ORIGINAL ARTICLE

Evaluation of oxidative stress using exhaled breath 8-isoprostane levels on chronic kidney disease

BS Tiryaki, T Tasliyurt, BM Yelken[1], S Sahin, F Kutluturk[2], HI Koseoglu[3], B Ozturk, A Yilmaz, S Sahin[4]

Departments of Internal Medicine, [1]Nephrology, [2]Endocrinology, [3]Chest Diseases, [4]Biochemistry, Faculty of Medicine, Gaziosmanpasa University, Tokat, Turkey

Abstract

Background: There have been limited numbers of studies on patients with chronic kidney disease (CKD) to determine oxidative stress in exhaled breath condensate (EBC). Those two studies have been carried out on hemodialysis patients, and hydrogen peroxide and nitric oxide have been studied in order to show oxidative stress on EBC.
Aims: We investigated oxidative stress in EBC evaluating 8-isoprostane levels on different stages of CKD.
Materials and Methods: A total of 81 patients with 2-4 CKD stages have been evaluated prospectively. The patients have been categorized into three groups according to their CKD stages. For biochemical analysis, blood and breathing air samples were taken. 8-isoprostane has been measured using immunoassay method as the indicator of oxidative stress in EBC.
Results: 8-isoprostane values were 8.19 ± 4.56, 13.89 ± 8.70, and 14.20 ± 10.68 pg/min group 1, 2, and 3, respectively; and the EBC 8-isoprostane levels increased significantly as CKD stages advanced ($P = 0.018$). There was a statistically significant reverse correlation between 8-isoprostane and glomerular filtration rate (GFR; $r = -0.275$; $P = 0.014$), but not between 8-isoprostane and C-reactive protein ($r = -0.183$; $P = 0.177$).
Conclusions: We determined the level of 8-isoprostane in EBC of patients with different stages of CKD and showed that the level of 8-isoprostane significantly increased through the progress of CKD. We consider that our study is important because there have been limited number of studies that evaluate oxidative stress in CKD using EBC which is a noninvasive method.

Key words: 8-isoprostane, chronic kidney disease, exhaled breath condensate, oxidative stress

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Introduction

Chronic kidney disease (CKD) is a chronic, progressive disease presenting with irreversible renal damage depending upon reduction at glomerular filtration rate (GFR) and is a major cause of morbidity and mortality around the world.[1] Various mechanisms such as uncontrolled blood pressure, dyslipidemia, insulin resistance (IR), proteinuria, and oxidative stress (OS) have been suggested to express the progressive loss of function.[2,3]

The normal intracellular balance between reactive oxygen species (ROS) produced during aerobic metabolism and antioxidant defense mechanisms performing the function of free radical inactivation were modified by OS. The upregulation of OS mainly occurring during increased flux of free radicals/ROS and/or reduced antioxidant levels has now been accepted to play a critical role in the pathogenesis of obesity, atherosclerosis, type-2 diabetes mellitus (T2DM), and IR.[4,5] The antioxidant system has been reported to decrease and OS to increase with CKD progression in studies that have been carried out before.[6,7] Due to several clinical conditions, various oxidants can increase in serum or tissues. 8-isoprostane, a stable prostaglandin-like product, is formed from arachidonic acid by the nonenzymatic action

Address for correspondence:
Dr. Turker Tasliyurt,
Department of Internal Medicine
Gaziosmanpasa University, 60100 Tokat, Turkey.
E-mail: turtasliyurt@hotmail.com

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of ROS, and therefore, it has been suggested to be a marker of OS and oxidative activities.[8] In a study evaluating OS in CKD patients with 8-isoprostane, an inverse correlation was found between GFR and OS.[7]

Exhaled breath condensate (EBC) is a noninvasive method used for taking a sample from lungs in order to measure several biological indicators.[9] There have been limited number of studies that studied OS in CKD on EBC.[10,11] Those two studies have been carried out on hemodialysis patients, and hydrogen peroxide (H_2O_2) and nitric oxide (NO) have been studied in order to shown OS on EBC. Unlike those studies, we have searched for OS in different phases of CKD on EBC studying the level of 8-isoprostane.

