Ukrainian Journal of Nephrology and Dialysis
Scientific and Practical, Medical Journal

**Founders:**

- State Institution «Institute of Nephrology NAMS of Ukraine»
- National Kidney Foundation of Ukraine

**ISSN 2304-0238;**
**eISSN 2616-7352**

**Journal homepage: https://ukrjnd.com.ua**

**Research Article**

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doi: 10.31450/ukrjnd.2(74).2022.04

# Serum soluble Fas-ligand levels and flow-mediated vasodilation in patients undergoing peritoneal dialysis

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Citation:

Huddam B, Azak A, Karakus V, Alp A, Genek DG, Kocak MGK, et al. Serum soluble Fas-ligand levels and flow-mediated vasodilation in patients undergoing peritoneal dialysis. Ukr J Nephr Dial. 2022;2(74): 25-32. doi: 10.31450/ukrjnd.2(74).2022.04.

**Abstract.** *Flow-mediated vasodilation (FMD) has been demonstrated to be a useful, non-invasive tool for the detection of endothelial dysfunction in atherosclerotic cardiovascular disease, the leading cause of mortality in end-stage kidney disease. The Fas/Fas ligand system of apoptosis resulting from activation of the caspase cascade- contributes to the pathophysiology of atherosclerosis. This 'apoptotic' system plays a central role in immune homeostasis. Vascular endothelial cells and inflammatory cells are the main resources of the Fas ligand. In this study, we aimed to investigate the role of soluble Fas ligand (sFasL) as a marker of FMD in peritoneal dialysis (PD) patients.*
*Methods. A total of 43 patients undergoing maintenance PD and 40 healthy donors were enrolled in this cross-sectional observational study. Demographics, anthropometric measurements and clinical examinations were obtained. Endothelial function was evaluated by FMD of the brachial artery with high-resolution ultrasonography. Serum sFasL concentrations were measured with an enzyme-linked immunosorbent assay kit.*
*Results. The enrolled partisipants were devited on 2 groups: PD patients who had been treated at least 12 weeks (group 1; mean age 41±14 years, M/F: 22/21) and gender matched 40 healthy controls (group 2; mean age 50±12 years, M/F: 19/20). The forearm FMD and serum sFasL levels were significantly lower in PD patients (3.95±2.01 vs 8.83 ± 6.17; p<0.001 and 54 ± 24 vs 73 ± 30; p=0.001). Forearm FMD was correlated with sFasL (r=0.289; p=0.008), age, BMI and uric acid (r= 0,32; p=0.003, respectively), hemoglobin (r= 0,293; p=0.007), calcium (r= 0,26; p=0.016), phosphate (r=- 0,250; p=0.023), magnesium (r= 0,255; p=0.020), 24 h SBP (r=- 0,257; p=0.019), creatinine and iPTH (r=- 0.50 and r=- 0,45; p<0.001, respectively). After adjustment for age, the stepwise multivariate analysis showed sFasL was independently associated to FMD (β: 0.180; p=0.03, CI: 0.078-0.314). vs 73 ± 30; p=0.001).*
*Conclusions. sFasL may be used as a simple screening marker for endothelial dysfunction in PD patients.*

**Key words:** *peritoneal dialysis, flow-mediated vasodilation, soluble Fas-ligand, endothelial dysfunction.*

**Article history:**

*Received January 11, 2022*
*Received in revised form February 24, 2022*
*Accepted February 30, 2022*

**Conflict of interest statement.** The authors declare no competing interest.



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УДК 616.61:616.381-089.819]:616.15-07

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# Концентрація розчинного Fas-ліганду в сироватці та потоко-опосередкована вазодилатація у пацієнтів, які лікуються методом перитонеального діалізу

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**Резюме.** *Потоко-опосередкована вазодилатація (ПОВ) є корисним, неінвазивним інструментом для виявлення ендотеліальної дисфункції у пацієнтів з атеросклеротичними серцево-судинними захворюваннями, які є основною причиною смертності хворих на хронічну хворобу нирок. Лігандна система Fas/Fas апоптозу, яка виникає в результаті активації каспазного каскаду, сприяє патофізіології атеросклерозу. Ця «апоптотична» система відіграє центральну роль в імунному гомеостазі. Ендотеліальні клітини судин і прозапальні клітини є основними ресурсами Fas-ліганду. У цьому дослідженні ми мали на меті дослідити роль розчинного ліганду Fas (sFasL) як маркера ПОВ у пацієнтів, які лікуються методом перитонеального діалізу (ПД).*

