

Original Article

Determination of Serum Perfluorooctanoic Acid and Perfluorooctanesulfonic Acid Levels with Different Stages of Chronic Kidney Disease

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ABSTRACT. The aim of this study is to investigate the perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS) levels in patients with different stages of chronic kidney disease (CKD). Sixty-one CKD stage 1–4 patients who applied to the nephrology outpatient clinic were recruited. A control group consisting of 26 age- and sex-matched healthy controls were also included in the study. Concentrations of PFOA and PFOS were determined by comparing their peak areas with their standard curves. All samples were analyzed three times. The average values of blank samples were subtracted from the detected PFOA and PFOS values. PFOA and PFOS levels were significantly higher in CKD group than the controls (11.4 ± 7.47 , 0.45 ± 0.55 ; 0.13 ± 0.17 , 0.19 ± 0.4 ng/mL, respectively) ($P = 0.001$). Hemoglobin, serum albumin, and estimated glomerular filtration rate (eGFR) levels were significantly lower and potassium and uric acid levels were higher in the CKD group than the controls. PFOA and PFOS levels were significantly higher in all stages of CKD patients than healthy controls. However, there was no correlation between eGFR, and PFOS and PFOA. We have demonstrated significantly increased PFOA and PFOS concentrations in different stages of CKD patients. We could not find an association between eGFR, age, and serum PFOS and PFOA concentrations.

Introduction

Perfluoroalkyl and polyfluoroalkyl compounds

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are varied large groups of chemicals composed of distinct polymer and nonpolymer substances.¹ These compounds are applied to alter the physicochemical properties of various commercial and industrial materials, resulting in increased resistance to water, fire, and oil.² They are chiefly used in textile surface treatments, leather products, paper and packaging, cleaning tools, and firefighting foams.^{3,4} The perfluoroalkyl acids (PFAAs) are a member of

perfluorinated chemicals composed of a carbon backbone. Perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS) which consist of eight carbon backbone is the most commonly known PFAAs. These compounds are found mainly in the environment and also detected in human blood.⁵ Due to the very strong carbon fluorine bonds; these compounds are highly stable in the environment. These chemicals are known to be transmitted to humans via the ingestion of contaminated water and foods, use of commercial products, and inhalation of dust in the environment.⁶ Despite the widespread use of PFAAs for 50 years, it has recently been shown to be biologically inactive.⁷ Within the certain level of PFOA and PFOS have not been shown to have negative effects on human health, but some studies have shown that they may have adverse effects on fatty acid metabolism and induce liver damages.^{5,8} However, in toxicology studies, PFOS and PFOA have been shown to be easily absorbed after oral exposure and accumulate mainly in serum, kidney, and liver.⁷ These compounds have different half-life in humans. Researchers have been shown the half-life of PFOA between 2.3 and 8.5 years, while the half-life of PFOS has been predicted approximately in five years.^{9,10} PFOA and PFOS also have a long half-life in serum due to their binding properties to serum proteins.⁹ Studies suggest that one of the target organs affected by PFAAs is the kidney.⁸ Besides, these compounds are excreted through the kidneys and studies showed high levels of protein-bound PFOA in kidney cytosol.¹¹⁻¹³

Chronic kidney disease (CKD) is a widespread public health problem in the world and its prevalence is increasing day by day.¹⁴ CKD is defined as a glomerular filtration rate (GFR) of less than 60 mL/min/1.73 m² for three months or more or damage to kidney structure and functions. CKD was classified in five stages according to GFRs by Kidney Disease Improving Global Outcomes published in 2012, and stage five was defined as end-stage renal disease.¹⁵ PFOS and PFOA are excreted into the tubular lumen through the

tubular epithelial cells. They may accumulate in the body and may cause many adverse effects on both the kidney itself and the whole body in CKD. Therefore, in this study, we aim to investigate the PFOA and PFOS levels in patients with different stages of CKD.

