.7 (2.5 - 4.5)	<0.001
CRP (mg/dl)	0.2 (0.05 - 7.49).	0.455 (0.2 - 18.04)	1.295 (0.2 - 11.2)	<0.001
PTH (ng/ml)	35.09 (11.49 - 171.6)	78.45 (12.46 - 489)	145.95 (13.7 - 1148)	<0.001
Ferritin (ng/ml)	57.4 (2.14 - 783.91)	91 (5.4 - 888.15)	1225.41 (12-2000)	<0.001
Vit. D (ng/ml)	15 (5.3 – 39.77)	15.9 (5.2-230)	14 (4.35-107)	0.382
Mikroalbumin/creatinine ratio (µ/ml)	7 (0-32)	117.5 (0-7700)	648.75 (36 – 3455.3)	<0.001
PON-1 (U/ml)	67 (55 – 78.7)	63.1 (52.3 – 72.5)	62 (50.7 – 72.5)	<0.001
PAF-AH (nmol/min/ml)	115.7 (95.8-1117)	116.95 (98.4 – 130.3)	117.4 (98.5-132.8)	0.469
MDA (nmol/ml)	4 (2.7-5.2)	4.3 (3.2 – 5.7)	4.4 (3.3 - 5.8)	0.001

Table 2. Gender distribution by groups

	Control (n=37)	non-HD CKD (n=44)	Dialysis (n=40)	p
Female (n = 55)	19 (51.4)	16 (36.4)	20 (50)	0.314
Male (n = 66)	18 (48.6)	28 (63.6)	20 (50)	

Table 3. Results of the correlation analysis

			AGE	BMI	e-GFR	CRP	PTH
Control	PAF-AH	R	-0.194	-0.018	0.320	0.045	-0.010
		P	0.250	0.917	0.053	0.791	0.952
	MDA	R	-0.188	-0249	0.235	0.108	0.028
		P	0.265	0.138	0.162	0.523	0.871
	PON-1	R	-0.092	0.068	0.378*	-0.248	-0.125
		P	0.588	0.687	0.021	0.139	0.461
non-HD CKD	PAF-AH	R	-0.050	-0.119	-0.125	-0.241	-0.050
		P	0.745	0.440	0.423	0.115	0.748
	MDA	R	0.014	-0.073	-0.054	-0.085	-0.012
		P	0.926	0.636	0.732	0.585	0.939
	PON-1	R	-0.108	-0.054	0.054	0.038	-0.002
		P	0.484	0.729	0.731	0.813	0.990
Dialysis	PAF-AH	R	-0.015	0.050	-0.079	0.053	0.145
		P	0.928	0.759	0.644	0.744	0.371
	MDA	R	0.025	-0.059	0.087	0.033	-0.384*
		P	0.881	0.716	0.608	0.842	0.014
	PON-1	R	-0.144	0.191	-0.226	-0.356	0.033
			0.374	0.237	0.179	0.024	0.838

r: Spearman's rank correlation

DISCUSSION

In our study, the PON-1 values were significantly different between the groups. It had the highest value in the control group. It was lower in the non-HD CKD group, but the lowest value was in the dialysis group. We determined that the PON-1 level decreased with the increase of the disease stage. The serum MDA levels were also significantly different between the groups. We found out that it was lowest in the control group, relatively higher in the non-HD CKD group but the highest value was in the dialysis group. However, there was no significant difference between the groups in respect of PAF-AH levels. The MDA levels was increased and PON-1 levels was decreased with the increase of the disease stage. Thus we suggested that MDA (an oxidant biomarker) and PON-1 (an antioxidant biomarker) can be used as markers in all stages of the CKD. We concluded that PAF-AH had no predictive value in the CKD patients.

In a study conducted by Papavasiliou et al, they showed that PAF-AH levels were increased with the increase of the disease stage in CKD. They included 13 stage 1-2 CKD patients, 23 stage 3-5 CKD patients and 15 healthy volunteers (control group) in the study. After the subcutaneous erythropoietin treatment with a dose of 50 IU/kg/week, they compared the PAF-AH and glutathione peroxidase levels between the groups. They observed that the PAF-AH levels were increased parallel to the increase of the disease stage (14). Our results were not consistent with the results of Papavasiliou et al. In our study, we did not detect any significant difference between the groups considering the PAF-AH levels. This conflicting result might depend on the erythropoietin treatment administered in the study of Papavasiliou et al. In another study conducted by Papavasiliou et al, they determined that plasma PAF-AH levels were increased in stage 3-4 CKD patients, who received erythropoietin treatment. The authors stated that erythropoietin had an anti-atherogenic activity in the CKD patients (15).