Materials and Methods

Study subjects

A total of 81 CKD patients over 18-years-old who had GFR values between 15 and 90 ml/min were evaluated prospectively between October 2010 and February 2011. All of the patients were consecutive and recruited from Department of Internal Medicine and Nephrology outpatient clinics at Gaziosmanpasa University. The patients with GFR < 15 ml/min, GFR > 90 ml/min, active infection symptom and finding, diabetes mellitus diagnosis, history of malignity, asthma or chronic obstructive lung disease (COPD), allergic rhinitis, smoking, gastroesophageal reflux symptoms, diseases requiring anti-inflammatory treatment, and the ones using antioxidant drugs were all excluded from the study. GFR values of the patients were measured with MDRD (The Modification of Diet in Renal Disease) formula, and according to National Kidney Foundation guidelines. The patients who had GFR = 60-89 ml/min were categorized as group 1 (stage 2), GFR = 30-59 ml/min as group 2 (stage 3), and GFR = 15-29 ml/min as group 3 (stage 4).[1]

Blood pressure was measured using a calibrated aneroid sphygmomanometer. Hypertension (HT) was defined as systolic blood pressure (SBP) ≥ 130 mmHg or diastolic blood pressure (DBP) ≥ 80 mmHg and/or use of antihypertensive medications.[12] The body mass index (BMI) was calculated from the measured weight (in kilograms) and height (in meters). Demographic parameters of the patients such as age and gender were recorded. For the analysis, blood and breathing air samples were collected. The study protocol was approved by the local ethics committees and informed consent was obtained from all the participants.

Measurement of 8-isoprostane in exhaled breath condensate

EBC was collected from the patients in each group using a condensing device (Ecoscreen, Jaeger, Germany). The samples were taken through nose-clip method model putting a saliva trap within the condenser. Patients were asked to breathe out spontaneously through a mouthpiece equipped with a saliva trap for 5-15 min. Amylase level that was normally controlled routinely for salivary contamination was controlled due to the use of salivary filter and disallowance to the salivary contamination. The liquid formed as result of the condensation of respiratory air was collected; the collected liquid was split into tubes in 600 µl pieces and stored at -80°C until measurement. The collection of EBC was performed following available recommendations.[9] All EBC samples were collected at the same time of day, between 2.00 and 3.00 PM. Samples were stored for 2 months and analyzed at the same time. 8-isoprostane as the indicator of OS was measured through enzyme immunoassay using Cayman Chemical 8-isoprostane kits. The detection limit was 2.7 pg/ml.

Serum samples were taken from the patients for analysis of chemical parameters including triglyceride (TG), high density lipoprotein-cholesterol (HDL-C), and C-reactive protein (CRP) levels. Serum HDL-C and TG were determined enzymatically (Olympus Diagnostica, Lismeehan, Ireland). For all laboratory parameters, venous blood samples were taken between 8.00 and 9.00 AM following 12-hour fasting.

Statistical analyses

All statistical analyses were conducted using Statistical Package for Social Sciences (SPSS) 12 software. Analysis of variance (ANOVA) method was used in order to determine whether there was difference among the three groups or not. The ANOVA table was analyzed to determine whether or not those differences were random. Bonferroni test was performed to see among which groups there were differences. Descriptive statistics of the all parameters in the study were measured. All variables were checked for their normality and the variables distributed normally. The data were transformed and because transformed data did not distribute normally, use of nonparametric statistical method were decided using original data. Then, Spearman's rho correlation was used in order to find the relationships between 8-isoprostane value and other variables and the degrees of relationships. The value of $P < 0.05$ was considered significant.

Results

The average age of the 81 patients included into the study was 56 ± 15. Forty of the patients were female and 41 male. The patients were classified into three equal groups according to CKD stages 2, 3, and 4. The group 1 (average age 57 ± 12 years; 15 females and 12 males), group 2 (average age 56 ± 16 years; 10 females and 17 males), and group 3 (average age 55 ± 17 years;

15 females and 12 males) included 27 patients each. The groups were concordant in terms of age and gender.

