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C-REAKTIVNI PROTEIN KAO NEZAVISNI FAKTOR RIZIKA ZA KAROTIDNU ATEROSKLEROZU KOD BOLESNIKA NA HEMODIJALIZI

C-REACTIVE PROTEIN AS AN INDEPENDENT RISK FACTOR FOR CAROTID ATHEROSCLEROSIS IN HEMODIALYSIS PATIENTS

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Sažetak - C-reaktivni protein je poznati faktor rizika za kardiovaskularne bolesti, ali povezanost C-reaktivnog proteina sa ranom fazom ateroskleroze je slabije proučena. Cilj našeg rada je bio ispitivanje povezanosti C-reaktivnog proteina sa indikatorima supkliničke ateroskleroze u karotidnim arterijama bolesnika na hemodijalizi. *B mode* ehosonografijom utvrđene su značajno više vrednosti ultrazvučnih markera rane ateroskleroze: površine preseka intime-medije i lumen-dijametra, mada ne i debljine intime-medije kod bolesnika na hemodijalizi u odnosu na kontrolnu grupu. Inflamacija (CRP>10 mg/l) bila je prisutna kod 58,1% bolesnika na hemodijalizi i bila je udružena sa višim vrednostima svih ultrazvučnih markera ateroskleroze, kao i sa većom učestalošću klinički manifestne aterosklerotične vaskularne bolesti. Na vrednost PPIM najjači uticaj je ispoljio nivo C-reaktivnog proteina (r = 0,619, p = 0,000), zatim starost (R = 0,704, p = 0,005) i pušenje (R = 0,742, p = 0,039). Rezultati našeg istraživanja podržavaju koncept o ključnoj ulozi inflamacije u razvoju ateroskleroze kod bolesnika na hemodijalizi.
Ključne reči: C-reaktivni protein + analiza; Bolesti karotidnih arterija; Zajednička karotidna arterija + ultrasonografija; Hronična bubrežna insuficijencija + komplikacije; Dijaliza

Summary *- C-reactive protein is a known risk factor for cardiovascular diseases, but the association of CRP with the early phase of atherosclerosis has been insufficiently investigated. The aim of the present study was to investigate the relationship between CRP and indicators of subclinical atherosclerosis in hemodialysis patients. Intima-media thickness, lumen-diameter and cross-sectional intima-media area (CSIM area) of the common carotid artery were established using B-mode ultrasound imaging in 43 HD patients and 21 age- and gender-matched healthy controls. CSIM area and LD were significantly higher in HD patients compared with control subjects. Inflammation (CRP>10 mg/l) was present in 58.1% of HD patients and was associated with higher values of all ultrasonographic markers of atherosclerosis, as well as higher prevalence of atherosclerotic vascular diseases. Higher CSIM area was associated with elevated CRP level (r=0.619, p=0.000), advanced age (R=0.704, p=0.005) and smoking habits (R=0.742, p=0.039). Results of our investigation support the concept on key role of inflammation in the development of atherosclerosis in HD patients.*
Key words: *C-Reactive Protein - analysis; Carotid Artery Diseases; Carotid Artery, Common - ultrasonography; Kidney Failure, Chronic - complications; Renal Dialysis*

Uvod

Kardiovaskularne bolesti (KVB) su glavni uzrok morbiditeta i mortaliteta bolesnika u terminalnoj fazi hronične bubrežne insuficijencije (HBI). Godišnja stopa mortaliteta usled KVB iznosi 9%, što je 10-20 puta više nego u opštoj populaciji nakon prilagođavanja za starost, pol, rasu i *diabetes mellitus* [1]. Visoka incidencija smrti od infarkta miokarda kod bolesnika srednje životne dobi na hroničnoj hemodijalizi (HD) zapažena je još ranih sedamdesetih godina i objašnjena je hipotezom o "akcelerantnoj aterosklerozi" koja je i danas podjednako aktuelna [2].

Razlozi za brzu kliničku progresiju ateroskleroze u HBI su verovatno mnogobrojni. Klasični faktori rizika kao što su starija životna dob, hipertenzija, dislipidemija, *diabetes mellitus*, fizička neaktivnost i pušenje nisu mogli zadovoljavajuće objasniti visoku prevalenciju koronarne bolesti u HBI [3]. Kasnija istraživanja su istakla doprinos inflamacije i oksidativnog stresa endotelnoj disfunkciji i aterogenezi [4]. Prema mišljenju Stenvinkela i saradnika,

Introduction

Cardiovascular disease (CVD) remains the main cause of morbidity and mortality in patients with end-stage renal disease (ESRD). The annual mortality rate due to CVD is 9%, which is 10- to 20-fold higher than in the general population, when adjusted for age, gender, race and diabetes mellitus [1]. High incidence of deaths from myocardial infarction in middle-aged patients on regular hemodialysis (HD) was observed in early 70s and was explained by a hypothesis of "accelerated atherosclerosis" which is actual today [2].

