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Which one is the predictor of carotid intima media thickness in patients with glomerulonephritis; neutrophil-to-lymphocyte ratio or proteinuria?

Glomerülonefritli hastalarda karotis intima media kalınlığının prediktörü hangisidir; nötrofil-lenfosit oranı mı proteinüri mi?

Aysun Toraman, Berna Eren, Fatih Düzgün, Fatma Taneli, Seyhun Kürşat

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Abstract

Purpose: Glomerulonephritis is inflammation of the glomeruli and proteinuria itself is a reflection of the glomerular inflammation. In this study we aimed to investigate the relationship between carotid intima media thickness (CIMT) one of the earliest findings of atherosclerosis and markers of glomerular inflammation.

Materials and Methods:40 patients with biopsy proven glomerulonephritis and 46 healthy controls were enrolled in the study. Human Growth arrest spesific protein 6 (Gas6) levels in serum samples were studied by ELISA. CIMT measurement was performed by the same radiologist. Neutrophil-lymphocyte ratio (NLR) was calculated by dividing the number of neutrophils by the number of lymphocytes.

Results: The mean age was 42.88±15.41 in patient group and 38.26±9.04 in controls. The mean duration of illness was 29.07±52.90 mounts, proteinuria was 4027.05±4030.22 mg/day, Modification of Diet in Renal Diseases Study Glomerular Filtration Rate (MDRD-GFR) was 53.80±48.32 mL/min/1.73m2. CIMT was 0.62±0.17 mm in patient group, 0.46±0.10 mm in controls. Neutrophil-to-Lymphocyte Ratio (NLR) was 3.69±4.46 in patient group and significantly higher than control group (1.74±0.63). Gas6 levels were statistically higher in control group. CIMT was positively correlated with age, fibrinogen, ferritin, proteinuria and NLR and negatively correlated with HDL cholesterol and Gas6 in glomerulonephritis group. Age was the predictor for CIMT in the logistic regression model. In all group CIMT was positively correlated with age, creatinine, uric acid, fibrinogen, ferritin, CaxP product, proteinuria and NLR and negatively correlated with hemoglobin level, Gas6. In lineer logistic regression analysis carotid IMT was significantly associated with age and ferritin and proteinuria.

Conclusion: We have shown that proteinuria is one of the main determinants of increased CIMT independent from the GFR levels in glomerulonephritis with relatively preserved glomerular filtration rate.

Key Words: Carotid ıntima media thickness, glomerulonephritis, neutrophil-to-lymphocyte ratio, growth arrest specific protein 6, proteinuria.

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Özet

Amaç: Glomerülonefrit glomerüllerin inflamasyonu olup proteinüri glomerüler inflamasyonun klinik bir yansıması olarak karşımıza çıkar. Bu çalışmada, aterosklerozun en erken bulgularından biri olan karotis intima media kalınlığı (KIMK) ile glomerüler inflamasyon belirteçleri arasındaki ilişkiyi araştırmayı amaçladık.

Gereç ve Yöntem: Biyopsi ile kanıtlanmış 40 glomerülonefrit hastası ve 46 sağlıklı kontrol çalışmaya dahil edildi. Serum büyüme durdurucu spesifik protein 6 (Gas6) düzeyleri ELISA ile çalışıldı. KIMK ölçümü aynı radyolog tarafından yapıldı. Nötrofil-lenfosit oranı (NLO) tam kan test sonucundan elde edilen nötrofil sayısının lenfosit sayısına bölünmesiyle hesaplandı.

Bulgular: Hasta grubunda ortalama yaş 42,88±15,41, kontrol grubunda 38,26±9,04 di. Ortalama hastalık süresi 29,07±52,90 ay, proteinüri 4027,05±4030,22 mg/gün, Modification of Diet in Renal Diseases Çalışması-Glomerüler Filtrasyon Hızı (MDRD-GFH) 53,80±48,32 mL/dk/1,73 m2 idi. KIMK hasta grubunda 0,62±0,17 mm, kontrol grubunda 0,46±0,10 mm idi. NLO hasta grubunda 3,69±4,46 olup kontrol grubundan anlamlı derecede yüksekti (1,74±0,63). Kontrol grubunda Gas6 düzeyleri istatistiksel olarak anlamlı yüksek saptandı. KIMK glomerülonefrit grubunda yaş, fibrinojen, ferritin, proteinüri ve NLO ile pozitif korelasyon, HDL kolesterol ve Gas6 ile negatif korelasyon gösterdi. Lojistik regresyon modelinde yaş KIMK için belirleyiciydi. Tüm grupta KIMK

