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Association between vascular calcification, atherosclerosis and inflammatory markers in end-stage renal disease patient and simple method for detecting vascular calcification (direct radiography)

Son dönem böbrek yetmezlikli hastalarda vasküler kalsifikasyon, ateroskleroz ve inflamatuar belirteçler arasındaki ilişki ve vasküler kalsifikasyonun saptanmasında basit bir yöntem (direkt radyografi)

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Abstract

Purpose: In our study, we planned to investigate the relationship of malnutrition with inflammation, atherosclerosis and calcification in dialysis patients.

Materials and methods: 140 Chronic kidney disease (CKD) patients and 44 healthy controls were included in the study. Carotid artery intima-media thickness (CIMT) was measured by doppler ultrasonography. Valvular calcification was assessed by echocardiography and vascular calcification scores (VCS) were done based on the radiograms. Biochemical parameters were assessed using routine laboratory methods. Subjective global assessment (SGA) was used to evaluate malnutrition.

Results: In the study, VCS showed no differences between hemodialysis (HD) and peritoneal dialysis (PD) patients (1.84±2.35 for HD, 1.77±1.64 for PD; *p*=0.83). CIMT, Osteopontin (OPN), interleukin-6 (IL-6) and homocysteine were significantly different in both dialysis groups compared to healthy controls. The Mean carotid intima-media thickness (m-CIMT) was higher in HD patients compared to PD group. CIMT, vascular calcification and SGA scores showed positive correlation with age, dialysis duration and valvular calcification grades, and negative correlation with albumin levels. A positive correlation between SGA scores and high-sensitive C-reactive protein (hs-CRP) levels was also noted. On multiple regression analysis, m-CIMT was independently associated with age, VCS and albumin levels. VCS was found to be independently associated with only albumin levels.

Conclusion: Vascular and valvular calcification, an indicator of cardiovascular mortality and morbidity in dialysis patients, was found to be significantly associated with malnutrition. We found higher rates of valvular calcification in patients with vascular calcification. Malnutrition was more prominent in these patients.

Keywords: Atherosclerosis, calcification, chronic renal failure, inflammation, malnutrition.

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Öz

Amaç: Çalışmamızda diyaliz hastalarında malnütrisyonun; inflamasyon, ateroskleroz ve kalsifikasyonla olan ilişkisini araştırmayı planladık.

Gereç ve yöntem: Çalışmaya 140 kronik böbrek yetmezlikli (KBH) hasta ve 44 sağlıklı kontrol dahil edildi. Karotis intima-media kalınlığı (KİMK) doppler ultrasonografi ile ölçüldü. Kapak kalsifikasyonu ekokardiyografi ile değerlendirildi ve direkt grafi ile vasküler kalsifikasyon skorları (VKS) hesaplandı. Biyokimyasal parametreler rutin laboratuvar yöntemleri kullanılarak değerlendirildi. Malnütrisyonun değerlendirilmesinde subjektif global değerlendirme skorlaması (SGA) kullanıldı.

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Bulgular: Çalışmada hemodiyaliz (HD) ve periton diyalizi (PD) hastaları arasında VKS açısından fark bulunmadı (HD için 1,84±2,35, PD için 1,77±1,64; *p*=0,83). KİMK, Osteopontin (OPN), interlökin-6 (IL-6) ve homosistein her iki diyaliz grubunda sağlıklı kontrollerle karşılaştırıldığında anlamlı olarak farklıydı. Ortalama karotis intima media kalınlığı (m-KİMK) HD hastalarında PD grubuna göre daha yüksekti. KİMK, vasküler kalsifikasyon ve SGA skorları yaş, diyaliz süresi ve kapak kalsifikasyon dereceleri ile pozitif, albümin seviyesi ile negatif korelasyon göstermekteydi. SGA skorları ile yüksek duyarlı C-reaktif protein (hs-CRP) seviyeleri arasında da pozitif yönde korelasyon kaydedildi. Çoklu regresyon analizinde m-KİMK bağımsız olarak yaş, VCS ve albümin seviyesi ile ilişkili bulunurken; VCS'nun bağımsız olarak sadece albümin seviyesi ile ilişkili olduğu bulundu.
Tartışma: Çalışmamızda diyaliz hastalarında kardiyovasküler mortalite ve morbiditenin önemli bir göstergesi olan vasküler-valvüler kalsifikasyonla malnütrisyonun önemli ilişkisinin olduğunu gördük. Vasküler kalsifikasyonu olan hastalarda valvüler kalsifikasyon belirgindi. Bu grup hastada malnütrisyon da belirgindi.
Sonuç: Diyaliz hastalarında kardiyovasküler mortalite ve morbiditenin bir göstergesi olan vasküler ve kapak kalsifikasyonunun malnütrisyon ile anlamlı ilişkisi bulundu. Vasküler kalsifikasyonu olan hastalarda daha yüksek oranda kapak kalsifikasyonu bulduk. Bu hastalarda malnütrisyon daha belirgindi.