The causes of atherosclerotic CVD in ESRD patients are probably multifactorial. Classsic risk factors such as old age, hypertension, dyslipidemia, diabetes mellitus, physical inactivity and smoking are prevalent in many patients with ESRD, but studies have shown that excess CVD is not explained adequately by traditional risk factors [3]. Thus, it has been postulated that non-traditional risk factors, such as inflammation and oxidative stress, may be important for the development of endothelial

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Skraćenice

KVB	- Kardiovaskularna bolest
HBI	- Hronična bubrežna insuficijencija
HD	- Hemodijaliza
CRP	- C-reaktivni protein
SGP	- Subjektivna globalna procena
DIM	- Debljina intime-medije
LD	- Lumen-dijametar
PPIM	- Površina preseka intime-medije

ubrzana ateroskleroza kod bolesnika sa HBI se pojavljuje usled sinergizma različitih mehanizama koji obuhvataju malnutriciju, inflamaciju, oksidativni stres i genetske komponente [5]. Uprkos brojnim radovima koji se bave ispitivanjem povezanosti inflamacije i KVB, još uvek nije u potpunosti razjašnjeno da li je inflamacija samo epifenomen ili su različiti reaktanti akutne faze neposredno uključeni u započinjanje i/ili dalji razvoj ateroskleroze.

Tokom protekle decenije, nekoliko velikih epidemioloških studija je identifikovalo CRP kao snažan i nezavistan faktor rizika za budući infarkt miokarda, moždani udar i periferni arterijsku bolest kod prividno zdravih osoba [6]. U populaciji bolesnika sa stabilnom ili nestabilnom anginom, serumski nivo CRP je pozitivno povezan sa akutnim infarktom miokarda ili iznenadnom srčanom smrću [7]. Poslednjih godina je dokazano da povećani nivo CRP predstavlja snažan faktor rizika za ukupni i kardiovaskularni mortalitet bolesnika na dijalizi [8]. Medutim, povezanost između CRP i supkliničke ateroskleroze je slabije proučena.

Ciljevi ovog istraživanja bili su: a) ultrazvučno određivanje ranih markera ateroskleroze u zidu karotidnih arterija kod bolesnika na HD u odnosu na kontrolnu grupu; b) ispitivanje faktora povezanih sa prisustvom inflamacije kod bolesnika na HD i c) ispitivanje povezanosti površine preseka intime-medije kao markera supkliničke ateroskleroze sa serumskom koncentracijom CRP kao markerom inflamacije i ostalim faktorima rizika za brzu progresiju ateroskleroze.

Materijal i metode

U studiju su uključena 43 bolesnika sa terminalnom bubrežnom insuficijencijom, koja se nalaze na hroničnom programu lečenja hemodijalizom u Centru za hemodijalizu Zdravstvenog centra "Zaječar" u Zaječaru, najmanje 6 meseci. Bolesnici (27 muškaraca i 16 žena), prosečne starosti 57,8±12 godina, a prethodno su lečeni hemodijalizom od 6 do 243 meseca. Indeks telesne mase (*Body mass index* - BMI), definisan kao količnik telesne težine izražene u kilogramima i kvadrata telesne visine izražene u metrima, iznosio je 24,1±4,5 kg/m^2. U vreme ispitivanja bolesnici nisu imali kliničke znake interkurentnih infekcija. Kao osnovni uzroci terminalne bubrežne insuficijencije navedeni su: hipertenzija kod 11 bolesnika (26%), hronični pijelonefritis kod 9 bolesnika (21%), hronični glomerulonefritis kod 7

Abbreviations

CVD	- Cardiovascular disease
ESRD	- End-stage renal disease
HD	- Hemodialysis
CRP	- C-reactive protein
SGA	- Subjective Global Assessment
IMT	- Intima-media thickness
LD	- Lumen-diameter
CSIMa	- Cross-sectional intima-media area

dysfunction and possibly atherogenesis [4]. According to Stenvinkel at al, accelerated atherogenesis in ESRD patients occurs due to synergism of various mechanisms including malnutrition, inflammation, oxidative stress and genetic components [5]. Although the association between CVD and inflammation in the dialysis patient population is well documented, it has not been fully clarified if the acute-phase response merely reflects an epiphenomenon accompanying established atherosclerotic disease or whether different acute-phase reactants themselves are involved in the initiation and/or progression of atherosclerosis.