Aysun Toraman, Assistant Professor. Division of Nephrology, Department of Internal Medicine University of Celal Bayar, MANİSA, e-mail: aystoraman@hotmail.com (orcid.org/0000-0003-2290-3342) (Sorumlu yazar)

Berna Eren, Specialist of Nephrology, Department of Internal Medicine University of Celal Bayar, MANİSA, e-mail: bernagursel@yahoo.com (orcid.org/0000-0003-1459-3663)

Fatih Düzgün, Assistant Professor, Celal Bayar University, Department of Radiology, MANİSA, e-mail: fatihdzgn@icloud.com (orcid.org/0000-0003-2199-0640)

Fatma Taneli, Professor, Celal Bayar University, Department of Biochemistry, MANİSA, e-mail: fatmataneli@gmail.com (orcid.org/0000-0002-5194-0460)

Seyhun Kürşat, Professor, Celal Bayar University, Department of Internal Medicine, Division of Nephrology, MANİSA, e-mail: seyhun.kursat@gmail.com (orcid.org/0000-0001-9437-8474)

yaş, kreatinin, ürik asit, fibrinojen, ferritin, CaxP çarpımı, proteinüri ve NLO ile pozitif ve hemoglobin düzeyi, Gas6 ile negatif korelasyon gösterdi. Lineer lojistik regresyon analizinde karotis IMK, yaş ve ferritin ve proteinüri ile anlamlı şekilde ilişkiliydi.
Sonuç: Çalışmamızda proteinürinin glomerüler filtrasyon hızı göreceli olarak korunmuş glomerülonefritte GFH düzeylerinden bağımsız olarak artmış KIMK'nın temel belirleyicilerinden biri olduğunu gösterdik.

Anahtar Kelimeler: Karotis intima media kalınlığı, glomerülonefrit, nötrofil-lenfosit oranı, growth arrest specific protein 6, proteinür**i.**

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Introduction

Glomerular diseases can result from many inherited or acquired disorders and can manifest in different forms, ranging from asymptomatic urinary abnormalities to acute kidney injury or end-stage renal disease. Glomerulonephritis is one of the most important causes of chronic kidney failure after diabetes and hypertension. A kidney biopsy is often required to diagnose the underlying pathology in patients with suspected glomerular disease, particularly in those with nephrotic/nephritic syndrome or suspected glomerulonephritis. Clinical manifestations associated with glomerular disease include hematuria and/or proteinuria, renal insufficiency, hypertension, edema, hypercoagulability and systemic findings related to underlying disease.

It has been shown in recent studies that the risk of atherosclerosis increases from early stages in chronic renal failure. Chronic inflammation also plays a significant role in the pathogenesis of atherosclerosis. Neutrophil-to-lymphocyte ratio (NLR) which calculated by dividing the number of neutrophils by number of lymphocytes, is a novel simple and inexpensive marker ofsubclinical inflammation in end stage renal disease and chronic diseases and cardiovascular diseases. Li et al. [3] have shown that higher NLR in hemodialysis patients was a predictor of increased intima-media thickness, pulse pressure and left ventricular mass index. Glomerular proteinuria observed during the course of glomerulonephritis is another indicator of inflammation. It is well known that long-term exposure of protein to renal tubular cells results in apoptosis and fibrosis [4, 5]. Growth arrest spesific protein 6 (Gas6) is a member of vitamin-K dependent proteins family. Gas6 is known to protect endothelial cells and vascular smooth muscle cells (VSMCs) against apoptosis by inhibiting BAD and Bcl-2 induced Caspase 3 activation in apoptotic pathway [6]. There are conflicting results regarding proteinuria and Gas6 relation in diabetic patients. In a study Li et al. [7] showed that Gas6 levels were lower then controls in diabetic macroalbuminuric patients although there was a moderate increase in creatinine levels and no relation was found between creatinine and Gas6 values. Erek-Toprak et al. [8] showed that plasma Gas6 levels were higher in diabetic patients with micro or macroalbuminuria compared to diabetic patients with normoalbuminuria and healthy controls. Hyde et al. [9] have shown that the up-regulation of Gas6/Axl signalling has a protective mechanism which reduces tubulo-interstitial apoptosis and slows progression to end stage renal failure in the partial nephrectomy and high phosphate diet model of mice.