Anahtar kelimeler: Ateroskleroz, kalsifikasyon, kronik böbrek yetmezliği, inflamasyon, malnütrisyon.

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Introduction

Accelerated cardiovascular calcification is one of the most important causes of morbidity and mortality in chronic kidney disease (CKD) [1]. Besides, increased levels of calcification stimulators such as hyperphosphatemia, hypercalcemia, increased levels of oxidized low-density lipoprotein (LDL)-cholesterol and hyperleptinemia, decreasing levels of calcification inhibitors like matrix G1a protein, fetuin-A, osteoprotegerin and osteopontin (OPN) play fundamental role in vascular calcification [2, 3].

The presence of plaques and increased intima-media thickness in the carotid arteries are strong predictors for cardiovascular events in general population [4]. Carotid artery intima-media thickness (CIMT) is a simple, reliable and non-invasive method, widely used in clinical trials for detecting asymptomatic atherosclerosis [5]. CIMT is increased in patients with renal failure and may help to predict patients that are at a higher risk of future cardiovascular events [5, 6].

In patients with end-stage renal disease (ESRD), malnutrition is a multifactorial condition with significantly poor clinical outcome [7, 8]. Malnutrition and cardiovascular diseases, especially atherosclerosis and related complications are linked to each other [8]. CKD is characterized by chronic inflammation which is responsible for the manifestation of malnutrition [9-11]. Malnutrition and conditions leading to excessive weight loss can cause inflammation [10]. Oxidative stress might be the main underlying cause in both conditions [12]. Inflammation in stage 5 CKD is multifactorial; impaired renal cytokine clearance, the presence of persistent infections such as oral cavity and gingival infections, vascular access infections, peritonitis, exogenous factors such as usage of bioincompatible dialysate, dialysis membranes and endotoxin exposure from contaminated dialysate especially in ESRD patients on renal replacement therapy (RRT) are the potential causes of inflammation [12]. Increased mortality and morbidity caused by this pathological condition is called malnutrition-inflammation-atherosclerosis (MIA) syndrome [8]. Pro-inflammatory cytokines play an important role in the pathogenesis of MIA syndrome [12]. Studies have shown that proinflammatory cytokines are 8 to 10 times higher in HD patients when compared to healthy controls [10-12].

OPN is produced by cells involved in bone morphogenesis and one major physiological role of OPN is to be in the control of biomineralization and calcification [13-15]. In addition, significant increase of OPN expression has been shown in smooth muscle cells of renal failure patients with vascular calcification [16, 17].

Prospective cohort studies conducted in stage 5 CKD patients have revealed the relationship between high levels of high-sensitivity C-reactive protein (hs-CRP), interleukin-6 (IL-6) and all cause-and cardiovascular cause-deaths [18-22].

Cohen et al. [21] stated that homocysteine elevation in renal insufficiency was first reported in 1977. Results of the studies on the role of hyperhomocysteinemia in dialysis patients and increased atherosclerosis vary. Two studies have shown that hyperhomocysteinemia was associated with increased cardiovascular morbidity in dialysis patients [23, 24]; while another study showed no correlation between increased homocysteine levels and cardiovascular events [25].

The aims of our study were: 1) to point out the importance of calcification (vascular and valvular), atherosclerosis, malnutrition and inflammation cascade on CKD 2) to identify markers that might affect atherosclerosis and vascular calcification 3) to figure out whether inflammatory markers and CIMT are different or not in dialysis groups compared to healthy controls of the same age group 4) to evaluate whether direct radiography, accessible and inexpensive method in routine practice, can be used in routine for vascular calcification scoring in these patients.

Materials and methods

In this cross-sectional single center study, after informed consent was obtained, stage 5 CKD patients treated with maintanence dialysis for at least 2 years period and healthy individuals were included. The patients were admitted from the department of Nephrology of Gazi University Medical Faculty, Ankara, Türkiye, from January to December 2007.