In the study of Neelofar K et al; the oxidative stress parameters and the levels of pro-inflammatory cytokines in the healthy volunteers, diabetic patients, and CKD patients originating from Northern India were investigated. The serum levels of oxidative stress parameters (malondialdehyde, nitric oxide, superoxide dismutase, and catalase and glutathione reductase) were evaluated. The authors reported that MDA and nitric oxide levels were higher in the patients with CKD and diabetes mellitus compared to the healthy volunteers (16). Our results were consistent with the results of Neelofar K. et al. We also determined that the MDA levels were higher in CKD patients compared to the healthy volunteers.

A study by Ogunleye A. et al., evaluated the total antioxidant capacity (TAC) and MDA levels in 36 hemodialysis patients. While the TAC level was lower in the hemodialysis patients compared to the healthy volunteers, there was no significant difference between the groups regarding the MDA levels. In our study, the MDA levels were increased in the non-HD CKD and dialysis groups (17). On the other hand, PON-1 levels, which is an antioxidant biomarker, was lower in the hemodialysis patients compared to the healthy volunteers. These results were partly consistent with the results of Ogunleye A et al. The conflicting results between our study and Ogunleye's study in respect of MDA levels, could be explained with small subject size (n=36) in the Ogunleye's study. In addition, all subjects were hemodialysis patients in this study.

In a study conducted by Kennedy DJ et al, the PON-1 and PAF-AH levels were investigated in 630 CKD patients. Following the matching process for the age and gender, the PON-1 and PAF-AH levels were measured in CKD patients and healthy volunteers. The levels of these parameters were high in the healthy volunteers and rather low in the CKD patients. The authors suggested that the low PON-1 and PAF-AH levels in the CKD patients might lead to atherosclerotic heart diseases in the long term (18). Our results were partially in concordance with the literature. Although the PON-1 levels were also low in CKD patients in our study, the PAF-AH levels were comparable between the groups. The conflicting PAF-AH levels of our study might be explained with the small subject size and the lack of age homogenization. The study of Kennedy et al. had a much larger subject size (n=630), they followed up the patients for 3 years and selected healthy volunteers after the age and gender matching.

In their study, Abdallah A. et al. investigated the serum PON-1 levels, transthoracic echocardiography and epicardial fat tissue thickness in 72 hemodialysis patients. They reported that compared to the healthy volunteers, the PON-1 levels were decreased and epicardial fat tissue thickness was increased with the increase of the disease severity in CKD patients (19). Likewise, Karatas A. et al. determined that ischemia-modified albumin and myeloperoxidase levels, oxidant biomarkers, were increased in correlation with the severity of the disease in all stages of CKD compared to the healthy participants. They also reported that the epicardial fat tissue thickness was increased with the increase of the severity of the disease (20). According to the results of our study, the MDA – an oxidant biomarker – levels were increased in correlation with the severity of the disease. Our results were in concordance with the literature.

There were certain limitations in our study. We could not achieve an age homogenization between our groups. The median age of the healthy volunteers in the control group was lower than the patients in the other two groups so that a relatively younger population was enrolled in the study. This difference could be explained with the fact that usually, younger individuals apply to an outpatient clinic of internal medicine for a check-up in our country. In our country, the elderly do not have a check-up culture. Another limitation was the unequal number of subjects in the groups.

CONCLUSION

In conclusion, we might suggest that PON-1 and MDA are useful biomarkers regarding the follow-up of the CKD patients. In our study, it was noteworthy that PON-1 levels, which are indicators of oxidant system, decreased as CKD stage progresses, and MDA levels which are indicative of antioxidant system, oxidant-antioxidant mechanisms reflect the predominance of destructive effects in advanced CKD patients. In order to demonstrate the correlation between PAF-AH and CKD, larger subject sizes are needed. We believe that our study will be a starting point for larger studies focused on CKD severity and antioxidant/oxidant biomarkers.

Competing interests: The authors declare that they have no competing interest.
Financial Disclosure: There are no financial supports
Ethical approval: Our study was approved by the Ethics Committee for Clinical Research of the Ordu University Medical Faculty (Date: 01.11.2018; Approval Nr: 2018/223). The patient consent was obtained from all participants.