BMI was 27.17 ± 4.69 kg/m^2 in group 1, 27.13 ± 5.08 kg/m^2 in group 2, and 26.22 ± 5.72 kg/m^2 in group 3. There were no statistically significant differences among the groups in terms of body weight (P = 0.742). About 85% of the patients were hypertensive. There was HT in 81.48% (n = 22) of the patients in group 1, in 85.18% (n = 23) in group 2, and in 88.88% (n = 24) in group 3. Average SBP/DBP of the was 136 ± 22/84 ± 12 mmHg in group 1, 136 ± 25/81 ± 13 mmHg in group 2, and 146 ± 29/87 ± 15 mmHg in group 3. No difference was observed among the groups in terms of SBP (P = 0.264) and DBP (P = 0.274).

Serum, GFR, low density lipoprotein (LDL), HDL, TG, CRP, and 8-isoprostane levels of the groups were given in Table 1. There were no significant differences among the groups in terms of LDL, HDL, TG, and CR*P* values. There were no differences between the groups in terms of the drugs such as angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, and statins used for the treatment of HT and hyperlipidemia.

8-isoprostane values were 8.19 ± 4.56, 13.89 ± 8.70, and 14.20 ± 10.68 pg/min group 1, 2, and 3, respectively, and the EBC 8-isoprostane levels increased significantly as CKD stages advanced (P = 0.018). The 8-isoprostane levels in groups 2 and 3 were significantly higher than that in group 1 (P = 0.047 and 0.035). 8-isoprostane level of group 3 was higher than that of group 2, but this difference was not significance (P = 0.99) [Figure 1].

A significant inverse correlation was found between 8-isoprostane and GFR (r = −0.275; P = 0.014), but not between 8-isoprostane and CRP (r = −0.183; P = 0.177).

Discussion

In relation to renal parenchymal damage and decreased GFR, presence of OS in CKD depends upon the increases in the level of oxidation in plasma and tissues, decreases in antioxidant capacity, and deterioration of antioxidant enzymes.[13] Increase of OS in CKD has been shown in some studies.[6,7,14,15] In a study carried out by Nagane *et al.*,[6] on different phases of CKD to prove OS, investigators proved that superoxide dismutase and nitric oxide (NO) significantly decrease and malondialdehyde and homocysteine significantly increase compared to the control group in CKD patients separated into five stages. This study revealed that antioxidant system weakens and OS increases with the progression of CKD. In their study, Oberg *et al.*,[15] have specified that several inflammations and OS biomarkers in blood samples of 60 patients having stage 3-5 CKD were higher than the healthy people. However, there was no signifcant relationship between GFR and any OS or infammation biomarker. In another study which included 87 patients with 1-4 phases CKD, plasma 8-isoprostane levels were measured. It was found that as the CKD phases progress, plasma 8-isoprostane levels significantly increase. A significant inverse correlation was also observed between GFR and plasma 8-isoprostane. OS was shown to increase with CKD progression and to have significant correlation with reduced kidney function.[7] Similarly, in present study 8-isoprostaten was used to evaluate of OS in different stages of CKD. However, in contrast to other studies, in our study, OS was evaluated in EBC which is known noninvasive method. In addition, it was detected that 8-isoprostane level significantly increased as the phase of CKD has progressed.

Majority of the studies carried out to evaluate OS in CKD were the ones in which OS indicators were measured in blood samples. EBC is a noninvasive method used for taking samples from the lungs in order to measure several biological indicators.[9] 8-isoprostane is one of the reliable indicators of OS. 8-isoprostane is an isomer of prostaglandin F2 alpha (PGF2α) and it is formed by a dominant nonenzymatic reaction from arachidonic acid peroxidation as result of the catalization of free radicals. It is chemically stabile, specific

Table 1: Comparison of the groups' laboratory values

	Group 1 (n=27)	Group 2 (n=27)	Group 3 (n=27)	P value
GFR (ml/min)	70.39±7.35	43.84±7.22	21.33±4.85	*
8-isoprostane (pg/ml)	8.19±4.56	13.89±8.70	14.20±10,68	0.018
CRP (mg/L)	13.98±22.45	9.08±12.10	7.21±6.43	0.376
LDL (mg/dl)	132.66±38.01	125.48±47.13	117.38±35.30	0.404
HDL (mg/dl)	53.24±20,58	46.25±9.70	48.62±11.19	0.241
TG (mg/dl)	157.60±59.18	160.58±94.17	149.69±67.28	0.867