A total of 140 patients [78 under haemodialysis (HD); 62 under peritoneal dialysis (PD)], diagnosed with chronic renal failure and under renal replacement therapy for at least two years, and 44 healthy controls were included in the study. Firstly, we planned to include more healthy controls at least as much as the number of the patients in our study. However, we couldn't reach our goal finding that much healthy participants, given our strict conditions on these individuals which must not have any comorbidities (diabetes, thyroid diseases, hypertension, renal and cardiac diseases) and must be drug free. An other reason was the fact that as the CIMT measurements are made two times with one week intervals, it caused difficulty for some of the participants continue to the study. Subjects aged <18 or >70 years, those with underlying malignancy, chronic liver disease, autoimmune disease, current or recent (<1month) active infection, a catheter or graft as vascular access (for HD group) and those with history or symptoms of cardiovascular disease and cardiovascular instability (myocardial infarction, congestive heart failure, arrhythmia, peripheral vascular disease, transient ischemic attacks or cerebrovascular accidents) were excluded from the study. All patients were informed about the study and their written informed consent was obtained.

Measurement of the thickness of the carotid intima media

Patients were placed in a supine position, and their heads were positioned in extension. The right and left carotid arteries were imaged with a 7 MHz linear probe of an ultrasonic device ATL (HDI 5000, Philips Bothell WA). Intima media thickness was measured after identification of a 1 cm-segment without atherosclerotic plaque at the 2 cm proximal of the main carotid artery bulb. The measurement was done on the echogenic line formed by the lumen and media layer. Mean carotid intima-media thickness (m-CIMT) was calculated by arithmetic averages of the right and left CIMT values. The CIMT measurements were performed (without knowing real identity of the participitants) twice with one week intervals by the radiology specialist Serap Gültekin, MD. The value of CIMT was calculated by taking the average of the two measurements.

Vascular calcification scoring

Adragao et al. [22] stated that this scoring was based on the publications. Pelvic X-ray image was evaluated in four areas by dividing the image by the upper horizontal line passing between the apex of the two femur bones, and a median vertical line passing on the vertebral column. Similarly, hand X-rays were examined in two sections, divided by a horizontal line passing on the metacarpal bones. In each section, the presence of linear calcification was scored as 1 and non-presence as 0, and the total score was presented between 0-8. Linear calcifications along the iliac and femoral arteries in pelvic X-rays, and those along radial and digital arteries in hand X-rays were included in the scoring.

Patch calcifications, phleboliths, and extra-vascular calcifications were excluded. Vascular calcification scorings were calculated according to the references by a Nephrologist and a Radiologist, seperately. Without sharing their data with each other, the averages of the two calculations were taken.

Evaluation of valvular calcification

Echocardiographic examination was performed in the left lateral decubitus position using Vingmed System Five (GEVingmed Sound; Horten, Norway) echocardiography device and a 1.5-3.6 mHzultrasound probe. Parasternal long-, short-axis, and apical views were recorded. The valvular calcification evaluation was carried out by a Cardiologist, without knowing the real idendities of the patients. All echocardiographic examinations were performed according to the recommendations of the American Society of Echocardiography [26, 27]. Valvular calcifications were assessed as >1 mm-spot brightness on the valves. Calcification grades were as follows: mild (point calcification <3 mm), moderate (multiple calcium dots of >3 mm), severe (wide calcification area in valvular annulus, semilunar cusps or both) [28].

Blood analyses

Blood samples of PD patients and controls were taken between 08.00-10.00 in the morning, and at the same hours but before the HD session in HD patients following at least an 8 h-fasting period. Serum/plasma were separated, and stored at -80°C until analysed (not longer than 3 months). Blood cells counts, levels of urea, creatinine, uric acid, calcium, phosphorus, albumin, total cholesterol, triglyceride, high-density lipoprotein (HDL), low-density lipoprotein (LDL), very low-density lipoprotein (VLDL) cholesterol, and fasting blood glucose were measured using standard laboratory techniques. Serum intact parathyroid hormone (iPTH) levels were measured by immunoradiometric assay (IRMA) with a commercially available kit (BioSource, Nivelles, Belgium).

