Eur J Gen Med 2015; 12(1):53-58

Original Article

DOI : 10.15197/sabad.1.12.09

The Relationship between Oxidative Stress and Coronary Artery Calcification in Patients Undergoing Peritoneal Dialysis or Hemodialysis

[1]Fatih Mehmet Erdur, [2]Kultigin Turkmen, [3]Hatice Kayikcioglu, [4]Fatih Ozcicek, [5]Aysun Toker, [6]Orhan Ozbek, [1]Suleyman Turk, [1]Halil Zeki Tonbul

ABSTRACT

Vascular calcification and increased oxidative stress are commonly seen in patients with end-stage renal disease (ESRD). Nitrotyrosine is one of the end products of nitric oxide metabolism and is accepted as an indicator of oxidative stress. Nitrotyrosine levels have been found to be high in ESRD patients. The aim of our study is to investigate the relation between coronary artery calcification and oxidative stress in peritoneal dialysis (PD) and hemodialysis (HD) patients. 46 PD and 34 HD patients are included in the study. Coronary artery calcification scoring (CACS) is made by multi slice computed tomography. Patients are divided into 4 groups according to their CACS values as Group 1 (CACS: 0), Group 2 (CACS:1-99), Group 3 (CACS:100-399) and Group 4 (CACS: ≥400). Serum nitrotyrosine levels were measured. Nitrotyrosine levels were significantly increased in HD patients compared to PD patients. Nitrotyrosine levels were found to be elevated in accordance with increased CACS in PD patients. However, we could not find this relationship in HD patients. There might be an important relationship between CACS and oxidative stress in PD patients.

Key words: *Hemodialysis, peritoneal dialysis, oxidative stress, vascular calcification*

Periton Diyaliz ve Hemodiyaliz Altındaki Hastalarda Oksidatif Stres ve Koroner Arter Kalsifikasyonu Arasındaki İlişki

ÖZET

Son dönem böbrek yetersizliği (SDBY) gelişen hastalarda vasküler kalsifikasyon (VK) ve oksidatif stres yaygın olarak gözlenmektedir. SDBY'li hastalarda oksidatif stres belirteci olan nitrotirozin düzeyinin yüksek olduğu tespit edilmiştir. Çalışmamızın amacı periton diyaliz (PD) ve hemodiyaliz (HD) hastalarında koroner arter kalsifikasyonu ile oksidatif stres arasındaki ilişkiyi incelemektir. Çalışmamıza 46 PD ve 34 HD hastası alınmıştır. Hastalara multi slice bilgisayarlı tomografi ile koroner arter kalsiyum skorlaması (KAKS) yapıldı. KAKS değerlerine göre hastalar Grup 1 (KAKS 0), Grup 2 (KAKS: 1-99), Grup 3 (KAKS:100-399) ve Grup 4 (KAKS: ≥400) olmak üzere 4 gruba ayrıldı. Serumda nitrotirozin düzeyleri ölçüldü. PD hastalarıyla kıyaslandığında, HD hastalarının nitrotirozin düzeyi anlamlı olarak yüksekti. PD ve HD hastaları KAKS göre gruplara ayrıldığında PD hastalarında KAKS arttığında nitrotirozin düzeyi de anlamlı olarak artmaktaydı, halbuki bu ilişki HD hastalarında tespit edilemedi.PD hastalarında KAKS ile oksidatif stres arasında anlamlı bir ilişki mevcuttur.