In present study we aimed to show whether there was a relation between carotid IMT as a predictor of asymptomatic atherosclerosis and vascular calcification andGas6, NLR, proteinuria and other markers of inflammation in patients with glomerulonephritis.

Materials and methods

Fourty patients with biopsy proven glomerulonephritis and 46 healthy controls were enrolled in the study. Control group consist of individuals who have no history of cardiac disease and kidney failure as well as matched individuals in terms of age and gender.

Patients with documented atherosclerotic cardiac artery disease, periferic artery disease and related symptoms were not included in the study. Those individuals with suspected acute renal failure, pregnant women, malignancy, infectious symptoms and hemodynamically unstable conditions were not included in the study.

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee at which the studies were conducted (IRB approval number 20478486-317) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Laboratory analysis

All patients' blood and urine samples were collected at 8 am after 12 hours of fasting. In dialysis patients blood samples were collected at midweek before the second dialysis session. Gas6 blood samples were taken into anti-coagulant free tubes and turned at a speed of 4000 rev/minutes for 7 minutes, after serum were separated, whole blood samples were stored at-80 ^{0}C.Human Gas6 levels in serum samples were studied by ELISA method with Shanghai YL Biotech Co., Jiading District, Shanghai kits. Intra-assay precision values <8%, inter- assay precision values <10 respectively. The readable range of the kit is 0.5-200 ng / mL and the sensitivity is 0.25 ng/mL.

Carotid intima-media thickness measurement

Carotid IMT measurement of all individuals participating in the study was performed by the same radiologist. For all patients, bilaterally one at the main carotid artery (2 cm proximal to bulb), one at the bulb level andone at the level of each of internal carotid artery measurements were performed and mean values were calculated. No measurement was performed locations where atherom plaque was seen.

Statistical analysis

SPSS for Windows version 15 Statistics was used for data input and statistical analysis. Normally distributed numerical parameters were expressed as mean±SD (standard deviation) and analyzed with parametric tests as analysis of variance (ANOVA: for more than two variables) and Student's t-test (for two variables). If both variables have continous data, again considering distribution of variable, parametric (Pearson r) or non-parametric (Spearmen p) correlation tests were used. Statistical significance was determined as p<0.05. Forward logistic regression analysis was performed to determine the model of independent predictors for CIMT.

Results

Baseline characteristics

Our study included 40 glomerulonephritis patients with mean age of 42.88±15.41 years and 46 individuals of control group with mean age of 38.26±9.04years.When the etiology of glomerulonephritis is examined; 12 patients had focal segmental glomerulosclerosis (FSGS), 9 patients had systemic lupus erythematozis (SLE), 6 patients had IgA nephropathy, 5 patients had amyloidosis, 4 patients had membranous GN, 2 patients had minimal change disease (MCD) and 2 patients had membranoproliferative glomerulonephritis (MPGN). Hyperlipidemia and hypertension ratios were higher in patients with glomerulonephritis than control group. The mean duration of illness was 29.07±52.90 months. Demographic characteristics of groups were given in Table 1.

In patient group there were 12 (30%) patients at stage I CKD, 6 (15%) patients at stage II CKD, 6 (15%) patients at stage III CKD, 3 (7.5%) patient at stage IV CKD and 13 (32.5%) patients at stage V CKD.

Laboratory parameters and correlations with CIMT and NLR

The serum creatinine, fibrinogen, CRP, ferritin, Ca, P, CaxP product and PTH parameters as indicators of inflammation and atherosclerosis were higher in glomerulonephritis group than control group. Carotid IMT as an early sign of atherosclerosis was 0.62±0.17 mm in patients with glomerulonephritis and 0.46±0.10 mm in the control group. NLR was 3.69±4.46 in patient group and significantly higher than control group (1.74±0.63). Gas6 levels were statistically higher in the control group than in the patient group. The comparative laboratory parameters of the groups were given in Table 2.

As seen in Table 3, CIMT was positively correlated with age, fibrinogen, ferritin, proteinuria and NLR and negatively correlated with HDL cholesterol and Gas6 in glomerulonephritis group. Age was the predictor for CIMT in the logistic regression model (β=0.637, t=4.680, p<0.001).There was a positive correlation between NLR and age, uric ascite, CRP and CIMT. Gas6 was negatively correlated with age, proteinuria and CIMT (Table 3).