During the last decade, several large epidemiological studies identified CRP as a strong and independent risk factor for future myocardial infarction, stroke and peripheral arterial disease in apparently healthy persons [6]. In population of patients with a stable or unstable angina, serum level of CRP is positively related with acute myocardial infarction or sudden cardiac death [7]. In recent years, higher level of CRP has been confirmed to represent a strong risk factor for overall and cardiovascular mortality in patients on dialysis [8]. But, the relationship between CRP and subclinical atherosclerosis is not well established.

The objectives of this investigation were: a) ultrasonographic identification of early markers of carotid atherosclerosis in HD patients compared with control subjects; b) examination of risk factors associated with inflammation in HD patients and c) evaluation of the relationship between CSIM area, as an ultrasonographic marker of subclinical atherosclerosis, and serum concentrations of CRP as an inflammatory marker and other risk factors for rapid progression of atherosclerosis.

Material and methods

The study included 43 patients with ESRD on regular HD treatment in the Hemodialysis Centre of the Zaječar Health Centre, for at least 6 months. Patients (27 men and 16 women) were of average age 57.8 ± 12 years, and they were previously treated with HD from 6 to 243 months. Their body mass index (BMI), defined as the weight in kilograms divided by square of height in meters, was 24.1 ± 4.5 kg/m^2.

bolesnika (16%), nefrolitijaza kod 5 bolesnika (12%), dijabetesna nefropatija kod 4 bolesnika (9%), policistična bolest bubrega kod 3 bolesnika (7%) i nepoznati uzrok kod 4 bolesnika (9%). Trinaest bolesnika (30,2%) su imala u anamnezi podatke o aterosklerotičnoj vaskularnoj bolesti: cerebrovaskularnoj bolesti (2 bolesnika), *angini pectoris* (7 bolesnika), infarktu miokarda (2 bolesnika) i perifernoj arterijskoj bolesti (4 bolesnika). Više od polovine bolesnika (65%) je uzimalo antihipertenzivne lekove (β-blokatore, inhibitore konvertaze angiotenzina, blokatore kalcijumskih kanala, diuretike). Nijedan bolesnik nije koristio inhibitore HMG-CoA reduktaze. Pušača je bilo 15 (34,9%). *Diabetes mellitus* je bio prisutan kod 6 ispitanika (13,9%), od kojih je 5 primalo insulin.

Kontrolna grupa je obuhvatila 21 zdravu osobu i bila je srodna po godinama starosti i po polu sa ispitivanom grupom. Osim ehosonografskog pregleda, kontrolnoj grupi su urađene i osnovne *skrining* analize radi isključivanja pre svega bubrežnih, ali i drugih oboljenja koja bi uticala na rezultate istraživanja.

Za definisanje nutritivnog statusa bolesnika na HD upotrebili smo modifikovani postupak subjektivne globalne procene (SGP) koji uključuje šest subjektivnih parametara. Tri se zasnivaju na anamnezi gubitka telesne težine, smanjenog unosa hrane i povraćanja, a tri na subjektivnom rangiranju propadanja mišića, prisustva edema i gubitka potkožnog masnog tkiva. Na osnovu ocene pojedinih parametara, svakom bolesniku je dodeljen ukupni SGP skor koji odražava njegov nutritivni status na sledeći način: 1=normalan nutritivni status, 2=blaga malnutricija, 3=umerena malnutricija i 4=teška malnutricija [9].

Krv za laboratorijske analize bolesnicima na HD je uzeta pre druge dijalize u nedelji i odmah dostavljena laboratoriji. Određivanje hemoglobina, albumina, fibrinogena, uree, kreatinina, ukupnog holesterola i triglicerida je izvršeno standardnim laboratorijskim metodama. HDL holesterol je određen enzimatskom procedurom posle precipitacije sa fosfovolframovom kiselinom i magnezijum hloridom, dok je LDL holesterol izračunat po Fridewaldovoj formuli. Koncentracija CRP je određena reakcijom aglutinacije *latex* partikula obloženih antitelima na humani CRP.

Ultrasonografski pregled karotidnih arterija je urađen *real-time* sonografijom visoke rezolucije sa linearnom sondom od 7,5 MHz na aparatu SIEMENS SONOLINE Sienna. Debljina intime-medije (DIM) i lumen-dijametar (LD) izmereni su na longitudinalnom preseku desne i leve *a. carotis communis*, 0,5 do 1 cm proksimalno od početka karotidnog bulbusa. DIM je definisana kao rastojanje između glavne ivice koje odgovara spoju lumen-intima i glavne ivice koja odgovara spoju medija-adventicija. LD je definisan kao rastojanje između glavne ivice koja odgovara spoju intima-lumen prednjeg zida i glavne ivice koja odgovara spoju lumen-