Anahtar kelimeler: *Hemodiyaliz, periton diyalizi, oksidatif stres, vasküler kalsifikasyonu*

[1]Department of Nephrology, Necmettin Erbakan University Meram School of Medicine, Konya, Turkey, 2Department of Nephrology, Mengucek Gazi Training and Research Hospital, Erzincan, University, Erzincan, Turkey, [3]Department of Internal Medicine, Necmettin Erbakan University Meram School of Medicine, Konya, Turkey-[4]Department of Internal Medicine, Mengucek Gazi Training and Research Hospital, Erzincan University, Erzincan, Turkey, [5]Department of Biochemistry, Necmettin Erbakan University Meram School of Medicine, Konya, Turkey, [6]Department of Radiology, Necmettin Erbakan University Meram School of Medicine, Konya, Turkey

Correspondence: *Fatih Mehmet Erdur,*
Necmettin Erbakan Universitesi, Meram Tip Fakultesi, Hemodiyaliz Sekreterligi, Meram, Konya, Turkey
Mobile phone: 90-530-8808072 E-mail: drfme@yahoo.com

Received: 04.11.2012, Accepted: 25.04.2014

INTRODUCTION

Cardiovascular diseases (CVD) are the most common causes of mortality in patients with chronic kidney diseases (CKD) (1). In the etiology of atherosclerotic CVD that have an increased frequency in this population, classical risk factors, such as diabetes mellitus, hypertension, dyslipidemia, obesity, etc., as well as increased inflammation, oxidative stress, anemia, and dysregulated bone and mineral metabolism play an important role (2). The profile of vascular diseases observed in patients with end-stage renal disease (ESRD) differs from general population. In general population, intimal atherosclerotic plaque pathologies are common, whilst among the patients with CKD, in addition to this pathology, medial calcification is determined (3,4).

Vascular calcification is a condition that is frequently encountered in diseases such as chronic kidney failure, diabetes, hypertension, atherosclerosis, in which endothelial damage occurs and is associated with increased morbidity and mortality in even younger ages (5). The studies performed within the last 25 years have demonstrated that inflammation and acquired immunity play a significant role in the development of atherosclerotic plaques (6). Coronary artery calcification is an index of the severity of atherosclerotic vascular disease, and may predict future adverse cardiovascular events especially in uremic patients undergoing hemodialysis (HD) (7). In patients with ESRD, vascular calcification was found to be associated with advanced age, male gender, non-black race, duration and adequacy of dialysis, serum calcium x phosphorus product and phosphorus level, vitamin D therapy and hyperparathyroidism (8,9). Moreover, the patients with ESRD are exposed to increased oxidative stress bearing (10). In this population, the defects in nitric oxide (NO) metabolism are considered to be a part of excessive atherosclerosis (11). Nitrotyrosine is one of the end products of NO metabolism and is accepted as an indicator of oxidative stress. In a study describing the autopsy results of the patients with ESRD, when the aorta specimens were dyed with immunologic stains, TNF-α and nitrotyrosine were found in the majority of these patients in comparison with the control group (12).

In our study, we aimed to ascertain the relationship between nitrotyrosine, an indicator of oxidative stress, and coronary artery calcification in HD and peritoneal dialysis (PD) patients.

MATERIALS AND METHODS

The study population consisted of 80 patients with ESRD who had been undergoing dialysis for at least 6 months. Of these 80 patients participated in the study, 46 patients (male/female=28/18) were undergoing peritoneal dialysis and 34 patients (male/female=20/14) were under hemodialysis treatment. The mean age of PD and HD patients were 50,43±15 and 47,74±12,33 years, respectively. After receiving the approval of the ethics committee, the study was started; all patients first read and signed the informed consent form to participate in the study. The exclusion criterias of the study were patients receiving hemodialysis for a period shorter than six months, a history of severe trauma, surgical operations or burns within the past one month and the patients having symptomatic and decompensated liver disease.