The hs-CRP levels were measured by the nephelometric method. Serum IL-6 Levels were measured by enzyme-linked immunosorbent assay (ELISA) using Human IL-6 kit (BIOSOURCE Immunoassay Kit, USA), and the results were expressed in pg/mL. Plasma OPN levels were measured by enzyme immunometric assay using Human OPN Enzyme Immunoassay kit (TiterZyme EIA, Kit-IBL [ImmunoBiological Laboratories] Co., Ltd., Japan), and results were expressed in ng/mL. Plasma homocysteine levels were measured by enzymatic assay using Homocysteine Enzymatic Assay kit (DIAZYME, USA), and results were given in micromol/L.

Nutrition status assessment

Kalantar Zadeh et al. [29] stated that malnutrition was assessed using the modified quantitative subjective global assessment (SGA) method defined. Scores up to 7 were considered as normal nutrition or mild malnutrition, while scores higher than 35 were defined as severe malnutrition [29]. Body mass indexes (BMI) of all patients and healthy controls were calculated by the formula, body weight (kg)/height(cm)^2.

Statistical analyses

The results were expressed as mean ± SD. In all cases, comparisons were two-tailed and *p*-value of <0.05 was considered statistically significant. Parametric variables (age, BMI, m-CIMT, OPN, IL-6, homocysteine, hs-CRP, haemoglobin, BUN, creatinine, uric acid, calcium, phosphorus, albumin, total, HDL and LDL-cholesterol) were evaluated by ANOVA and Tukey HSD tests. Non-parametric variables were examined by Chi-square test. For the binary group comparisons of the parametric variables, t-test; for OPN, IL-6, and hs-CRP values, due to large standard deviation, Mann-Whitney U test; and for correlation analyses Pearson and Spearman correlation tests were performed. Backward multiple linear regression analyses were performed to test the associations between several possible associated factors and CIMT and VCS evaluation scoring separately. The SPSS version 10.0 package program was used for all the baseline descriptive statistics, hypothesis tests and other analyses (SPSS, Inc., Chicago, IL, USA).

Results

The study group consisted of 78 HD (26 female, 52 male), 62 PD patients (32 female, 30 male) and 44 healthy controls (21 female, 23 male). Mean ages were 47.8±16.8 in the HD group, 45.9±13.2 in the PD group, and 45.1±12.3

years in the control group. Age distribution of the three groups were similar. As compared with PD group, HD group had higher smoking rate (32% vs 64%, respectively) (p=0.000). The dialysis pasts of HD patients were longer than of the PD patients (p=0.05). Demographic characteristics of patients and controls are given in Table 1. Significant differences were found in BUN, serum uric acid, triglyceride, serum albumin levels and BMI values when HD patients were compared with PD group (Table 2).

At the comparison of dialysis patients in terms of vascular calcification rates, the proportion of patients without calcification were higher in HD patiens than PD patients. Those with a calcification score above 5 were significantly higher in the HD group than PD group (p=0.001). More than the half of PD patients had a moderate VCS. The incidence of valvular calcification for both valves was higher in HD group compared to PD group (Table 3).

While the mean OPN (p=0.06), IL-6 (p=0.23) and homocysteine (p=0.17) levels of HD and PD patients did not show statistically significant difference, m-CIMT (p=0.000) values were significantly different in both groups (Table 2). When HD patients were compared with healthy controls, values of OPN (p=0.000), IL-6 (p=0.01), homocysteine (p=0.000) and m-CIMT(p=0.000) were significantly different. Similarly, when PD patients were compared with healthy controls, the values of OPN (p=0.000), IL-6 (p=0.01), homocysteine (p=0.000) and m-CIMT(p=0.000) were significantly different (Table 4).

Based on the modified SGA scoring method; on the HD group, 5 of the patients (6%) were normal, 71 of the patients (91%) were moderate and 2 of them (2.6%) were severely malnourished. On the PD group, 9 of the patients (14.5%) were normal and 53 of the group patients (85.5%) were moderately malnourished (p=0.05). When compared the two dialysis groups, the SGA score below 15 was 88% in PD group and 82% in HD group. In the current study, HD patients were found to be prone to malnutrition compared to PD patients.

Positive correlation was found between m-CIMT and age (p=0.000, r=0.35), dialysis period (p=0.04, r=0.14), VCS (p=0.004, r=0.23), SGA score (p=0.000, r=0.29), mitral valve calcification grade (p=0.002, r=0.26) and aortic valve calcification grade (p=0.008, r=0.22). Negative correlation was found between m-CIMT and between albumin (p=0.01, r=-0.18), calcium (p=0.05, r=-0.14) and phosphorus (p=0.04, r=-0.16) (Table 5). m-CIMT was independently associated with age (p=0.01, Beta:0.26), VCS (p=0.03, Beta:0.19) and albumin levels (p=0.01, Beta:-0.26) on multiple regression analysis (Table 6).