During the study patients had no clinical signs of overt intercurrent infections. Causes of ESRD were: hypertension in 11 patients (26%), chronic pyelonephritis in 9 patients (21%), chronic glomerulonephritis in 7 patients (16%), nephrolithiasis in 5 patients (12%), diabetic nephropathy in 4 patients (9%), polycystic kidney disease in 3 patients (7%) and unknown etiologies in 4 patients (9%). Thirteen patients (30.2%) had clinical signs of atherosclerotic vascular disease: cerebrovascular disease (2 patients), angina pectoris (7 patients), myocardial infarction (2 patients) and peripheral arterial disease (4 patients). More than half of the patients (65%) were on antihypertensive medications (β-blockers, angiotensin-converting enzyme inhibitors, calcium channel blockers, and diuretics). Not a single patient used HMG-CoA-reductase inhibitors. There were 15 smokers (34,9%). Diabetes mellitus was present in 6 subjects (13.9%), 5 of whom received insulin.

The control group included 21 age - and gender-matched healthy persons. Apart from ultrasonographic examination, the control group was subjected to basic "screening" analysis for the purpose of excluding renal before all, but also other diseases which might have influenced results of the research.

The nutritional status of HD patients was evaluated using a modified procedure of Subjective Global Assessment (SGA), which included six subjective parameters. Three were based on patient's history of weight loss, incidence of anorexia, and incidence of vomiting, and three were based on subjective grading of muscle wasting, the presence of edema, and subcutaneous fat loss. Based on these assessments, each patient had a score that reflected the nutritional status in the following way: 1 = normal nutritional status, 2 = mild malnutrition, 3 = moderate malnutrition, and 4 = severe malnutrition [9].

Blood for laboratory analysis of HD patients was taken before the second dialysis in the week and was promptly delivered to the laboratory. Levels of hemoglobin, albumin, fibrinogen, urea, creatinine, total cholesterol and triglyceride were established by standard laboratory methods. HDL cholesterol levels were determined by enzymatic procedure after precipitation of apo-B-containing lipoproteins with phosphotungstic acid and magnesium chloride, while LDL cholesterol levels were calculated using the Friedewald formula. CRP concentrations were determined by the latex agglutination method.

Ultrasonographic examination of carotid arteries was done by high resolution real-time sonography with linear probe of 7,5 MHz on SIEMENS SONOLINE Sienna. Intima-media thickness (IMT) and lumen-diameter (LD) were measured on longitudinal section of right and leftcommon carotid artery, 0,5 to 1 cm proximally from the beginning of the carotid bulbus. IMT was defined as the distance

rena po 3 puta unutar sekcije duge 20 mm, na mestu bez diskretnih plakova, gde je debljina zida vizuelno procenjena kao najveća. Iz prosečnih vrednosti izračunata je površina poprečnog preseka intima-medije (PPIM) upotrebom sledeće formule [11]:
PPIM = 3,14 [(LD/2 +DIM)2 - (LD/2)2]

Prikupljeni podaci su prvo obrađeni standardnim statističkim postupcima radi dobijanja mera centralne tendencije i mera varijabiliteta. Testiranje statističke značajnosti razlike izvršeno je pomoću t-testa za 2 nezavisna uzorka i Hi kvadrat testa. Model povezanosti između imenovanih nezavisnih varijabli i PPIM kao zavisne varijable testiran je pomoću linearne regresione analize. Vrednost verovatnoće $p < 0,05$ je prihvaćena kao statistički značajna.

Rezultati

Za procenu stepena uznapredovalosti ranih aterosklerotskih lezija u zidu zajedničke karotidne arterije koristili smo 3 ultrazvučna markera supkliničke ateroskleroze: DIM, LD i PPIM. Mada su kod bolesnika na HD prosečne vrednosti svih ultrazvučnih markera bile veće nego kod zdravih ispitanika, statistički značajna razlika između ispitivanih grupa utvrđena je za prosečne vrednosti LD ($p < 0,001$) i PPIM ($p = 0,001$), ali ne i DIM ($p > 0,05$) (Tabela 1).

Tabela 1. Značajnost razlike u prosečnim vrednostima ehosonografskih parametara supkliničke ateroskleroze između grupe bolesnika na hemodijalizi i kontrolne grupe

Table 1. *Significance of differences in mean values of ehosonographic parameters of subclinical atherosclerosis between hemodialysis patients and control subjects*

Ehosonografski parametri *Ultrasonographic parameters*	Kontrolna grupa *Control subjects* X ± SD	Bolesnici na HD *HD patients* X ± SD	p
DIM-IMT (mm)	0,073 ± 0,14	0,66 ± 0,13	0,06
LD-LD (mm)	7,56 ± 0,94	6,55 ± 0,74	0
PPIM-CSIM area (mm^2)	19,22 ± 4,81	15,05 ± 0,26	0

Legenda: X-aritmetička sredina; SD-standardna devijacija; DIM-debljina intime-medije; LD-lumen-dijametar; PPIM-površina preseka intime-medije

Legend: X - mean values; SD - standard deviation; IMT - intima-media thickness; LD - lumen-diameter; CSIM area - cross sectional intima-media area

Ukoliko se posmatra procentualna distribucija PPIM u ispitivanim grupama, evidentno je da se kod bolesnika na hemodijalizi PPIM najčešće nalazi u opsegu 15-19,9 mm^2, dok je kod više od polovine zdravih ispitanika PPIM manja od 15 mm^2 (Grafikon 1).