Table 1. Demographic characteristics of groups.

	Control Mean ±SD or % (n) (n=46)	Glomerulonephritis (n=40)		*p* value
Age (year)	38.26±9.04	42.88±15.41		0.193
Sex (%M)	52.2	50.0		0.867
Hypertension	13.0	40.5		**0.001**
Hyperlipidemia	17.3	71.4		**0.02**
Cigarette	43.5	56.3		0.292
SBP (mmHg)	122.95±11.74	123.57±17.91		0.883
DBP (mmHg)	77.34±9.04	74.88±10.02		0.330
CKD duration	-	29.07±52.90		-
BMI (kg/m^2)	25.81±4.30	25.24±3.67		0.578
Etiology	-	Ig A nephropathy(%)	15	-
		Lupus	22.5	
		Focal segmental glomerulosclerosis	30	
		Minimal change disease	5	
		Amiloidozis	12.5	
		Membranous GN	10	
		MPGN	5	

Tablo 2. Comparative laboratory finding of all groups.

	Control (n=46) (Mean ±SD)	Glomerulonephritis (n=40)	*p* value
WBC (K/µL)	6441.30±1528.36	7687.00±2544.99	0.036
Hb (gr/dl)	14.17±1.69	10.97±1.52	<0.001
Creatinine (mg/dl)	0.78±0.16	2.99±2.51	<0.001
Albumin (g/dl)	4.29±0.39	3.02±0.93	<0.001
Uric ascite (mg/dl)	5.30±1.70	6.72±2.35	0.013
Fibrinogen	293.23±81.90	461.54±195.82	<0.001
CRP (mg/dl) (0-5)	2.51±1.24	13.58±1.68	0.007
Total cholesterol (mg/dl)	195.73±37.41	243.33±117.63	0.065
LDL (mg/dl)	118.82±27.92	146.72±63.07	0.049
TG (mg/dl)	144.13±100.91	183.32±115.49	0.180
HDL (mg/dl)	47.60±11.20	48.25±16.40	0.868
Calcium (mg/dl)	9.40±0.60	8.97±0.86	0.036
Phosphor (mg/dl)	3.62±0.51	4.65±1.23	<0.001
Ca-P product (mg/dl)	34.14±5.61	42.03±12.29	0.005
MDRD-GFR (ml/dk)	105.85±20.86	53.80±48.32	<0.001
PTH (pg/ml)	57.13±26.87	191.02±226.01	0.006
ALP (IU/L)	74.90±26.16	99.20±115.77	0.348
Ferritin (ng/dl)	47.34±38.09	153.02±206.87	0.021
Proteinuria (mg/day)	62.15±47.80	4027.05±4030.22	<0.001
Gas6 (ng/mL)	98.84±53.32	66.28±45.37	0.013
CIMT	0.46±0.10	0.62±0.17	<0.001
NLR	1.74±0.63	3.69±4.46	0.010

(WBC: White bloodcount, Hb:hemoglobin,CRP:C-reactive protein, MDRD-GFR: Modification of diet in renal Deseases Glomeruler filtration rate, PTH: parathormone, ALP: Alkaline phosphatase)

Table 3. The correlation of demographic and laboratory parameters with CIMT, NLR and Gas6 inpatient with glomerulonephritis (n=40).

	CIMT		GAS6		NLR	
	r value	*p* value	r value	*p* value	*r* value	*p* value
Age	**0.666**	**<0.001**	**-0.329**	**0.041**	**0.311**	**0.043**
Smoking years	0.198	0.209	-0.057	0.719	0.025	0.880
SBP	**-0.346**	**0.025**	0.330	0.040	0.004	0.981
DBP	-0.156	0.324	0.085	0.592	-0.229	0.155
BMI	0.204	0.196	-0.214	0.173	0.041	0.801
Hb	-0.175	0.266	-0.091	0.568	-0.152	0.350
Creatinine	0.159	0.315	0.114	0.471	0.022	0.896
Albumin	-0.313	0.044	0.086	0.586	-0.011	0.946
Uric ascite	0.224	0.159	0.014	0.932	**0.310**	**0.045**
Fibrinogen	**0.363**	**0.032**	-0.221	0.201	0.195	0.270
CRP	0.092	0.600	-0.002	0.992	**0.320**	**0.039**
T cholesterol	0.245	0.128	-0.087	0.593	-0.177	0.286
LDL	0.187	0.249	-0.168	0.300	-0.263	0.110
TG	**0.307**	**0.054**	**-0.401**	**0.014**	-0.016	0.926
HDL	**-0.321**	**0.044**	0.317	0.056	-0.132	0.430
Ca	0.023	0.884	-0.255	0.107	-0.123	0.456
P	0.236	0.142	-0.193	0.232	0.168	0.314
MDRD-GFR	-0.188	0.234	-0.112	0.481	-0.008	0.962
PTH	0.068	0.682	0.331	0.049	0.062	0.716
Ferritine	**0.311**	**0.045**	0.084	0.595	0.198	0.220
Proteinuria	**0.370**	**0.016**	**-0.297**	**0.021**	-0.253	0.065
Gas6	**-0.439**	**0.005**		-	0.140	0.388
CIMT	**-**	**-**	**-0.439**	**0.005**	**0.157**	**0.047**
NLR	**0.157**	**0.047**	0.140	0.388	**-**	**-**