Positive correlation was found between VCS and age (p=0.01, r=0.20), dialysis period (p=0.001, r=0.27), m-CIMT (p=0.004, r=0.23), SGA score (p=0.001, r=0.27), mitral valve calcification grade (p=0.000, r=0.47) and aortic valve calcification grade (p=0.000, r=0.38). There was a negative correlation between VCS and albumin level (p=0.002, r=-0.25) (Table 5). VCS was independently associated with only albumin levels (p=0.02, Beta:-2.23) on multiple regression analysis (Table 6).

Patients' SGA scores showed positive correlation with age (p=0.000, r=0.45), duration of dialysis (p=0.000, r=0.34), m-CIMT (p=0.000, r=0.29), VCS (p=0.001, r=0.27), mitral valve calcification grade (p=0.000, r=.30), aortic valve calcification grade (p=0.003, r=0.24), hs-CRP (p=0.000, r=0.31); and negative correlation with albumin (p=0.004, r=-0.24), calcium (p=0.06, r=-0.13) and phosphorus (p=0.01, r=-0.21) (Table 5).

Duration of dialysis (p=0.01, r=0.2), phosphorus (p=0.001, r=0.27) and parathormone (p=0.000, r=0.35) showed positive correlation with OPN. Homocysteine levels showed positive correlation with uric acid (p=0.03, r=0.17), hs-CRP (p=0.009, r=0.22) and IL-6 (p=0.000, r=0.59). IL-6 levels had positive correlation with homocysteine (p=0.000, r=0.59), age (p=0.02, r=0.19) and hs-CRP (p=0.001, r=0.29).

Table 1. General characteristics of haemodialysis and peritoneal dialysis patients and controls

	HD group (n=78)	PD group (n=62)	Control group (n=44)
Male	52 [a,b]	30 [a,b]	23 [a]
Age (years)	47.8±16.8[c,d]	45.9±13.2[c,d]	45.1±12.3[c]
Dialysis duration (month)	69.76±41.10[e]	57.09±39.41[e]	-
Body mass index (kg/m²)	23.38±4.76[f,g]	25.21±5.67[f,g]	25.03±4.72[f]
Smokers (%)	50 (64.1%)[h,ı]	20 (32.3%)[h,ı]	15 (34.1%)[h]
Comorbidities			
Hypertension (%)	23 (29.5%)	20 (32.3%)	
Diabetes Mellitus (%)	14 (17.9%)	10 (16.1%)	
Glomerulonephritis (%)	15 (19.2%)	8 (12.9%)	
Nephrolithiasis (%)	2 (2.6%)	5 (8.1%)	
Amyloid (%)	3 (3.8%)	2 (3.2%)	
Polycystic renal disease (%)	1 (1.3%)	3 (4.8%)	
Pyelonephritis (%)	6 (7.7%)	1 (1.6%)	
Malignity (%)	1 (1.3%)	0	
Unknown (%)	13 (16.7%)	13 (21%)	

Data are presented as n (%) or mean±SD, HD, haemodialysis; PD, peritoneal dialysis, [a]*p*=0.07 (for all groups)
[b]*p*=0.02 (HD compared to PD group), [c]*p*=0.56 (for all groups), [d]*p*=0.46 (HD compared to PD group), [e]*p*=0.05 (HD compared to PD group)
[f]*p*=0.06 (for all groups), [g]*p*=0.04 (HD compared to PD group), [h]*p*=0.04 (for all groups), [ı] *p*=0.000 (HD compared to PD group)