Our study was designated as a cross-sectional study. The patients were performed whole blood count, routine biochemical analyses and coronary artery calcium score (CACS) analyses with multi-slice computerized tomography which were previously taken for coronary artery atherosclerosis screening. Venous blood samples for biochemical analyses were drawn after an overnight fast before first exchange in PD patients and before the mid-week session in patients receiving HD. The blood samples that were collected within plain tubes were centrifuged at 5000g for 5 minutes so that the serum was separated. The specimens were put into the eppendorf tubes and stored at -80°C until ELISA analyses. For all patients included in the study, age, gender, body weight, body mass index, primary diseases and duration of dialysis were recorded. The systolic blood pressure (SBP) and diastolic blood pressure (DBP) of patients were measured. Patients with SBP and DBP >140 mmHg and 90 mmHg, respectively, or who were already on antihypertensive treatment were accepted to be hypertensive. HD patients were receiving dialysis with bicarbonate using semisynthetic dialysis membrane for duration of 4 hours, 3 times a week. PD patients included in the study were performing 4 exchanges a day; 41 patients were using a low-calcium dialysate, whilst 5 patients were using high-calcium dialysate.

Coronary artery calcium scoring (CACS)

The coronary artery calcium scoring was done with 64-MDCT scanner (Somatom Sensation 64, Siemens Medical Solutions, Erlangen, Germany). During the examination, the heart was scanned in cranio-caudal direction, from the carina to the apex. During the procedure, it

was achieved 140 kV tube voltage, 190 mAseff effective tube current-time product, 24x1,2 mm collimation, 2.8 mm table support per rotation, and 33 ms tube rotation time. For every patient, 65% R-R reconstruction was prepared at a medium smooth convolution kernel (B35f) and a 512x512 reconstruction matrix. Then, all images were reconstructed and transferred to an external environment (Leonardo, Siemens Medical Solutions, Erlangen, Germany) for calcium scoring (Syngo Calcium Scoring CT, Siemens, Germany). The coronary calcium score was calculated considering a cut-off of 130 HU as described by Agatston et al. (13).

Depending on the CACSs, the patients were divided into 4 groups, those with CACS 0 (group 1), the patients with low calcium scores (CACS 1-9, Group 2), those with medium calcium scores (CACS 10-399, Group 3) and the patients with high calcium scores (CACS≥400, Group 4).

Statistical analysis

The data were entered on a computer using SPSS (statistical package for social science) software version 17.0. The results were presented as mean ± standard deviation and % percentage. The analyses for the compliance with normal distribution were done. For the comparison of two groups, group analyses were done with Freidman test after determination of median and standard deviation values with descriptive tests. To compare to percentages, Fischer's exact and chi-square tests were used. Any p value<0.05 was considered to be significant.

RESULTS

Tables 1 and 2 show demographic, clinic, and biochemical parameters of the patients with ESRD who were included in our study. There was no difference between demographic features of PD and HD patients. There was also no difference between the use of vitamin D and the use of calcium-containing phosphorus binders (Table 1). When the clinical features and biochemical values of PD and HD patients were compared, the mean Ca and P levels and Ca x P product of HD patients were significantly higher than PD patients. Similarly, the mean albumin, ferritin, LDL cholesterol and hemoglobin values of HD patients were significantly higher than PD patients (Table 2).

The mean age of 46 patients in the PD group was 50.43±15 years; 28 of these patients were men and 18 patients were women. The mean age of 34 HD patients (20 men and 14 women) was 47.74±12.33 years. According to the knowledge obtained from the medical records of the patients in the PD group, the causes of renal failure were polycystic kidney (n=1), amyloidosis (n=1), IgA nephropathy (n=1), chronic pyelonephritis (n=1), hypertension (n=5), diabetic nephropathy (n=4) and nephrolithiasis (n=2). For 31 PD patients, the reason of chronic renal failure was unknown. In the hemodialysis group, the causes of renal failure were amyloidosis (n=1), IgA nephropathy (n=1), nephrolithiasis (n=2), glomerulonephritis (n=2), polycystic kidney (n=3), diabetic nephropathy (n=3). The etiology of chronic renal failure was unknown in 22 patients. The mean CAC score of the patients with ESRD was found to be 241.1±339.7 cm3. When these patients were divided depending on their renal replacement treatment, the CAC scores of PD and HD patients were found to be 225.12±285.87 and 262.75±405.09 cm3, respectively (p=0.627) (Table 2).