Since control group also had traditional risk factors (hypertension, hyperlipidemia, smoking) in terms of atherosclerotic disease, all groups were evaluated together and when distribution was expanded and became heterogenous carotid IMT was positively correlated with age, creatinine, uric acid, fibrinogen, ferritin, CaxP product, proteinuria and NLR and negatively correlated with hemoglobin level, Gas6, MDRD-GFR and HDL cholesterol (Table 4). In lineer logistic regression analysis carotid IMT was significantly associated with age (β=0.462, t=4.401, p<0.001) and ferritin (β=-0.1213, t=2061, p=0.045) and proteinuria (β=0.272, t=2666, p=0.011).

NLR has positive correlation with age, uric acid, PTH, CRF duration, carotid IMT, ferritin, proteinuria and negatively correlated with hemoglobin level, albumin, Ca and MDRD-GFR. In lineer logistic regression analysis ferritine (β=0.753, t=7.881, p<0.001) and age (β=-0.208, t=-2.175, p=0.034) were predictors for NLR.

In addition, negative correlation was detected between Gas6 and age, TG, carotid IMT and proteinuria (Table 4). In the logistic regression analysis, Gas6 remained significantly associated with carotid IMT (β=-0.462, t=-2.159, p=0.038). 55% of the patients (22 patients) were receiving immunosuppressive treatment. When the treatment protocols of the patients were examined; 8 patients (20%) were using steroid, 7 patients (17.5%) were steroid+cyclophosphamide, 2 patients (5%) were cyclophosphamide, 2 patients were steroid+mycophenolate mofetil, 2 patients were steroid+azathioprine and 1 patient was steroid+methotrexate. There was no

significant difference in leucocyte, neutrophil, lymphocyte, platelet and NLR between patients receiving and not receiving immunosuppressive treatment.

Gas6 levels were significantly lower in patients treated with angiotensin-converting enzyme inhibitor/angiotensin receptor blocker (17 patients) for proteinuria and/or hypertension than those who did not use (51.39±39.07 vs 74.10±46.10). MDRD-GFR values were higher in patients using ARB or ACE inhibitors (Table 5).

CIMT was higher (0.67±0.13, 0.58±0.20, *p*=0.001) and Gas6 was lower (53.48±43.26, 71.76±44.19, *p*=0.002) in patients with nephrotic proteinuria when the glomerulonephritic patients were divided into two groups as nephrotic and non-nephrotic proteinuria.

Tablo 4. The correlation of demographic and laboratory parameters with CIMT, NLR and Gas6 in all group (n=86).