Table 2. Laboratory findings of haemodialysis and peritoneal dialysis patients

	HD group (n=78)	PD group (n=62)	*p*
BUN (mg/dl)	67.16±16.19	55.38±16.33	0.000
Creatinine (mg/dl)	8.76±2.62	9.15±3.01	0.41
Kt/V	1.28±0.28	1.68±0.94	0.001
Calcium (mg/dl)	8.82±0.81	8.95±0.70	0.33
Phosphate (mg/dl)	5.30±1.32	5.07±1.45	0.32
PTH (pg/ml)	433.38±412.61	468.08±419.75	0.62
Uric acid (mg/dl)	6.07±0.80	5.55±0.88	0.000
Fasting blood glucose (mg/dl)	112.12±53.96	109.61±52.62	0.78
Haemoglobin (g/dl)	10.71±1.35	10.97±1.75	0.31
Total cholesterol (mg/dl)	173.75±42.92	185.01±34.83	0.09
HDL-cholesterol (mg/dl)	41.46±12.80	46.38±18.41	0.06
LDL-cholesterol (mg/dl)	96.28±34.75	105.29±28.85	0.10
Triglyceride (mg/dl)	203.80±137.27	161.41±59.45	0.02
Total protein (g/dl)	6.93±0.58	6.85±0.47	0.41
Albumin (g/dl)	4.13±0.46	3.98±0.37	0.03
hs-CRP(mg/dl)	0.77±1.13	0.85±0.86	0.14
Osteopontin (ng/ml)	33.42±18.44	28.26±20.65	0.06
IL-6 (pg/ml)	10.42±23.80	6.02±11.60	0.23
Homocysteine (µmol/L)	29.57±11.78	26.88±11.30	0.17
m-CIMT (mm)	1.01±0.30	0.82±0.29	0.000
VCS	1.84±2.35	1.77±1.64	0.83
SGA	11.79±5.43	10.93±3.16	0.27
BMI (kg/m²)	23.38±4.76	25.21±5.67	0.04

Data are mean values±SD, BMI: Body Mass Index, BUN: Blood Urea Nitrogen, hs-CRP: high sensitive C reactive protein
HD: haemodialysis, Kt/V: fractional urea clearance, m-CIMT: mean carotid intima-media thickness
PD: peritoneal dialysis, SGA: subjective global evaluation score, VCS: vascular calcification score

Table 3. Vascular and valvular calcification values in haemodialysis and peritoneal dialysis patients

	HD Group n (%)	PD Group n (%)
Vascular calcification[a]		
None	39 (50%)	18 (29%)
Score between 1-4	27 (34.6%)	41 (66.1%)
Score ≥5	12 (15.4%)	3 (4.8%)
Mitral valve calcification present[b]	48 (61.5%)	26 (41.9%)
Atrioventricular valve calcification present[c]	28 (35.9%)	12 (19.4%)

HD: haemodialysis, PD: peritoneal dialysis, [a]p=0.001 HD compared to PD group, [b]p=0.02 HD compared PD group, [c]p=0.03 HD compared to PD group

Table 4. Osteopontin, interleukin-6, and homocysteine levels, and mean carotid artery intima-media thickness of haemodialysis and peritoneal dialysis patients and controls

	HD group (n=78)	PD group (n=62)	Control group (n=44)
Osteopontin (ng/ml)	33.42±18.44[a,x]	28.26±20.65[a]	9.58±8.88
IL-6 (pg/ml)	10.42±23.80[b,y]	6.02±11.60[b]	0.69±2.63
Homocysteine (µmol/L)	29.57±11.78[a,z]	26.88±11.30[a]	9.61±3.25
m-CIMT (mm)	1.01±0.30[a,c]	0.82±0.29[a]	0.184±0.25

Data are mean values ± SD, [a]p=0.000 compared to controls, [b]p=0.01 compared to controls [c]p=0.000 compared to PD group
[x]p=0.06 HD compared to PD group, [y]p=0.23 HD compared to PD group, [z]p=0.17 HD compared to PD group
m-CIMT: mean carotid artery intima-media thickness, IL-6: interleukin-6, HD: haemodialysis, PD: peritoneal dialysis

Table 5. Correlations of m-CIMT, VCS and SGA values with study parameters

	m-CIMT		VCS		SGA	
Variables	**r**	***p***	**r**	***p***	**r**	***p***
Age	0.35	0.000	0.20	0.01	0.45	0.000
Dialysis period	0.14	0.04	0.27	0.001	0.34	0.000
m-CIMT			0.23	0.004	0.29	0.000
VCS	0.23	0.004			0.27	0.001
SGA	0.29	0.000	0.27	0.001		
MV calcification	0.26	0.002	0.47	0.000	0.30	0.000
AV calcification	0.22	0.008	0.38	0.000	0.24	0.003
Albumin	-0.18	0.01	-0.25	0.002	-0.24	0.004
Calcium	-0.14	0.05	-0.01	0.82	-0.13	0.06
Phosphorus	-0.16	0.04	0.06	0.46	-0.21	0.01
Uric acid	0.07	0.41	0.12	0.06	0.17	0.03
Osteopontin	-0.06	0.47	-0.11	0.17	0.03	0.72
IL-6	0.14	0.09	0.12	0.08	0.09	0.27
Homocysteine	0.14	0.09	0.07	0.40	0.04	0.96
hs-CRP	0.14	0.08	0.13	0.16	0.31	0.000