The mean nitrotyrosine level of all patients was 13.37±12.3 nM. When PD and HD patients were compared with respect to nitrotyrosine levels, it was found that the hemodialysis patients had higher levels of nitrotyrosine (PD vs. HD, 9.58±3.28 nM, 18.49±17.47 nM, respectively,

Table 1. *Demographic Characteristics of Patients*

	ESRD Patients (n:80)	*PD Patients (n:46)*	*HD Patients (n:34)*	*p value***
Male/Female (n)	*38/42*	*18/28*	*20/14*	*0.85*
*Age (year)**	*49.29±13.93*	*50.43±15*	*47.74±12.33*	*0.48*
*BMI (kg/m2)**	*26.77±5.15*	*26.91±5*	*26.59±5.42*	*0.62*
*Duration of Dialysis (year)**	*4.85±3.37*	*4.61± 2.22*	*5.18±4.51*	*0.80*
Hypertension (n.%)	*56 (70%)*	*32 (69%)*	*24 (70%)*	*0.24*
Diabetes Mellitus (n. %)	*13 (16%)*	*9 (20%)*	*4 (12%)*	*0.35*
Usage of Vitamin D (%)	*78.6*	*78.9*	*78.3*	*0.95*
Usage of Ca Containing Phosphate binder (%)	*64.1*	*63.3*	*65.2*	*0.13*

*PD; peritoneal dialysis HD; hemodialysis, BMI; body mass index, Ca; calcium, *mean ± standart deviation, p**; comparison of peritoneal dialysis and hemodialysis patients*

Table 2. *Clinical and Biochemical Parameters of End-Stage Renal Disease Patients*

Parameters	*ESRD Patients (n=80) (Mean±SD)*	*PD Patients (n=46) (Mean±SD)*	*HD patients (n=34) (Mean±SD)*	*P value¥*
SBP (mmHg)	*139±29*	*134±28*	*145±28*	*0.13*
DBP (mmHg)	*86±16*	*84±17*	*89±17*	*0.24*
Calcium (mg/dL)	*9.2±0.98*	*8.92±0.87*	*9.6±1.0*	*0.002*
Phosphorus (mg/dL)	*4.63±1.32*	*4.24±1.0*	*5.17±1.53*	*0.004*
PTH (pg/mL)	*320.8±207*	*327±198*	*312±222*	*0.74*
Albumin (g/dL)	*3.85±0.48*	*3.6±0.45*	*4.17±0.3*	*0.0001*
Hemoglobin (g/dL)	*11,5±1,7*	*11,2±1,85*	*12,01±1,37*	*0.02*
LDL (mg/dL)	*112.2±28.4*	*108.5±26.9*	*117.2±29.9*	*0.117*
CRP (mg/L)	*14.8±17*	*15.5±18.9*	*14.1±13.9*	*0.96*
Kt/V	*-*	*2,08±0,66**	*1,49±0,26⁺*	*-*
Nitrotyrosine (nM)	*13.37±12.3*	*9.58±3.28*	*18.49±17.47*	*<0.0001*
CACS (cm^3)	*241.1±339.7*	*225.1±285.8*	*262.7±405.1*	*0.627*

PD; peritoneal dialysis, HD; hemodialysis, ESRD; end-stage renal disease, SBP; systolic blood pressure, DBP; diastolic blood pressure, iPTH; intact parathyroid hormone, CACS; coronary artery calcification score, Kt/V; dialysis adequacy, LDL; low density lipoprotein, CRP; C-reactive protein, SD; standart deviation,
** each dialysis adequacy (kt/v) measured weekly for PD patients*
⁺ each dialysis adequacy (kt/v) measured after a dialysis session for HD patients
¥ p values for comparison of PD and HD patients

p<0.0001) (Table 2). When PD patients were divided into 4 groups depending on their CACS, there was a significant relationship between the levels of nitrotyrosine and CACS. The nitrotyrosine level increased as CAC score became greater (r=0.343, p=0.031) (Table 3). No significant relationship was found between CACS and nitrotyrosine in HD patients as a result of comparison of 4 groups formed on the basis of CACS (p=0.95).