	CIMT		GAS6		NLR	
	r value	*p* value	r value	*p* value	*r* value	*p* value
Age	**0.624**	**0.001**	**-0.262**	**0.040**	**0.441**	**<0.001**
Smoking years	0.180	0.156	0.071	0.582	0.065	0.615
CRF duration	0.158	0.213	-0.136	0.292	**0.272**	**0.031**
SBP	**-0.278**	**0.026**	0.160	0.214	-0.079	0.537
DBP	-0.126	0.319	-0.113	0.384	-0.146	0.253
BMI	0.196	0.121	0.221	0.084	0.055	0.669
Hb	**-0.321**	**0.010**	0.161	0.212	**-0.274**	**0.030**
Creatinine	**0.378**	**0.020**	-0.025	0.848	0.149	0.244
Albumin	**-0.426**	**0.001**	**0.260**	**0.041**	**-0.285**	**0.023**
Uric ascite	**0.406**	**0.001**	-0.172	0.184	**0.255**	**0.046**
Fibrinogen	**0.560**	**0.001**	-0.197	0.157	0.144	0.293
CRP	-0.120	0.379	0.229	0.096	0.113	0.412
T cholesterol	**0.343**	**0.006**	-0.205	0.117	0.037	0.780
LDL	**-0.295**	**0.020**	-0.210	0.117	0.070	0.593
TG	**0.382**	**0.002**	**-0.379**	**0.003**	0.051	0.694
HDL	**-0.346**	**0.006**	0.239	0.065	-0.093	0.478
Ca	-0.081	0.526	-0.004	0.974	**-0.099**	**0.046**
P	**0.348**	**0.006**	-0.203	0.119	0.082	0.530
Ca-P product	**0.303**	**0.017**	-0.144	0.275	0.085	0.519
MDRD-GFR	**-0.449**	**0.001**	0.075	0.561	**-0.307**	**0.014**
PTH	0.182	0.161	0.000	0.999	**0.229**	**0.048**
ALP	0.135	0.302	-0.093	0.488	0.176	0.243
Ferritine	**0.438**	**0.001**	-0.118	0.367	0.623	<0.001
Proteinuria	**0.549**	**0.001**	**-0.426**	**0.001**	**0.192**	**0.032**
Gas6	**-0.333**	**0.009**	**-**	**-**	-0.025	0.849
CIMT	**-**	**-**	**-0,333**	**0,009**	**0.342**	**0.007**
NLR	**0.342**	**0.007**	-0.025	0.849	-	-

Table 5. Laboratory parameters according to angiotensin-converting enzyme inhibitor/angiotensin receptor blocker treatment or lack of treatment.

	Nontreated (n=23)	Treated (n=17)	*p*
NLR	4.32±5.76	2.83±1.21	0.013
Hb	10.62±11.48	11.48±1.38	0.079
Creatinine	4.05±1.32	1.32±1.03	<0.001
Albumin	3.15±0.99	2.78±0.87	0.049
Uric ascite	6.68±2.84	6.56±1.56	0.871
Fibrinogen	489.85±224.54	415.14±150.03	0.254
CRP	1.74±2.09	0.95±1.07	0.049
Ca-P product	45.22±13.86	37.41±8.87	0.044
MDRD-GFR	40.33±49.75	76.43±39.89	0.015
PTH	277.52±274.86	74.75±59.64	0.003
Proteinuria	3459.06±4290.54	4660.98±3869.94	0.360
Gas6	74.10±46.10	51.39±39.07	0.045
CIMT	0.60±0.19	0.64±0.16	0.459

Discussion

Glomerular diseases are processes leading to inflammation of the glomeruli that develop due to different etiologies. Proteinuria plays an important role in determining the prognosis independent from the etiology. The greater the amount of proteinuria is the faster the progression to chronic renal failure. In glomerulonephritis, the indicator of chronicity in renal biopsy is the degree of fibrosis and tubululointerstitial atrophy rather than glomerulosclerosis. It is well known that long-term exposure of protein to renal tubular cells results in apoptosis and fibrosis [4, 5]. In present study, we have shown the relation of CIMT with inflammation and apoptosis in patients with glomerulonephritis. CIMT was higher in glomerulonephritis group. Gas6 that prevents apoptosis and protects against fibrosis and vascular calcification, was found to be lower in glomerulonephritis group than control group. In addition, negative correlation with proteinuria and CIMT suggests that Gas6 may be a useful parameter in predicting prognosis and early atherosclerosis in glomerulonephritis. When the whole group assessed together, the detection of the CIMT as a predictor of Gas6 strengthens this relation. Proteinuria seen during the course of glomerulonephritis is also an indicator of glomerular inflammation. We found NLR twofold higher than control group and NLR showed positive correlation with CIMT in glomerulonephritis group and a statistically significant correlation with MDRD-GFR, PTH, proteinuria and CIMT. All these results support that NLR which is a cheap and easily obtainable parameter can be used in practice as a marker of atherosclerotic diseases in glomerulonephritis.