U odnosu na vrednost serumskog CRP, bolesnici na HD su podeljeni u 2 osnovne grupe. Grupu I (18 bolesnika, 41,9%), sačinjavali su bolesnici čiji je CRP bio manji od 10 mg/l i grupu II (25 bolesnika, 58,1%) sačinjavali su bolesnici čiji je CRP bio veći od 10 mg/l. Osnovni klinički podaci,

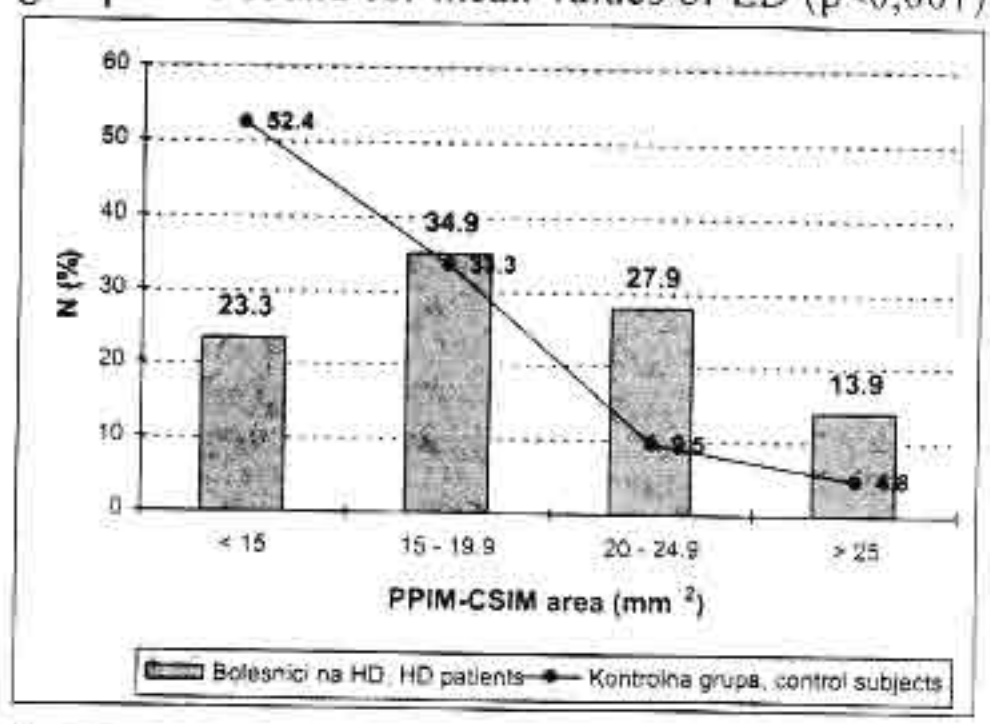
from the leading edge of lumen-intima echo of the far wall to the leading edge of the media-adventitia echo in the far wall. LD was defined as the distance between the leading edge of the intima-lumen echo of the near wall and the leading edge of the lumen-intima echo in the far wall [10]. Both parameters were measured 3 times inside a section 20 mm long, at the place without discreet plaques, where wall thickness was visually estimated as the greatest. Cross-sectional intima-media area (CSIM area) was calculated from mean values, using the following formula [11]:

CSIM area = 3, 14 [(LD/2 +DIM) 2 - (LD/2)2]

Collected data were first elaborated by standard statistical procedures for the purpose of obtaining measures of central tendency and measures of variability. Normally distributed data are presented as mean ± SD. For statistical analysis we used an unpaired Student's t-test to compare means between two groups and the 2 test to compare prevalences between groups. For multivariate linear regression, we used a stepwise model. All variables that were significantly correlated with CSIM area in the univariate analysis were included in the model. The P-value < 0.05 was considered statistically significant.