Materials and Methods

In this cross-sectional study, sixty-one CKD stage 1–4 patients who applied to the Nephrology outpatient clinic were recruited. Inclusion criteria for CKD patients were; age >18 years, no acute cardiovascular event, infection or surgical intervention during the previous three months, and no recent history of malignancy. A control group consisting of 26 age- and sex-matched healthy controls were also included in the study. Patients with a history of cardiovascular disease, and those patients with stage 5 CKD or receiving renal replacement therapy [hemodialysis (HD), peritoneal dialysis or renal transplantation] were excluded from the study. The stage of CKD was detected by the estimated GFR (eGFR), with respect to the CKD Epidemiology Collaboration (CKD-EPI) Equation. Fasting venous blood samples were collected from all participants and centrifuged at 1500 × *g* for 10 min. immediately after collection. After separation, the serum samples were stored in the freezer at –80°C until the time of the assay. Before analysis, frozen serum samples were thawed at 4°C and then vortexed for 30 seconds till homogeneous. Demographic and clinical data of the participants were obtained from the laboratory information system. Body mass index was calculated using kg/m² formula.

Biochemical parameters were measured using routine laboratory methods. PFOA (98%) and PFOS (96%) standards were purchased from Sigma Aldrich, other chemicals with analytical grade (99.5% pure) were purchased from Merck and used as received. 5 mL highly pure Milli-Q water was added on 1 g sample to homogenize. One milliliter of the homogenate samples were mixed with 0.5 M 1 mL tetrabutylammonium hydrogensulfate and 0.25

M 2 mL sodium carbonate buffer (pH 10) for the extraction procedure. Then, 5 mL methyl-tert-butyl ether (MTBE) was added to the mixture and stirred for 20 min after centrifugation, aqueous and organic phases were separated, and 4 mL MTBE was eliminated from the mixture. The aqueous phase was treated with MTBE and extracted twice; obtained extracts were combined. The solvent was evaporated under N₂ atmosphere and 0.5 mL of methanol was then added. The obtained extract was filtered in a vial. Blank samples were prepared using ultra-pure water.¹⁶ LC-MS/MS analysis was performed on an AB SCIEX 3200 QTRAP system with Betasil C18 column (50 × 2.1 mm i.d. 5 µm). Ten microliters of the extract was injected in the instrument at 25°C with 2 mM ammonium acetate/methanol mobile phase beginning from 10% methanol. At a flow rate of 300 µL/min, the gradient increased to 95% methanol at 10 min before reverting to original conditions at 15 min.¹⁶ Concentrations of PFOA and PFOS were determined by comparing their peak areas with their standard curves. All samples were analyzed three times. The average values of blank samples were subtracted from the detected PFOA and PFOS values. The limit of detection (LOD) was found to be three times of the standard deviation of the blanks. The limit of quantification (LOQ) was determined to be three times of the LOD value (Table 1).

Statistical Analysis

Analysis of study data was carried out using IBM SPSS Statistics version 21.0 version 21 for Windows (IBM Corp., Armonk, NY, USA). Descriptive statistics for numerical variables were given as mean, standard deviation, median, minimum, and maximum.

Data normality was examined by the Kolmogorov–Smirnov test. Qualitative data were evaluated using the Chi-square test and quantitative data were tested using the Kruskal–Wallis and Mann–Whitney U test. Pearson correlation test was used for correlation analysis. For all tests, $P < 0.05$ values were considered statistically significant.

Results

Sixty- one CKD patients [27 (44.3%) males and 34 (55.7%) females] and 26 age- and sex-matched healthy subjects [11 (42.3%) males and 15 (57.7%) females] were included in the present study. Demographic data and studied laboratory parameters were presented in Table 1. We found that PFOA and PFOS levels were significantly higher in CKD group compared to the controls (11.4 ± 7.47 , 0.45 ± 0.55 ; 0.13 ± 0.17 , 0.19 ± 0.4 ng/mL respectively) ($P = 0.001$, Table 2). As expected, hemoglobin, serum albumin, and eGFR levels were significantly lower and potassium and uric acid level were higher in CKD group compared to the controls (Table 2).