*Методи. До цього перехресного обсерваційного дослідження було включено 43 ПД пацієнти 40 умовно-здорових донорів. Досліджувались демографічні дані, антропометричні вимірювання та клінічні обстеження. Функцію ендотелію оцінювали за допомогою ПОВ плечової артерії та УЗД високої роздільної здатності. Концентрації sFasL у сироватці крові вимірювали за допомогою набору для імуноферментного аналізу.*

*Результати. Включені до дослідження учасники були розділені на 2 групи: ПД пацієнти, які лікувались ПД щонайменше 12 тижнів (група 1; середній вік 41±14 років, M/F: 22/21) та 40 здорових осіб контролю (група 2; середній вік) 50±12 років, M/Ж: 19/20). Рівні ПОВ передпліччя та концентрації sFasL у сироватці крові були значно нижчими у ПД пацієнтів порівняно з контролем (3,95±2,01 проти 8,83 ± 6,17; p<0,001 та 54 ± 24 проти 73 ± 30; p=0,001). ПОВ передпліччя корелював з sFasL (r=0,289; p=0,008), віком, індексом маси тіла та сечовою кислотою (r=0,32; p=0,003 відповідно), гемоглобіном (r=0,293; p=0,007), кальцієм (r = 0,26; p=0,016) та фосфором крові (r=- 0,250; p=0,023), магнієм сироватки (r= 0,255; p=0,020), систолічним артеріальним тиском (r=- 0,257; p=0,019), креатиніном та паратиреоїдним гормоном крові (r=- 0,50 і r=- 0,45; p<0,001 відповідно). Після коригування на вік поетапний багатофакторний аналіз показав, що концентрація sFasL сироватки незалежно асоціювалась з ПОВ ( : 0,180; p=0,03, CI: 0,078-0,314 проти 73 ± 30; p=0,001).*

*Висновки. sFasL можна використовувати як простий скринінговий маркер ендотеліальної дисфункції у ПД пацієнтів.*

**Ключові слова:** *перитонеальний діаліз, опосередкована потоком вазодилатація, розчинний Fas-ліганд, ендотеліальна дисфункція.*

**Introduction.** Endothelium-dependent vasodilation is a parameter that can be sued to assess endothelial function in atherosclerotic cardiovascular disease, as well as in end-stage renal disease (ESRD) patients in whom cardiovascular disease (CVD) is the leading cause of morbidity and mortality [1-4]. Vascular inflammation and endothelial injury play a role in the accelerated development of atherogenesis in uremia [1, 5]. Proteins such as Fas and Fas ligand (FasL) are expressed in atherosclerotic lesions indicating the signs of apoptosis and inflammation present in atherosclerotic plaques and are also found in the circulation in small amounts [5-7]. Membrane-bound Fas (mFas), a cell-surface receptor, transduces apoptosis after interaction with membrane-bound or soluble Fas ligand (sFasL), whereas FasL is a cytokine expressed on activated T cells, natural killer cells and vascular endothelial cells. Fas ligand is cleaved from the cell membrane by metalloproteinase enzyme to form sFasL [5-8]. Studies have reported that serum sFasL might be used as an independent novel marker of vascular injury related to endothelial function [5-7, 9]. To examine the endothelial function, high-resolution

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vascular ultrasound has been used to demonstrate the endothelium-dependent flow-mediated vasodilation (FMD), which depends largely on nitric oxide (NO) synthesis [10, 11].

**The present study aimed** to evaluate the forearm FMD and serum sFasL, a component of the apoptosis cascade, in patients undergoing peritoneal dialysis (PD), and compare the results with healthy control subjects.