Results

For evaluation of the degree of advancement of early atherosclerotic lesions in the vessel wall, 3 ultrasonographic markers of subclinical atherosclerosis were used: IMT, LD and CSIM area. Although mean values of all ultrasonographic markers were higher in HD patients than in healthy subjects, a statistically significant difference between examined groups was found for mean values of LD ($p<0,001$)



Grafikon 1. Distribucija PPIM kod bolesnika na hemodijalizi u odnosu na kontrolnu grupu

Graph 1. *Distribution of CSIM area in hemodialysis patients and in control subjects*

Legenda: PPIM-površina poprečnog preseka intime-medije; HD-hemodijaliza

Legend: CSIM area - cross-sectional intima-media area; HD-hemodialysis

Tabela 2. Uporedne karakteristike bolesnika grupe I (CRP < 10 mg/l) i bolesnika grupe II (CRP > 10 mg/l)
Table 2. *Comparative characteristics of patients belonging to group I (CRP < 10 mg/l) and group II (CRP > 10 mg/l)*

Ispitivane varijable *Examined variables*	CRP < 10 mg/l (N = 18)	CRP >10 mg/l (N = 25)	p
Pol (m/ž)/*Gender* (m/f)	12:6	16:10	n.s
Starost (godine)/*Age (years)*	54,6 ± 14,1	60,1 ± 11,2	n.s
Zastupljenost pušača (%) *Prevalence of smokers (%)*	28	40	n.s
Zastupljenost *diabetes mellitusa* (%) *Prevalence of diabetes mellitus (%)*	5	20	n.s
% zastupljenost PEM (SGP 2-4) *% prevalence of PEM (SGP 2-4)*	22	64	< 0,01
ITM (kg/m²)/*BMI (kg m²)*	24,7 ± 4,9	23,7 ± 4,2	n.s
Hemoglobin (g/l)/*Hemoglobin (g l)*	79 ± 7	77 ± 10	n.s
Albumin (g/l)/*Albumin (g l)*	37,7 ± 1,9	35,2 ± 2,8	< 0,01
Kreatinin (μmol/l)/*Creatinine (μmol l)*	819 ± 186	702 ± 221	n.s
Urea (mmol/l)/*Urea (mmol l)*	26,8 ± 6,3	28,6 ± 7,4	n.s
Holesterol (mmol/l) *Cholesterol (mmol l)*	5,7 ± 1,8	5,2 ± 1,81	n.s
HDL holesterol (mmol/l) *HDL cholesterol (mmol l)*	0,89 ± 0,26	0,93 ± 0,29	n.s
LDL holesterol (mmol/l) *LDL cholesterol (mmol l)*	3,8 ± 1,5	3,3 ± 1,6	n.s
Trigliceridi (mmol/l) *Triglycerides (mmol l)*	3,0 ± 1,9	2,6 ± 1,1	n.s
SKP(mmHg)/*SBP (mmHg)*	139 ± 12	153 ± 16	< 0,01
DKP (mmHg)/*DBP (mmHg)*	81 ± 5	85 ± 7	n.s
DIM (mm)/*IMT (mm)*	0,65 ± 0,12	0,79 ± 0,12	< 0,001
LD (mm)/*LD (mm)*	7,16 ± 1,02	7,85 ± 0,79	< 0,05
PPIM (mm²)/*CSIM area (mm²)*	16,8 ± 3,86	21,48 ± 4,15	< 0,001
Zastupljenost AVB (%) *Prevalence of AVD (%)*	11	44	< 0,05
SE/*ESR*	47 ± 24	71 ± 39	< 0,001

Legenda: N-broj bolesnika; PEM-proteinsko-energetska malnutricija; ITM-indeks telesne mase; SKP-sistolni krvni pritisak; DKP-dijastolni krvni pritisak; DIM-debljina intime-medije; LD-lumen-dijametar, PPIM- površina poprečnog preseka intime-medije; AVB-aterosklerotična vaskularna bolest; SE-sedimentacija eritrocita;

Legend: N - number of patients; PEM - protein-energy malnutrition; BMI - body mass index; SBP - systolic blood pressure; DBP - diastolic blood pressure; IMT - intima-media thickness; LD - lumen-diameter; CSIM area - cross-sectional intima-media area; AVD - atherosclerotic vascular disease; ESR-erythrocyte sedimentation rate

laboratorijski i ultrazvučni parametri koji su ispitivani u navedenim grupama, prikazani su u Tabeli 3.

Tabela 3. Regresioni model PPIM
Table 3. *Regression model of CSIM area*

Model/*Model*	B	SE	Beta	t	P
Constant/*Constant*	5,76	2,79		2,07	<0,05
CRP/*CRP*	0,15	0,04	0,46	3,88	<0,001
Starost/*Age*	0,13	0,04	0,34	3,02	< 0,01
Pušenje/*Smoking*	2,42	1,13	0,24	2,13	< 0,05

Ispitivane grupe bolesnika su bile ujednačene u odnosu na pol i starost. Distribucija pušenja i *diabetes mellitusa* se nije statistički značajno razlikovala između dve grupe. Primenom SGP je dokazana veća zastupljenost malnutricije kod bolesnika u grupi II

and CSIM area (p = 0.001), but not for IMT (p > 0,05) (Table 1). If distribution of CSIM area is considered in examined groups, it is evident that in HD patients CSIM area is most frequently in the range of 15-19,9 mm², while in more than half of healthy subjects CSIM is less than 15 mm² (Graph 1).