In a second step, we divided CKD patients into four stages based on eGFR levels. PFOA and PFOS levels were significantly higher in all stages of CKD patients compared to healthy controls (Table 3). However, there was no correlation between eGFR, and both PFOS and PFOA. Interestingly, the most obvious high level of PFOA was found in stage 2 (13.4 ± 8.9 ng/mL). Nevertheless, the most prominent high level of PFOS was found in stage 4 (1.08 ± 0.55 ng/mL) (Table 3). Finally, we examined the correlation between PFOA and uric acid levels in CKD patients and found a very weak negative correlation ($r = -0.350$, $P = 0.046$) (Figure 1). However, we could not find any

Table 1. The values of retention time, correlation coefficient, recovery, limit of detection, limit of quantification of perfluorooctanoic acid and perfluorooctanesulfonic acid.

Compounds	Retention time (min)	Correlation coefficient (R ²)	Recovery (%)	LOD (ng/g)	LOQ (ng/g)
PFOA	2.57	0.9997	90.6–101.2	0.038	0.125
PFOS	2.63	0.9998	89.2–98.4	0.002	0.007

PFOA: Perfluorooctanoic acid, PFOS: Perfluorooctanesulfonic acid, LOD: Limit of detection, LOQ: Limit of quantification.

Table 2. Demographic information of the study groups.

Parameters	Control (n=26)	CKD (n=61)	P
Age (years) (x±s)	47.2±9.2	51.1 ±10.4	0.043
Gender	-	-	
Male	11 (42.3%)	27 (44.3%)	
Female	15 (57.7%)	34 (55.7%)	
BMI (kg/m ²)	28.2±6.4	30.1±5.9	0.398
Glucose (mg/dL)	100.2±26.4	113.8±41.2	0.206
eGFR (mL/min/1.73 m ²)	101.4±8.3	63.8±30.9	0.001
BUN (mg/dL)	12.4±3.8	25.1±15.1	0.001
Creatinine (mg/dL)	0.8±0.1	1.4±0.8	0.001
Uric acid (mg/dL)	5.3±1.9	6.6±2.0	0.062
Sodium (mmol/L)	138.4±1.8	138.4±3.3	0.773
Potassium (mmol/L)	4.4±0.5	5.5±5.3	0.033
Calcium (mg/dL)	8.8±1.7	8.8±0.8	0.212
Phosphor (mg/dL)	3.3±0.4	3.7±0.9	0.268
AST (U/L)	23.6±16.2	24.9±27.7	0.423
ALT (U/L)	21.3±9.4	22.8±20.8	0.479
Albumin (g/dL)	4.3±0.3	3.9±0.5	0.030
Hb (g/dL)	13.8±1.9	11.7±2.3	0.001
PFOA (ng/mL)	0.03±0.17	11.38±7.47	0.001
PFOS (ng/mL)	0.198±0.4	0.45±0.55	0.001

P <0.05 is significant. eGFR: Estimated glomerular filtration rate, PFOA: Perfluorooctanoic acid, PFOS: Perfluorooctanesulfonic acid, BMI: Body mass index, BUN: Blood urea nitrogen, ALT, Alanine transaminase, AST: Aspartate transaminase, Hb: Hemoglobin.

Table 3. Parameters of the control and study groups.