AV: atrioventricular valve, m-CIMT: mean carotid artery intima-media thickness, hs-CRP: high sensitive C reactive protein, IL-6: interleukin-6
MV: mitral valve, SGA: Subjective global evaluation score, VCS: vascular calcification score

Table 6. Multiple regression analyses of factors affecting m-CIMT and VCS

Dependent	Independent	Beta	*p*
m-CIMT	Age	0.26	0.01
	VCS	0.19	0.03
	Albumin	-0.26	0.01
VCS	Albumin	-2.23	0.02

VCS: vascular calcification score, m-CIMT: mean carotid artery intima-media thickness

Discussion

In our study, m-CIMT levels were found significantly higher in HD patients compared to PD patients and healthy controls. Levels of m-CIMT were also higher in PD patients than healthy controls. Recent studies have shown that m-CIMT is an independent marker of cardiovascular mortality in HD patients [30, 31]. One of the reasons of the higher levels of m-CIMT in HD patients might be the better protection of PD patients for their residual renal function (RRF). This claim would become more important if RRF of PD patients could have been evaluated in our study. Rroji et al. [32] stated that RRF is an independent predictor for atherosclerosis proposed. Dialysis duration of HD patients were longer than those of PD patients. That could be explanation of the increased atherosclerosis in the HD group. The high smoking rate of HD patients may also play a role for this difference. Smoking is a well-documented cardiovascular risk factor [33, 34].

There is also a positive link between age and atherosclerosis. Preston et al. [35] stated that m-CIMT increases with age and LDL-cholesterol, and decreases with HDL-cholesterol. In our study, CIMT values significantly increased with increasing age. Majority of studies showed negative correlation between m-CIMT and albumin, and positive correlation between m-CIMT and CRP [29-31]. In the present study, a considerable negative correlation was established between the m-CIMT and albumin, as cited at previous studies. Though there are studies in the literature reporting the correlation between higher inflammatory levels and m-CIMT, we could not confirm this in our study. We found that m-CIMT values were associated with age, VCS and albumin levels in dialysis patients. There may be many reasons why we did not find a direct relationship between m-CIMT, and inflammatory parameters; atherosclerosis is a chronic pathology and aging and long dialysis time may be more predictive factors for atherosclerosis. Perhaps malnutrition plays a more important role in atherogenesis than inflammation. Inflammation may trigger malnutrition, leading to atherosclerosis.

Although the relationship between calcification and inflammation has been shown in many studies [36, 37], we could not present significant association between calcification scores and the two inflammation markers, IL-6 and hs-CRP. In contrast, there was a significant linear relationship between vascular calcification grades and clinical (i.e. SGA), and laboratory findings (*i.e.* albumin) of malnutrition. SGA and m-CIMT were significantly associated in patients with vascular calcification. Therefore, it can be assumed that the effect of inflammation on vascular calcification may be via indirect pathways. As in our patient groups, inflammation might lead to vascular calcification by triggering malnutrition.

In our study, higher SGA scores were associated with positively correlated m-CIMT and vascular calcification. We also observed that malnutrition was more pronounced and severe in elderly long-duration dialysis patients. We found that malnutrition was associated with higher hs-CRP, however, we could not confirm that with IL-6 levels. While no significant association was found between SGA scores and IL-6, the observation of a positive association with hs-CRP contradicts the knowledge that IL-6 is a more sensitive cytokine than other cytokines [36]. At the previous studies, high levels of hs-CRP and IL-6 were proposed to be the best predictors of malnutrition in end stage renal failure patients, even IL-6 was proposed to be a better predictor particularly on cardiovascular events and mortality [19, 20]. At present, proinflammatory cytokines, which can be used for different diagnostic purposes, are influenced by many factors, and are not sufficient to distinguish the relationship between vascular

calcification, atherosclerosis and malnutrition. Moreover, since this study is not a prospective mortality and morbidity study, it is impossible to anticipate which cytokine is more closely related to malnutrition, and what could be its future consequences. However, the close association between the inflammation indicator hs-CRP and malnutrition score reminds once again the tight link between inflammation and malnutrition. Many publications reported a linear relationship between inflammation and malnutrition [7-9, 36-42]. We have also achieved similar results.