According to the value of serum CRP, HD patients were divided into 2 main groups. Group I (18 patients, 41.9%), consisted of patients whose CRP was lower than 10 mg/l and group II (25 patients, 58.1%) of patients whose CRP was higher than 10 mg/l. Basic clinical data, laboratory and ultrasonographic parameters examined in these groups are presented in table 2.

Examined groups were age - and gender-matched. Distribution of smoking and diabetes mellitus was not significantly different between these two groups. According to SGA, higher prevalence of malnutrition was confirmed in group II than in group I (p=0.007), although this difference for BMI was not statistically significant. Statistically significant difference between two groups was not registered for pre-dialysis values of hemoglobin, creatinine, urea and cholesterol, but albumin levels were significantly lower in group II than in group I (p=0.002).

In HD patients in group II, there were statistically higher values of systolic (p=0.004), but not diastolic blood pressure compared with patients in group I. Prevalence of atherosclerotic vascular disease was significantly more frequent in group II, than in group I (p=0.022). A statistically significant difference of all ultrasonographic markers of subclinical atherosclerosis was established between examined groups: patients in group II had statistically higher values of IMT (p=0.000), LD (p=0.018) and CSIM area (p= 0.000) than those in group I. In accordance with expectations, patients with CRP beyond 10 mg/l had significantly higher values of ESR (p= 0.000) and fibrinogen (p=0.000) in comparison with patients whose CRP was lower than 10 mg/l.

For the purpose of determining the significance of individual variables [age, gender, length of dialysis, smoking, lipid status, malnutrition (SGA), albumin, fibrinogen, CRP] in explaining CSIM area as a dependent variable, stepwise method of multiple linear regression analysis was used. R square of regression model (R2=0.550) suggests that approximately 55% of variability of outcome variable-CSIM area is explained through coexistence of variables from the model presented in table 3, while isolated level of CRP can explain almost 38% varying in CSIM area values. The linear relationship model between CSIM area as a dependent variable and CRP as an independent variable is graphically presented in diagram 2.