**Methods.** Forty-three consecutive patients referred to our hospital undergoing maintenance PD for at least 12 weeks were enrolled in this cross-sectional observational study. The study was approved by the local ethics committee (Ankara Training and Research Hospital on 28.12.2011 with protocol no: 0446/3721). Written informed consent was obtained from the patients and control subjects enrolled in the study. Patients with a history of ischemic heart disease, overt heart failure with an ejection fraction below 45%, severe hypertension with average systolic blood pressure (SBP) ≥160 mm Hg or average diastolic blood pressure (DBP) ≥100 mm Hg, diabetes mellitus, obesity with a BMI above 30 kg/m$^2$, chronic or acute inflammatory diseases, chronic or acute infectious diseases, and malignancy were excluded. Among the patients and control subjects, current smokers and those who quit within the past six months were also excluded. Data on demographics were recorded; anthropometric measurements and clinical examinations including rest electrocardiogram and 24 hours ambulatory blood pressure monitoring (ABPM) were obtained. Ischemic conditions, such as angina pectoris, peripheral vascular disease, myocardial infarction, percutaneous coronary angiography/artery bypass grafting, and stroke were analyzed. Body Mass Index (BMI) was calculated, [(weight in kg) / (height in m$^2$)], according to WHO recommendations [12]. Diabetes was defined according to the American Diabetes Association recommendations [13]. We used ABPM-Oscar-2 24-Hr ABP (Sun Tech Medical, Glenwood Avenue Raleigh, NC 27617 USA)-instead of of ce blood pressure measurement [14]. Fifteen patients revealed average blood pressures of systolic and diastolic ≥140/90 mm Hg. These patients were classified to be in stage 2 of hypertension. Eight control subjects revealed average blood pressures of systolic and diastolic between 120/80 mm Hg and134/86 mm Hg, and they were classified as being in stage 1 [14, 15].

Endothelial function was evaluated by FMD of the brachial artery with high-resolution ultrasonography [6, 10, 11, 16]. ATL 5000 ultrasound system (Advanced Technology Laboratories Inc., Bothell, WA) with a 12-MHz probe was used to take single two-dimensional frames of brachial artery end-diastolic diameter, and the ultrasound images were recorded on a S-VHS videotape. After three consecutive measurements, the average value was calculated to find the baseline diameter of the vessel. Then, FMD was calculated by the formulae:100 (maximum diameter during blood flow in hyperemia- baseline diameter)/(baseline diameter). ELISA kit (Human soluble FasL/Bender MedSystems, BMS260GmbH, Vienna, Austria) was used to measure serum sFasL concentrations.

***Statistical analysis.*** All calculations were carried out using SPSS, version 16.0 (SPSS Inc, Chicago, IL, USA) for Windows. The normally distributed data were expressed as mean ± standard deviation (SD). Data without normal distribution were expressed as median (range). The Mann-Whitney test or Student's t-test were used to compare differences in numeric data. The $\chi^2$ test was used to compare differences in categorical data. Correlations between normally distributed variables were determined by Pearson's linear regression analysis. Correlations between variables without normal distribution were determined by Spearman's rank correlation analysis. A stepwise multiple regression analysis with a forward elimination procedure was performed to assess the influence of variables on endothelial function. Results were expressed with a regression coefficient of beta (β) in 95% confidence intervals (CI). A two-tailed p-value < 0.05 was considered to be statistically significant.

**Results.** The patients' baseline characteristics are shown in Table 1.

*Table 1*

**Baseline characteristics of the study population**

| | Patient Group (n=43) | Control Group (n=40) | p |
|---|---|---|---|
| Age (years, mean) | 41±14 | 50±12 | <0.01 |
| Gender (M, %) | 52 | 48 | 0.21 |
| FMD (%) | 3.95 ± 2.01 | 8,83 ± 6.17 | < 0.001 |
| sFasL (pg/ml) | 54 ± 24 | 73 ± 30 | 0.001 |
| CRP (mg/l) | 2.67 (0.08-8.17) | 0.50 (0.12-1.34) | 0.003 |
| BMI (kg/m$^2$) | 23.5 ± 4.33 | 28.8 ± 2.73 | < 0.001 |
| Hemoglobin (g/dl) | 11.6 ± 1.9 | 14.0 ±1.5 | < 0.001 |
| Creatinine (mg/dl) | 9.19 ± 3.4 | 1.11 ± 0.2 | < 0.001 |
| Uric acid (mg/dl) | 5.54 ± 1.07 | 6.64 ± 1.6 | < 0.001 |
| Sodium (mmol/L) | 135.15 ± 21.07 | 139.93 ± 2.99 | 0.159 |
| Potassium (mmol/L) | 4.42 ± 0.72 | 4.48 ± 0.35 | 0.595 |