*The statistical analysis was not performed. GFR=Glomerular filtration rate, CRP=C-reactive protein, LDL=Low density lipoprotein, HDL=High density lipoprotein, TG=Triglyceride

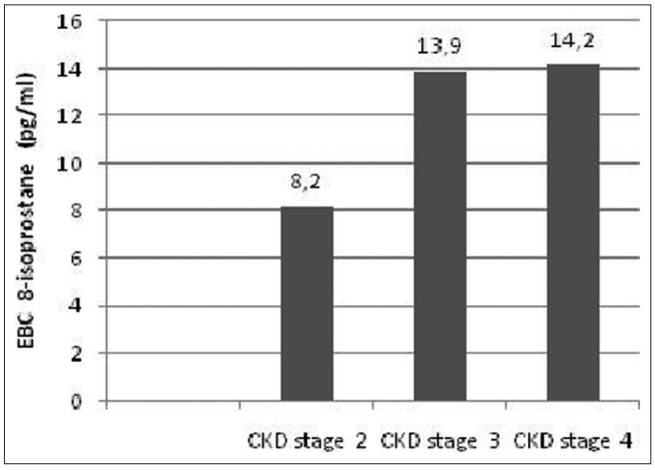

Figure 1: Mean exhaled breath condensate 8-isoprostane levels in the chronic kidney disease stages 2-4 (analysis of variance, P=0.018) (a) Average EBC 8-isoprostane levels in the groups (ANOVA P=0.018)

for lipid peroxidation, and has been suggested to be a marker of oxidative activities and oxidative stress.[16] There have been studies showing that 8-isoprostane level increases in EBC in some diseases such as COPD, asthma, interstitial lung disease, pulmonary sarcoidosis, obstructive sleep apnea syndrome and cystic fibrosis.[17-22] We also studied 8-isoprostane a chemically stabile indicator of OS. There have been limited number (two) of studies that evaluate OS in CKD with EBC.[10,11] Rysz *et al*.,[10] have compared H_2O_2 level in EBC of 29 chronic hemodialysis (HD) patients and 40 healthy people. They found significantly higher H_2O_2 levels in patient group compared to the control group. Rolla *et al*.,[11] have studied NO and NO metabolites with EBC in 12 chronic HD patients and suggested that oxidative stress was the most probable cause of increased NO metabolites in end stage renal disease patients before HD. Those two studies are the ones carried out with chronic HD patients. The present study is the first to evaluate OS in different stages of CKD as measured by EBC 8-isoprostane.

It has been shown that reactive oxygen production increase along with the age. OS increases as a result of oxidative phosphorylation change and mitochondrial DNA damage.[23] In a study in which the relationship of essential HT and OS was researched, it was determined that high blood pressure had negative correlation with antioxidant level of the plasma and 8-isoprostane measured in the plasma and urinary had a positive correlation with the blood pressure.[24] Moreover, some other studies have reported that obesity, IR, and hyperlipidemia were correlated with OS.[25-27] In our study, age, blood pressure, BMI, and lipid values were similar among the groups, revealing the absence of any association between them and OS in different CKD stages.

There are some limitations of this current study. Differences in procedures such as collection, storage, and analysis of the samples affect 8-isoprostane levels. The same technical analysis was used to carry out all procedures by the experienced personnel for eliminating these aforementioned differences. So as to eliminate the diurnal rhythm effect, samples were taken at the same time intervals of the day. In addition, a many diseases and drugs may negatively effect on 8-isoprostane levels. Therefore, in patient selection, some disorders such as COPD, asthma and antioxidant usage were excluded from the study.

Conclusion

In conclusion, in the present study we showed that 8-isoprostane level as an indicator of OS significantly increased in patient groups in different phases of CKD and that as the CKD stage progressed, the level of 8-isoprostane significantly increased. We consider that our study is important because it is one of the limited number of studies that evaluate OS in CKD with noninvasive EBC method and because it is the first study to evaluate OS on different stages of CKD using this method.

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