DISCUSSION

In our study, we investigated the relationship between nitrotyrosine, an indicator of oxidative stress and vascular calcification in patients with ESRD undergoing either PD or HD treatment. The main consequences of our study can be summarized as follows: i) the nitrotyrosine levels of HD patients were significantly higher in comparison of PD patients, ii) when PD and HD patients were divided into subgroups depending on their CACS, nitrotyrosine levels increased as CACS became greater in PD patients, but this association was not found in HD patients. In patients with CKD, a significant correlation was found between increased oxidative stress, impaired NO metabolism and endothelial dysfunction. Endothelium-derived NO is an effective vasodilator and also has anti-atherogenic properties. Increased oxidative stress causes the formation of superoxide and this oxygen radical generates peroxynitrite, a product of NO metabolism, interacting with NO. The nitrotyrosine, an indicator of vascular oxidative stress, is generated as a result of the interaction of peroxynitrite with tyrosine residues (14). Massy et al. (11) demonstrated that nitrosative stress increased in

Table 3. *Nitrotyrosine Levels According to Coronary Artery Calcification Score Groups in Patients receiving Peritoneal Dialysis and Hemodialysis*

CACS Groups	*Nitrotyrosine Levels of PD Patients (nM) (mean±SD)*	*Nitrotyrosine Levels of HD Patients (nM) (mean±SD)*
CACS Group 1	*8.16±1.03*	*17.32±15.02*
CACS Group 2	*8.49±1.17*	*22.30±26.87*
CACS Group 3	*10.65±3.54*	*24.69±30.22*
CACS Group 4	*11.04±4.70*	*15.85±10.40*
*p value**	*0.031*	*0.95*

PD; peritoeal dialysis HD; hemodialysis, CACS; coronary artery calcification score, SD; standart deviation,
*; p values for comparison of between CACS groups of peritoneal dialysis and hemodialysis patients

chronic HD patients, but plasma nitrotyrosine levels of these patients were within normal ranges. On the other hand, Guilgen et al. (4) evaluated the external iliac and renal arteries of 27 patients with ESRD, who underwent renal transplantation as to oxidative stress and showed immunohistochemically that these patients had much more higher nitrotyrosine in comparison with the control group. In our study, it was found that serum nitrotyrosine levels of HD patients were significantly higher in comparison with PD patients. In the past, inflammation and oxidative stress that occur due to prolonged use of catheters as vascular access and the use of non-biocompatible membranes were more common in HD patients than PD patients; currently, with the widespread availability of biocompatible membranes, inflammation and oxidative stress occur less frequently in HD patients in comparison with PD patients (15).

Hye In Kim et al. found that prevalence of vascular calcification on plain radiography was not different according to dialysis modality (16). In our previous study (17) we found that coronary artery calcification and thoracic periaortic calcification was not differ in HD and PD patients. In present study also we couldn't find significant relation between HD and PD patients according to vascular calcification. In an autopsy study carried out by Koleganova et al. on 31 ESRD patients, the aortas of these patients were compared to the control group and it was found immunohistochemically that aorta specimens of these patients contained very high amounts of TNF-α and nitrotyrosine (12). In another autopsy study evaluating vascular calcification of renal and iliac arteries, it was found a positive correlation between immunohistochemically increased nitrotyrosine and vascular calcification (4). In our study, it was found that nitrotyrosine levels increased in correlation with elevated CACS in PD patients. The reason of why the same correlation wasn't demonstrated in HD patients is thought to be due to the small number of HD patients participating in the study.

The most important limiting factor in our study is the small number of patients. Moreover there isn't a control group in our study. Additionally, computed tomography techniques are not successful enough to distinguish medial from intimal calcification. We think that testing other markers in addition to nitrotyrosine as the indicators of oxidative stress could be more convenient to assess oxidative stress. In conclusion, in our study, oxidative stress was found to be increased in patients with ESRD. Particularly in PD patients, oxidative stress increased as CACS became greater. This condition indicates that there is a close relationship between vascular calcification and oxidative stress in this population.