*Continuation of Table 1*

| | Patient Group (n=43) | Control Group (n=40) | p |
|---|---|---|---|
| Calcium (mg/dl) | 10.43 ± 1.15 | 9.77± 0.42 | 0.721 |
| Phosphate (mg/dl) | 4.75 ± 1.61 | 3.35 ± 0.63 | < 0.001 |
| Magnesium (mmol/L) | 0.97 ± 0.25 | 0.84 ± 0.07 | 0.03 |
| iPTH (pg/ml) | 466 (36-891) | 68 (24-92) | < 0.001 |
| Albumin (g/dl) | 3.63 ± 0.44 | 4.18 ± 0.39 | < 0.001 |
| Total cholesterol (mg/dl) | 190.1 ± 60.5 | 199.3 ± 37 | 0.413 |
| LDL-cholesterol (mg/dl) | 117.6 ± 47.6 | 116 ± 32.1 | 0.861 |
| HDL-cholesterol (mg/dl) | 38.8 ± 11.2 | 47.2 ± 11.4 | < 0.01 |
| Triglycerides (mg/dl) | 192 (44-300) | 172 (54-290) | 0.376 |
| Glucose (mg/dl) | 87.5 ± 9.4 | 91.4 ± 7.5 | 0.042 |
| 24h systolic BP (mmHg) | 129 ± 9.8 | 120 ± 12.0 | < 0.01 |
| 24h diastolic BP (mmHg) | 78 ± 8.1 | 76 ± 8.4 | 0.423 |

Data are shown as mean±SD or median (range) depending on their distribution.

p value < 0.05 indicates statistical significance.

Abbreviations: BMI, body mass index; BP, blood pressure; FMD, flow-mediated vasodilation; HDL-cholesterol, high-density lipoprotein cholesterol, hsCRP, high sensitivity C-reactive protein; iPTH, intact parathyroid hormone; LDL-cholesterol, low-density lipoprotein cholesterol; sFasL, soluble Fas ligand.

Patients undergoing PD showed markedly lower forearm FMD and serum sFasL levels, whereas hsCRP showed markedly higher levels. The patient group had significantly lower means of BMI, uric acid, HDL cholesterol, and albumin than the control group. Both the calcium and phosphate were high with very high iPTH levels in the patient group. In each group, the 24hr consecutive readings of systolic and diastolic blood pressures were recorded. There was a clear difference for systolic blood pressure, but none for diastolic blood pressure. The forearm FMD, sFasL, hsCRP, iPTH and triglyceride values revealed nonuniform distribution in the patient group. The nonparametric Spearman's rank correlation analysis was used to assess the relationship between FMD, sFasL, hsCRP, iPTH, triglycerides and other variables.

Forearm FMD revealed direct, moderate and significant correlations with sFasL (r=0.289; p=0.008), age (r= 0,32; p=0.003), BMI (r= 0,32; p=0.003), Hb (r= 0,293; p=0.007), uric acid (r= 0,32; p=0.003), calcium (r= 0,26; p=0.016) and magnesium (r= 0,255; p=0.020). The inverse and strong correlations with creatinine (r=- 0.50; p<0.001) and iPTH (r=- 0,45; p<0.001) and inverse, moderate correlations with phosphate (r=- 0,250; p=0.023), albumin (r=- 0.251; p=0.022), 24 h SBP (r=- 0,257; p=0.019) were remarkable (Table 2).