Parameters	Control (n=26) Mean SD	Stage 1 (n=14) Mean SD	Stage 2 (n=18) Mean SD	Stage 3 (n=18) Mean SD	Stage 4 (n=11) Mean SD
Glucose (mg/dL)	100.2±26.4	129.3±48.0	110.9±35.8	111.3±45.6	102.5±31.4
eGFR (mL/min/1.73 m ²)	101.4±8.3	104.9±8.8	76.7±12.9	43.7±8.02	23.2±4.5
Albumin (g/dL)	4.3±0.3	3.82±0.80	4.26±0.39	3.91±0.41	4.0±0.4
AST (U/L)	23.6±16.2	32.5±45.8	22.0±7.5	26.4±32.9	16.2±2.0
Median (min; max)	19 (15; 83)	18 (11;154)	22 (11; 39)	17 (10; 130)	16 (13; 18)
ALT (U/L)	21.3±9.4	20±10.6	25.8±16.3	16.7±10.7	29.4±44.5
Median (min; max)	19 (6; 37)	17 (7;36)	20 (9; 74)	14 (7; 47)	15 (6; 130)
Hemoglobin (g/dL)	13.8±1.9	12.8±1.2	13.4±1.8	11.2±1.8	9.7±2.7
PFOS (ng/mL)	0.19±0.4	0.27±0.32	0.41±0.57	0.23±0.38	1.08±0.55
Median (min; max)	0.087 (0.002; 1.63)	0.151 (0.078; 1.09)	0.156 (0.036; 1.81)	0.093 (0.013; 1.59)	1.31 (0.174; 1.75)
PFOA (ng/mL)	0.13±0.17	10.8±6.24	13.4±8.9	7.2±8.4	11.9±7.07
Median (min; max)	0.0 (0.0; 0.87)	12.35 (0.0; 19.7)	10.7 (0.0; 30.6)	3.24 (1.4; 21.8)	9.2 (5.16; 25.9)

eGFR: Estimated glomerular filtration rate, PFOA: Perfluorooctanoic acid, PFOS: Perfluorooctanesulfonic acid, ALT: Alanine transaminase, AST: Aspartate transaminase.

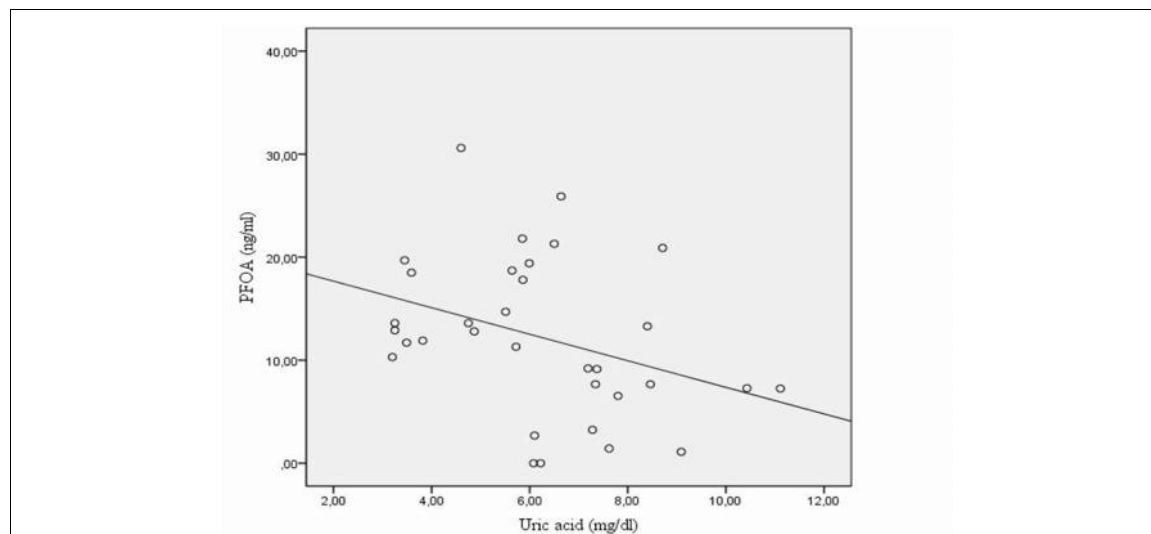


Figure 1. The correlation between perfluorooctanoic acid and uric acid levels in chronic kidney disease patients.