In the literature it has been demonstrated that acute or chronic renal failure may cause elevated NLR by the underlying inflammation in the development of renal failure. NLR has a prognostic valuefor cardiovascular disease in renal diseases [10, 11]. Abe et al. [10] have shown that higher NLR is associated with increased risk of cardiovascular disease events and is a stronger predictor of future events in hemodialysis patients. In a recent study, Kucuk et al. [12] have found that NLR was significantly higher in the granulomatosis with polyangiitis (GPA) group compared with the control group. Although NLR was different and serum creatinine was similar between non-renal GPA group and controls, non-renal GPA group had higher NLR than controls. Also NLR has been demonstrated to be an independent risk factor for cardiovascular events in general population and in diseases other than chronic renal failure [13, 14]. Corriere et al. [15] have shown NLR as a strong predictor of the presence and the number of carotid atherosclerotic plaques in 324 patients older than 65 years old without hematopoietic disorders, malignancies, acute infections and chronic inflammatory diseases. In a study Li et al. [3] have shown that higher NLR in hemodialysis patients was a predictor of increased pulse pressure, left ventricular mass index and intima-media thickness. In our study, NLR in the glomerulonephritis group

was found twofold higher than control group and there was a significant positive correlation between NLR and age, uric acid, CRP and CIMT. When the whole group was evaluated together NLR had a positive correlation with age, uric acid, PTH, CRF duration, carotid IMT, ferritin, proteinuria and negative correlation with hemoglobin level, albumin and MDRD-GFR. All of these parameters were atherosclerosis associated parameters in chronic renal failure. Yılmaz et al. [16] have shown that NLR was higher in CKD than control group and correlated with uric acid and proteinuria in patients with stage 3-4 CKD. Emokpae et al. [17] showed that NLR was positively correlated with urinary protein levels in sickle cell anaemia patients with macroalbuminuria and/or renal failure. All of these studies and our study support the close relation between NLR, CIMT and proteinuria.

Immunosuppressive drugs used in the treatment of glomerulonephritis may cause changes in NLR values. Although 55% of the patients were receiving immunosuppressive treatment, no significant difference was found between the groups receiving and not receiving immunosuppressive treatment in terms of leukocyte, neutrophil, lymphocyte, platelet and NLR.

Carotid IMT is an indicator of early atherosclerosis both in the general population and chronic renal failure. Increased CIMT is a reflection of systemic atherosclerosis in patients with cardiovascular risk factors. Lai et al. [18] have shown that CIMT was higher in stage 2-3 chronic renal failure patients than controls and releated to worsen cardiovascular outcomes. In our study, CIMT was significantly higher in glomerulonephritis patients with mean MDRD-GFR of 53.80±48.32 mL/min than control group. Proteinuria and NLR as systemic and glomerular inflammation markers were positively correlated with CIMT. Regression analysis revealed age as a predictor for CIMT. Van den Munckhof et al. [19] have specified significant positive correlation between age and CIMT in a systematic review of studies in the general population without CVD/risk factors. Although it was mentioned that studies including patients with CVD had higher CIMT compared to patients without CVD, a linear relation between age and CIMT was also present. Gustafsson et al. [20] have shown that mean IMT was higher in systemic lupus erythematosus (SLE) patients than control group in a study of 281 SLE patients. SLE subgroup with nephritis had plaques twice as often as age-matched non-nephritis SLE patients and controls. In present study when all group evaluated together carotid IMT was positively correlated with age, creatinine, uric acid, fibrinogen, ferritin, CaxP product, proteinuria and NLR and negatively correlated with Gas6. In lineer logistic regression analysis carotid IMT was significantly associated with age and ferritin and proteinuria. Falaschi et al. [21] have shown that mean IMT of the SLE patients was significantly higher than control group and patients with nephrotic-range proteinuria had a significantly higher IMT than without nephrotic-range proteinuria. In our study, 16 patients had nephrotic range proteinuria and CIMT was significantly higher in patients with nephrotic-range proteinuria than sub-nephrotic proteinuria group.