*Table 2*

**Relationships between variables in the patient group of ESRD under PD treatment**

| | sFasL (pg/ml) r | FMD (%) r |
|---|---|---|
| FMD (%) | 0,289** | 1 |
| sFasL (pg/ml) | 1 | 0,289** |
| hsCRP (mg/dl) | -0,010 | -0,139 |
| Age (years) | 0,132 | 0,317** |
| BMI (kg/m$^2$) | 0,156 | 0,322** |
| Hemoglobin (g/dl) | 0,252* | 0,293** |
| Creatinine (mg/dl) | -0,342** | -0,501** |
| Uric acid (mg/dl) | 0,144 | 0,318** |
| Sodium (mmol/l) | -0,032 | 0,034 |
| Potassium (mmol/l) | -0,081 | 0,121 |
| Calcium (mg/dl) | 0,177 | 0,264* |
| Phosphate (mg/dl) | -0,188 | -0,250* |
| Magnesium (mmol/l) | -0,222* | 0,255* |
| iPTH (pg/ml) | -0,326** | -0,452** |
| Albumin (g/dl) | 0,154 | -0,251* |

*Continuation of Table 2*

| | sFasL (pg/ml) r | FMD (%) r |
|---|---|---|
| Total cholesterol (mg/dl) | -0,041 | -0,152 |
| LDL-cholesterol (mg/dl) | -0,023 | 0,12 |
| HDL-cholesterol (mg/dl) | 0,092 | 0,146 |
| Triglycerides (mg/dl) | -0,067 | -0,073 |
| Glucose (mg/dl) | 0,089 | 0,054 |
| 24h systolic BP (mmHg) | -0,145 | -0,257* |
| 24h diastolic BP (mmHg) | -0,094 | -0,136 |

Results written in bold indicate statistical significance *p < 0.05, and ** p< 0.01. 'r' represents the coefficient of correlation.

Abbreviations: BMI, body mass index; FMD, flow-mediated vasodilation; hsCRP, high sensitivity C-reactive protein; iPTH; intact parathyroid hormone; sFasL, soluble Fas Ligand.

Serum sFasL was inversely and moderately correlated with creatinine (r= 0,34; p=0.002), iPTH (r= 0,33; p=0.003), hemoglobin (r= -0,25; p=0.021) and magnesium (r= 0,22; p=0.043) (see Table 2). However, no correlation was observed between sFasL level and other variables. hsCRP did not correlate with sFasL or the forearm FMD (p=0,926 and p=0,211 respectively). Multiple regression analysis with a forward elimination procedure was used to assess the combined influence of variables on patients' endothelial function through forearm FMD. After adjustment for age, no association was observed between forearm blood flow and hsCRP, BMI, hemoglobin, creatinine, uric acid, phosphate, magnesium, iPTH and 24hr SBP. We observed that only sFasL was independently and associated to FMD ( = 0.18; p=0.03, CI:0.078-0.314) (Table 3).

*Table 3*

**Evaluation of the variables predicting plasma endothelial function through forearm FMD in PD patients**

| | Control | p value |
|---|---|---|
| sFasL | 0,347 | 0,03* |
| BMI (kg/m$^2$) | 0,278 | 0,285 |
| Glucose (mg/dl) | 0,456 | 0,729 |
| Creatinine (mg/dl) | 0,447 | 0,713 |
| Uric acid (mg/dl) | 0,974 | 0,421 |
| Phosphate (mg/dl) | 0,833 | 0,562 |
| Magnesium(mmol/l) | 0,431 | 0,699 |
| iPTH (pg/ml) | 0,774 | 0,723 |
| 24hr SBP (mmHg) | 0,763 | 0,755 |

*p < 0.05 represents statistical significance.

Abbreviations: BMI, body mass index; hsCRP, high sensitivity C-reactive protein; iPTH; intact parathyroid hormone, sFasL, soluble Fas Ligand; SBP, systolic blood pressure.