PFOA: Perfluorooctanoic acid.

correlation between PFOS and PFOA and all other studied laboratory parameters.

Discussion

In the present study, we observed significantly higher concentrations of PFOA and PFOS in different stages of CKD patients. In addition, no correlation was found between eGFR and PFOA, and PFOS concentrations. We only found a weak correlation between PFOA concentration and uric acid levels in CKD patients. To the best of our knowledge, there are no other reports in the literature evaluating serum PFOA and PFOS concentrations at different stages of CKD patients. These findings provide valuable information for understanding PFOA and PFOS levels at different stages of CKD patients.

Recently, there is a growing body of concern for human exposure of perfluorinated compounds (PFCs), including PFOA and PFOS, due to its persistence in the environment. People can be exposed to these chemicals not only by using consumer products but also by inhaling air in the environment. PFOA and PFOS are excreted from the body through the kidneys and accumulate mainly in the kidneys

due to their long half-life.^{11,13}

In the literature, Liu et al evaluated the PFOA and PFOS levels of the uremic patients receiving HD. They showed that PFOA levels were lower, but PFOS levels were higher in the serum of the uremic patients. They speculated that lower PFOA levels might have resulted from less exposure to products that contain PFOA in the living environment. They also showed high PFOA levels in older uremic patients (age >50) and they associated this increase with the accumulation of PFOA in the human body. They also evaluated the effect of dialysis treatment on the levels of PFOS in these patients. They showed low levels of PFOS even after in one single dialysis session and concluded that PFOS levels might be removed by dialysis treatment.¹⁷ In our study, we found high levels of PFOA and PFOS in the serum of CKD patients in accordance with the literature. The reasons for these high levels may increase due to food intake and exposure to gases and products containing PFCs.

PFOA and PFOS levels have been evaluated in different populations over the past years. In another study by Hsu et al determined the background levels of PFOA and PFOS in the serum samples of the general population of

Taiwan. They found high concentrations of PFOS than PFOA in 59 samples of individual volunteers in Taiwan.¹⁸ They speculated that these findings were similar to previous studies of PFOA and PFOS levels in the general population from other countries. They also showed that PFOS levels were increased with age in line with previous studies.^{5,19} However, we could not find any association between serum PFOS level and age in both healthy subjects and CKD patients. In the present study, we also investigated PFOA and PFOS concentrations at different stages of CKD. We found a high level of PFOA in stage 2 and a high level of PFOS in stage 4 in CKD patients compared to the control subjects.

Studies have shown that high levels of PFCs have adverse effects on human health including neurotoxicity, cell membrane disruption, developmental toxicity, genetic damage and kidney disease-related pathways.^{6,20-22} In addition, other studies have shown that exposure to PFOA and PFOS have disrupted oxidative balance and resulted in oxidative damage.²³ Moreover, epidemiological and toxicological studies indicate that PFOS and PFOA levels may be associated with low birth weight, increased blood cholesterol concentrations, testicular cancer, and hyperuricemia.²⁴⁻²⁷ Therefore, the determination of PFCs levels is clinically important for human studies.

There are some limitations in this study. First of all, the number of studied subjects was small, therefore, it is an important shortcoming for predicting correlation with other parameters. Second, since a single determination of PFOS and PFOA levels, the power for the findings was not high. Thirdly, the concentration of PFOA and PFOS may also be related with the dietary food intake and different lifestyles of the patients. We could not evaluate these parameters in our study group. Nevertheless, PFOA and PFOS concentrations were significantly high in CKD patients.

Conclusion

In conclusion, we have demonstrated significantly increased PFOA and PFOS concen-

trations in different stages of CKD patients. Most probably, because of the small number of patients, we could not find an association between eGFR, age and serum PFOS and PFOA concentrations. Further prospective and larger studies are needed to clarify the determinants of PFOS and PFOA, and to highlight their effects on human health and renal functions in CKD patients.

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Conflict of interest: None declared.

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