Gas6 is a member of vitamin K dependent proteins family [22, 23]. Vitamin K acts as a coenzyme in the γ-carboxylation of Gas6 and Gas6 is activated. Gas6 binds to Axl receptor in VSMC surface and passes anti-apoptotic signal into the cell and by this way promotes cell survival and migration [24]. Son et al. [25] have shown that inorganic phosphate downregulated the expression of Gas6 and Axl and so induced VSMC apoptosis and calcification. In our study Gas6 level was significally lower in glomerulonephritis group than healty controls. If it was thought that patients with glomerulonephritis are on a severe and tight diet due to both renal failure, nephrotic syndrome and medications used in the treatment, lack of vitamin K may be a contributory factor beside hyperphosphatemia for lower Gas6 levels. Hemodialysis (HD) patients have higher risk of vitamin K deficiency because of lowering dietary potassium and phosphorus, they limit foods including main sources of vitamin K [26, 27]. However, significantly reduced vitamin K intake was also reported in a study of kidney transplant recipients with a glomerular filtration rate of 61 ml/min, who did not have significant diet limitations [28]. Jiang et al. [29] investigated the effect of vitamin K2 on aortic calcification induced by warfarin via Gas6/Axl survival pathway in rats. They have found that warfarin decreased Gas6 and Axl, p-Akt, and the expression of Bcl-2 protein levels. After 100μg/g vitamin K2 treatment calcium depositions, ALP activity and apoptosis were significantly decreased, Gas6,

Axl, p-Akt and Bcl-2 expression were increased and calcification was reversed by 44%.

The relationship between Gas6 and cardiovascular diseases has been demonstrated in many mouse models and cell cultures. However, there are conflicting reports whether Gas6 levels are increasing or decreasing in human studies of diabetic and/or chronic renal diseases. In a recent study Gas6 levels were found to be higher in patients with SLE than controls and it was said that Gas6 could be regarded as disease activity marker [30]. Gas6 levels were obtained to be higher in Ig A nephropathy but it can be explained by the proliferation of mesangium in Ig A nephropathy and the increased expression of Gas6 in mesangial cells [31]. Our study group included patients with proteinuria and inactive disease activity. Gas6 was negatively correlated with age, proteinuria and CIMT. Also Gas6 level was decreased in nephrotic range proteinuria group compaired with sub-nephrotic range proteinuria group. Gas6/Axl signalling potentiates toxin-induced glomerular inflammatory disease but has a protective function within the tubulointerstitium in the podocyte ablation model [32]. It has been shown that upregulation of Axl has a protective mechanism in animal model of CKD and reduced tubulointerstitial apoptosis and slowed the progression of renal failure. Considering that the Gas6/Axl system is involved in many different pathways, new comprehensive and high patient number of studies are needed to explain these contradictory results in the literature.

Angiotensin-converting enzyme (ACE) inhibitors and angiotensin receptor blockers (ARBs) are used as an adjuvant treatment in glomerulonephritis with proteinuria. Tselios et al. [33] had shown no significant differences in the cumulative occurrence of atherosclerotic cardiovascular events in a study involving 144 patients with lupus nephritis who were treated with ACE inhibitors/ARBs for at least 5 years versus 301 LN patients with no such treatment. In our study 17 patients were treated with ACE inhibitors/ARBs. There wasn't any significant difference in CIMT and proteinuria but NLR and Gas6 levels were lower than nontreated patients. Melaragno et al. [34] have shown both expression of Gas6 and Axl increased in rat VSMC culture after exposure to angiotensin II. Treatment with ACE inhibitors/ARBs may have a role at decreased Gas6 levels in our treated group.

As a conclusion glomerular diseases have an increased risk of atherosclerosis even from the early stages. It is supported by the fact that CIMT, the best indicator of early atherosclerosis, is higher in our patient group than control group. NLR and proteinuria are two parameters that can routinely use following glomerular diseases, showed significant correlation with CIMT. Proteinuria is particularly important in glomerular diseases, because with the effective treatment the disease enters the remission and at the same time the risk of atherosclerosis is also reduces. There are conflicting results with Gas6 levels in the literature and in our study reduced Gas6 levels were found to be in contrast to existing studies on renal failure, but decreased Gas6 levels have been shown to be associated with cardiovascular diseases and/or cardiovascular risks. Gas6 has a complex mechanism because of its role in many different routes. In addition, hyperphosphatemia due to renal failure and K vitamin deficiency due to inadequate nutrition may also contribute to a decrease in Gas6 levels. As a result, proteinuria appears to have the most important role in determining CIMT. NLR and Gas6 levels are the reflection of proteinuria.

Our study has two main limitations. First, many different types of glomerular diseases evaluated together, the sample wasn't homogeneous and sample size was relatively small. Another limitation is the ımmunsuppressive drugs used in the treatment may effect NLR. Prospective studies with higher number of patients and homogenous subgroups are needed to assess these relations.

Conflict of Interest: The authors have declared that no conflict of interest exists.

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