**Discussion.** In this study, we aimed to evaluate the relationship between FMD-which can be used to predict endothelial dysfunction-and classical risk factors/clinical parameters and serum FasL levels in chronic renal failure patients under peritoneal dialysis. Inflammation, protein-calorie malnutrition, high levels of uremic solutes and cardiovascular risk factors contribute to cardiovascular mortality in uremic patients. Data suggest that the burden of cardiovascular disease has already accompanied to the loss of renal function before patients start dialysis [1]. Endothelial function is accepted as a potential indicator of vascular risk factors [2]. A change in endothelial function from regulation by nitric oxide to the regulation by reactive oxygen species-dominated in ammatory environment leads to endothelial dysfunction. In endothelial dysfunction, the balance is changed from vessel relaxation to vessel contraction [2, 17, 18]. In ESRD patients, endothelium dysfunction might be associated with abnormal responses to shear stress changes [18]. Strong relationships were detected between mechanistically diverse risk factors and endothelial dysfunction [2]. Endothelial dysfunction is an early sign of atherosclerosis and reveals the increased cardiovascular mortality in patients with CKD 5D [1].

We examined endothelial function from the forearm conduit arteries of the peripheral circulation by high-resolution vascular ultrasound, which provided us with non-invasive, repeatable, and cheap measures. The inflation and subsequent deflation of a sphygmomanometer cuff on the distal forearm caused a physi-

ological stimulus on the brachial artery diameter by an increase in blood flow, which we assessed by percent change in FMD [17]. We observed that the response of forearm blood flow to reactive hyperemia (RH) was impaired in the PD patients compared to the response in the control subjects. We found that the FMD of the patients on PD was markedly lower than the FMD of the control subjects. We think the underlying reason for this is the alterations in endothelial function in patients with ESRD on dialysis. In these patients, the endothelium participates in inflammation and the alterations in the endothelial function cause morphological changes through the vessels, which later contribute to the development and progression of atherosclerosis [17].

Apoptosis is not only a physiologic process but also involved in pathologic conditions, as in the case of atherosclerotic lesions with a large number of apoptotic cells and apoptosis-related proteins [9]. Apoptosis is induced by a variety of extrinsic and intrinsic pathways by different molecules, which might have interconnections between them. The extrinsic is known as the death-receptor-initiated pathway of apoptosis with the engagement of plasma membrane death receptors on a variety of cells [19, 20]. One of the death receptors is the type 1 TNF receptor and a related protein called Fas (Apo-1 or CD95), which is expressed in many cell types. Fas has a specific ligand called the FasL, which is expressed on T cells, some cytotoxic lymphocytes, natural killer cells and vascular endothelial cells. The Fas, upon ligation by FasL (CD178), rapidly induces the apoptosis cascade [6-9, 19,20]. The Fas and FasL act as pro-inflammatory proteins and are related to inflammation as well as apoptotic responses in atherosclerotic plaques [5, 7]. Both Fas and FasL have soluble forms. The soluble Fas (sFas) is generated by the alternative splicing of a single gene. The soluble Fas ligand (sFasL) is generated by a metalloproteinase-like enzyme, which cleaves the Fas ligand from the cell surface [5-8, 19-21]. Soluble FasL can mediate non-apoptotic functions, including migration of inflammatory cells and cytokine responses, whereas its excessive amounts were detected to stimulate autoimmunity and tumorigenesis through sFasL-induced non-apoptotic activities. This has been explained by NF- B mediated stimulation of cell proliferation, survival and inflammation within an elevated cytokine milieu [20, 22]. It has been emphasized that sFasL-Fas play an important role in apoptosis in patients with ESRD. Recently, sFas has been reported to associate with coronary and peripheral atherosclerosis in patients with ESRD, whereas sFasL has been reported as a novel marker of endothelial dysfunction and vascular disease in atherosclerosis [5, 7-9, 21]. Reports of a population study detected that subjects at higher cardiovascular risk had markedly lower sFasL levels than the healthy subjects [5].

In ESRD, it was shown that the prevalence of inflammation was quite high in patients both prior to initiation of dialysis and in dialysis, and therefore inflammation might be a potential risk factor for cardiovascular morbidity and mortality [20]. In ESRD patients, the inflammatory marker of hsCRP may provide information about the impaired endothelial function, but it may not be exactly accepted as a marker of systemic inflammation affecting the vascular network [2].