OPN levels were significantly higher in dialysis groups compared to healthy controls. The expression of OPN increases in case of hyperphosphatemic, parathyroid hormone-elevated, and calciphylaxed vascular bed [43]. The role of OPN on the development of atherosclerosis is unclear. Because of its role in the pathogenesis of vascular calcification, the relationship between plasma OPN levels and m-CIMT was investigated in patients with essential hypertension, and it was found that there was a positive correlation between the two [44]. We did not find a significant relationship between m-CIMT and OPN. Duration of dialysis, higher phosphorus and parathormone levels showed positive correlation with OPN. Nitta et al. [45] stated that in a study in 2001, reported a relation between high levels of OPN and presence of vascular calcification in HD patients but we could not confirm this relation in our study.

In our study, we found increased levels of homocysteine in CKD patients. Renal failure is a well-known cause of hyperhomocysteinemia [46]. Yet, it is not clear whether hyperhomocysteinemia is an independent risk factor for atherosclerosis. IL-6 and hs-CRP, predictors of inflammation, were positively corralated with homocysteine levels. Similar to our results, previous studies confirmed that elevated inflammation is associated with hyperhomocysteinemia [47]. We did not find significant relation between m-CIMT, vascular calcification and homocysteine levels in our study. The results of studies emphasizing the relationship between m-CIMT and homocysteine levels are also contradictory. Similar findings to our results could be found in the literature [48].

IL-6 levels were also significantly higher in dialysis patients than in healthy controls. We did not find significant association between m-CIMT, VCS, and SGA and IL-6 levels. It has been shown that IL-6 is a good predictor of mortality in dialysis patients [10, 20], and that there is positive correlation between m-CIMT and IL-6 [49]. IL-6 and CRP levels are 8 to 10 times higher in dialysis patients [41]. In our patient group, IL-6 levels were 6 to 10 times higher than the healthy population, supporting the literature. In our study, there was a strong association between IL-6 and hs-CRP. Elevated hs-CRP levels significantly predicted malnutrition in dialysis groups. However, we could not show positive correlation between malnutrion and calcification. No significant correlation between m-CIMT and IL-6 might suggest a weak relationship between inflammation and atherosclerosis for our patient group. As mentioned previously, although the relationship between m-CIMT and IL-6 has not been fully clarified, IL-6 is a strong predictor of mortality for dialysis patients. Prospective follow-up of our dialysis population would be needed to test the accuracy of this information.

In our study, we did not see the relationship between osteopontin and vascular calcification, which has been shown in some other studies. Again, although IL-6 and homocysteine levels were higher in our dialysis patients than in healthy controls, we did not find a significant relationship between them and CIMT and calcification. We observed that hs-CRP, which we frequently use in daily practice, is higher especially in dialysis patients with significant malnutrition.

Study limitations: RRF has a major influence upon the efficacy of m-CIMT. If we knew the RRF of PD patients, we could have more detailed explanations about the different m-CIMT measurements between PD and HD patients. Of course, it would have been more valuable if the number of our patients was more and we could reach a conclusion by making repetitive measurements of these patients over the years. Demonstrating vascular calcification and atherogenesis with more detailed methods would also strengthen our study.

In conclusion, inflammation is an important factor triggering malnutrition in dialysis patients.

Moderate to severely malnourished dialysis patients have vascular/valvular calcification and atherosclerosis is more common. Direct radiography, which is an easy and inexpensive method in routine practice, can be used to predict vascular calcification in these patients. Moreover, there is a strong association between vascular and cardiac valve calcification. Both atherosclerosis and calcification are more common in dialysis patients with low serum albumin levels. Comprehensive nutritional treatment protocols should be established and implemented for these patients.

Conflicts of interest: No conflict of interest was declared by the authors.

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Authors' contributions to the article

R.M. and U.D. conceived the idea, R.M., E.I.S. and C.K.D. collected the data. C.M. and N.T. performed the calculations, data analysis and created tables. S.G. evaluated direct radiographs. G.T. evaluated echocardiograms. S.G. made and evaluated laboratory measurements. R.M. wrote the manuscript with input from all authors. All authors discussed the results, reviewed and commented on the manuscript.