Conflict of Interest

The authors declare that they have no conflict of interest.

REFERENCES

1. *Foley RN, Parfrey PS, Sarnak MJ. Clinical epidemiology of cardiovascular disease in chronic renal disease. Am J Kidney Dis 1998; 32: 112-9.*
2. *Derici U, El Nahas AM. Vascular calcifications in uremia: old concepts and new insights. Semin Dial 2006; 19: 60-8.*
3. *Shao JS, Cai J, Towler DA. Molecular mechanisms of vascular calcification: lessons learned from the aorta. Arterioscler Thromb Vasc Biol 2006; 26: 1423-30.*
4. *Guilgen G, Werneck ML, de Noronha L, Martins AP, Varela AM, Nakao LS, et al. Increased calcification and protein nitration in arteries of chronic kidney disease patients. Blood Purif 2011; 32: 296-302.*
5. *Proudfoot D, Skepper JN, Shanahan CM, Weissberg PL. Calcification of human vascular cells in vitro is correlated with high levels of matrix Gla protein and low levels of osteopontin expression. Arterioscler Thromb Vasc Biol 1998; 18: 379-88.*
6. *Ross R. Atherosclerosis an inflammatory disease. N Engl J Med 1999; 340: 115-26.*
7. *K Taki, F Takayama, Y Tsuruta, T Niwa. Oxidative stress, advanced glycation end product, and coronary artery calcification in hemodialysis patients. Kidney International 2006; 70: 218-24.*
8. *Raggi P, Boulay A, Chasan-Taber S et al. Cardiac calcification in adult hemodialysis patients. A link between end-stage renal disease and cardiovascular disease? J Am Coll Cardiol 2002; 39: 695-701.*
9. *Turkmen K, Tonbul HZ. Vascular calcification in chronic renal failure. Turkish Nephr Dialysis Transplant J 2010; 19: 82-7.*
10. *Namiduru ES, Tarakcioglu M, Tiryaki O, Usalan C. Evaluation of oxidative and nitrosative stress in hemodialysis patients. Minerva Med 2010; 101: 305-10.*
11. *Massy ZA, Borderie D, Nguyen-Khoa T, Drueke TB, Ekindjian OG, Lacour B. Increased plasma S-nitrosothiol levels in chronic haemodialysis patients. Nephrol Dial Transplant 2003; 18: 153-7.*
12. *Koleganova N, Piecha G, Ritz E, et al. Arterial calcification in patients with chronic kidney disease. Nephrol Dial Transplant 2009; 24: 2488-96.*
13. *Agatston AS, Janowitz WR, Hildner FJ, Zusmer NR, Viamonte M Jr, Detrano R. Quantification of coronary artery calcium using ultrafast computed tomography. J Am Coll Cardiol 1990; 15: 827-32.*

14. Loscalzo J. Nitric oxide insufficiency, platelet activation, and arterial thrombosis. Circ Res 2001; 88: 756-62.

15. Canivet E, Lavaud S, Wong T, Guenounou M, Willemin JC, Potron G, et al. Cuprophane but not synthetic membrane induces increases in serum tumor necrosis factor-alpha levels during hemodialysis. Am J Kidney Dis 1994; 23: 41-6.

16. Hye In Kim, Won Suk An. Comparison of Fetuin-A, Vitamin D, Monounsaturated Fatty Acid, and Vascular Calcification on Plain Radiography Between Dialysis Modalities. IJKD 2013; 7: 453-60.

17. Turkmen K, Ozcicek F, Ozcicek A, Akbas EM, Erdur FM, Tonbul HZ. The relationship between neutrophil-to-lymphocyte ratio and vascular calcification in end-stage renal disease patients. Hemodial Int 2014; 18:47-53.