In our study, we identified patients had some traditional and non-traditional risk factors. These factors were high blood pressure (15 of our patients had untreated essential hypertension), low serum HDL cholesterol and high triglyceride levels, male gender, anemia, high serum levels of calcium, phosphate, iPTH, and hsCRP. Among these risk factors, considerable differences between patients and control subjects were observed in 24 hr systolic blood pressure, serum hsCRP, hemoglobin, HDL cholesterol, phosphate, and iPTH levels (Table1), such that, the patients had the less favorable results.

The patients undergoing maintenance peritoneal dialysis had lower forearm FMD and serum sFasL values than the forearm FMD and serum sFasL values of control subjects. This finding was following the observation that the endothelial function is impaired in the brachial circulation in the presence of traditional and novel risk factors [2]. In ESRD, the uremic serum has been claimed to be apoptogenic as a result of retained uremic toxins, which might cause accelerated apoptosis with overexpression of Fas-Fas ligand present on the cell surface, and require serum sFasL to catalyze this expression [8]. The sFasL, by binding to Fas and specifically blocking the apoptotic activity of membrane-bound FasL may explain the low levels of serum sFasL levels in our patients. In the correlation analysis, we observed some cardiovascular and uremic risk factors were correlated with sFasL and FMD in the patient group, and also a direct, moderate correlation was observed between forearm FMD and sFasL.

The association between endothelial dysfunction and apoptosis was evaluated by comparing the FMD with sFasL level. Troyanov et al reported positive predictive results in terms of serum sFas levels and cardiovascular disease in ESRD patients [5, 7]. Hebert et al reported in 107 chronic hemodialysis patients that markedly higher plasma sFas but similar plasma sFasL levels were obtained in the patients with evidence of CAD compared to those without, and the sFas was obtained to be a novel marker of CAD by its independent association with CAD [23]. Supporting results of the above studies were obtained from patients in a wide spectrum, undergoing maintenance hemodialysis and peritoneal dialysis. These patients demonstrated significantly higher serum sFas levels without any difference in serum sFasL levels in comparison with the healthy control subjects [8].

Age, serum sFasL, uric acid, calcium and magnesium values, hemoglobin and the body mass index were directly and moderately correlated with the forearm FMD. It was remarkable that serum creatinine and iPTH levels showed strong, inverse correlations with FMD, whereas serum phosphate and albumin levels

and 24h SBP showed inverse and moderate correlations with forearm FMD. Serum creatinine, iPTH and magnesium levels and hemoglobin were inversely and moderately correlated with the serum levels sFasL.

The present study has several limitations. Residual kidney function is an important contributor to the advance of arteriosclerosis. However, we did not give any data about some important variables such as Kt/V, residual renal function, or creatinine clearance. Also, the detailed method of PD (APD or CAPD) and PD duration were missing. The small sample size can also be discussed. In this study, we only compared our PD patients with healthy controls. Including hemodialysis patients and CKD patients on pre-dialysis stages could also increase the scientific quality of the study.

**Conclusion.** In this observational cross-sectional study, despite the moderate and strong correlations between risk factors and FMD, only serum sFasL level showed an independent association with the forearm FMD. It can be reassessed, but the sFasL per se was significantly better than the classic risk factors at identifying endothelial dysfunction in PD patients. Our result suggests that sFasL may represent a novel and independent marker of endothelial dysfunction in PD patients. Although this situation suggests that apoptosis might pose a risk for cardiovascular diseases by acting on endothelial function in PD patients, this in itself is no proof of a causal relationship, as there may be a common third cause, such as having at least one or more of the risk factors which can impact endothelial dysfunction.

**Conflict of interest.** All the authors declared that they have no conflicts of interest.

**Funding.** None

**Authors contributions:**

***Bülent Huddam:*** the idea for the research and article/hypothesis generation, reagents, space and supervision;

***Alper Azak, Volkan Karakus:*** planning the methods;

***Alper Alp, Dilek Gibyeli Genek:*** supervision and responsibility for the project organization and the manuscript preparation;

***Meral Gülay Kadioglu Kocak, Yelda Dere, Murat Duranay:*** supplying financial resources, equipment, reagents, space, and personnel vital to the project;

***Dilek Ersil Soysal:*** English translation.

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