Ahmet Karatas ORCID: 0000-0001-9095-6054
Ahmet Bayrak ORCID: 0000-0002-4431-0524
Tulin Bayrak ORCID: 0000-0002-3596-0488
Ebru Canakci ORCID: 0000-0003-2093-9229

REFERENCES

1. Levey AS, Eckardt KU, Tsukamoto Y, et al. Definition and classification of chronic kidney disease: a position statement from Kidney Disease: Improving Global Outcomes (KDIGO). Kidney Int 2005;67:2089-100.
2. Ketteler M, Block GA, Evenepoel P, et al. Executive summary of the 2017 KDIGO Chronic Kidney Disease-Mineral and Bone Disorder (CKD-MBD) Guideline Update: what's changed and why it matters Kidney Int 2017;92:26-36
3. Mihai S, Codrici E, Popescu ID, et al. Inflammation and chronic kidney disease: current approaches and recent advances, in: Chronic Kidney Disease, Ed:Thomas Rath, Intechopen, Rijeka, Croatia; 2018. pp. 31-51.
4. Henderson LW, Koch KM, Dinarello CA, et al. Hemodialysis hypotension: the interleukin hypothesis. Blood Purification1983;1:3-8.
5. Vaziri ND. Oxidative stress in chronic renal failure: the nature, mechanism and consequences. Semin Nephrol 2004;24:469-73.
6. Stenvinkel P, Alvestrand A. Inflammation in end-stage renal disease: sources, consequences, and therapy. Semin Dial 2002;15:329-37.
7. Himmelfarb J, Stenvinkel P, Ikizler TA, et al. The elephant in uremia: oxidant stress as a unifying concept of cardiovascular dis- ease in uremia. Kidney Int 2002;62:1524-38.
8. Imaizumi TA, Stafforini DM, Yamada Y, et al. Platelet - activating factor: A mediator for clinicians. J Intern Med 1995;238:5-20.
9. Camussi G, Salvidio G, Tetta C. Platelet-activating factor in renal disease. Am J Nephrol 1989;9:23-6.
10. Church DF, Pryor WA. Free radical chemistry of cigarette smoke and its toxicological implications. Environ Health Perspect 1985;64:111-26.
11. Ruperez AI, Gil A, Aguilera CM. Genetics of oxidative stress in obesity. Int J Mol Sci 2014;15:3118-44.
12. Kao MPC, Ang DSC, Pall A, et al. Oxidative stress in renal dysfunction: Mechanisms, clinical sequelae and therapeutic options. Journal of Human Hypertension. 2010; 24:1-8.
13. Panth N, Paudel KR, Parajuli K. Reactive Oxygen Species: A Key Hallmark of Cardiovascular Disease. Adv Med 2016;2016:9152732.
14. Papavasiliou EC, Gouva C, Siamopoulos KC, et al. Erythrocyte PAF-acetylhydrolase activity in various stages of chronic kidney disease: effect of long-term therapy with erythropoietin. Kidney Int 2005;68:246-55.
15. Papavasiliou EC, Gouva C, Siamopoulos KC, et al. PAF-acetylhydrolase activity in plasma of patients with chronic kidney disease. Effect of long-term therapy with erythropoietin. Nephrol Dial Transplant 2006;21:1270-7.
16. Neelofar K, Arif Z, Arafat MY, et al. A study on correlation between oxidative stress parameters and inflammatory markers in type 2 diabetic patients with kidney dysfunction in north Indian population. J Cell Biochem 2018;27.
17. Ogunleye A, Akinbodewa AA, Adejumo OA, et al. Changes in antioxidant status associated with haemodialysis in chronic kidney disease. Ghana Med J 2018;52:29-33.
18. Kennedy DJ, Tang WH, Fan Y, et al. Diminished antioxidant activity of high-density lipoprotein-associated proteins in chronic kidney disease. J Am Heart Assoc. 2013;2:e000104.
19. Abdallah E, El-Shishtawy S, Sherif N, et al. Assessment of the relationship between serum paraoxonase activity and epicardial adipose tissue in hemodialysis patients. Int Urol Nephrol 2017;49:329-35.
20. Karatas A, Canakci E, Bektas O, et al. Relationship of epicardial fat tissue thickness with oxidant biomarkers in chronic kidney disease. Bratisl Lek Listy. 2018;119:566-71.