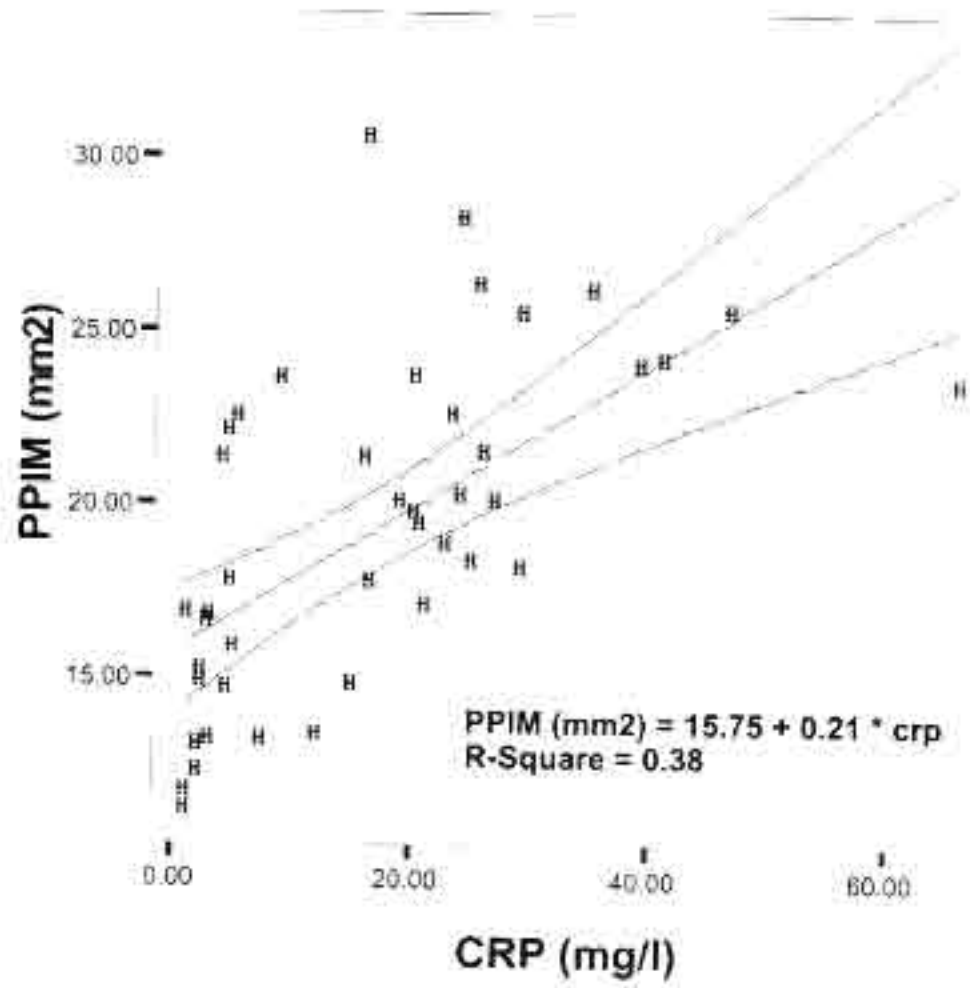
Discussion

Results of ultrasonographic examination show that HD patients have greater severity and spread of

nego kod bolesnika u grupi I (p = 0,007), iako ostvarena razlika za BMI nije bila statistički značajna. Statistički značajne razlike između ispitivanih grupa nije bilo ni u predijaliznim vrednostima hemoglobina, kreatinina, uree i holesterola, ali serumske koncentracije albumina su bile značajno niže kod bolesnika u grupi II nego kod bolesnika u grupi I (p = 0,002).

Kod bolesnika iz grupe II bile su značajno veće vrednosti sistolnog (p = 0,004), ali ne i dijastolnog krvnog pritiska u odnosu na bolesnike iz grupe I. Prisustvo klinički manifestne ateroskleroze je zabeleženo značajno češće kod bolesnika grupe II, nego kod bolesnika grupe I (p = 0,022). Između ispitivanih grupa ustanovljena je statistički značajna razlika u vrednostima svih ehosonografskih markera supkliničke ateroskleroze: bolesnici iz grupe II imali su značajno veće vrednosti DIM (p = 0,000), LD (p = 0,018) i PPIM (p = 0,000) nego bolesnici iz grupe I. U skladu sa očekivanjima, bolesnici sa nivoom CRP iznad 10 mg/l imali su značajno veće vrednosti SE (p = 0,000) i fibrinogena (p = 0,000) u poređenju sa bolesnicima čiji je CRP bio niži od 10 mg/l.

Radi određivanja značaja pojedinih varijabli [starosti, pola, dužine dijaliziranja, pušenja, lipidnog statusa, malnutricije (SGP), albumina, fibrinogena, CRP-a] u objašnjenju PPIM kao zavisne varijable, upotrebljen je *stepwise* metod višestruke linearne regresione analize. Koeficijent determinacije regresionog modela iznosi $R^2 = 0,550$, što govori da je približno 55% varijabiliteta ishodne varijable PPIM objašnjeno koegzistencijom regresora iz modela prikazanog u Tabeli 3, dok izolovani nivo CRP može objasniti čak 38% variranja u vrednostima PPIM. Model linearne povezanosti između PPIM kao zavisne varijable i CRP-a kao nezavisne varijable, grafički je prikazan u Grafikonu 2.



Grafikon 2. Povezanost između CRP i PPIM. Granice 95% intervala poverenja su prikazane sa obe strane regresione linije R = 0.619; p< 0,001

Graph 2. *Relationship between CRP and CSIM area. 95% confidence limits are shown on either side of the regression line. R = 0.619, P <0.001*

Diskusija

Rezultati ultrazvučnog pregleda ukazuju da bolesnici na HD imaju veću težinu i rasprostranjenost ranih lezija ateroskleroze u karotidnim arterijama iskazano preko površine preseka intime-medije i lumen-dijametra od zdravih osoba srodne starosti i pola. Bliska korelacija između stepena aterosklerotskih lezija u karotidnim arterijama vrata i u koronarnim arterijama dokazana je u autopsijskim studijama još pre nekoliko decenija [12]. Stoga, ehosonografska evaluacija karotidnih arterija ima dijagnostički značaj kod bolesnika sa suspektnom koronarnom bolešću, ali i značaj u predviđanju rizika akutnog infarkta miokarda [13]. Od ultrazvučnih markera rane ateroskleroze u dosadašnjim ispitivanjima je najčešće korišćena DIM, mada je Jogestrand sa sar. pokazao da se izračunavanjem PPIM kompenzuje distenzija arterijskog zida izazvana povišenim krvnim pritiskom, koja je ponekad razlog merenja lažno nižih vrednosti DIM [11]. Nedavno je

early atherosclerosis lesions in carotid arteries, expressed through CSIM area and LD, than healthy age- and gender- matched persons. Close correlation between the level of atherosclerotic lesions in carotid arteries and coronary arteries has been confirmed in autopsy studies several decades ago [12]. Hence, ultrasonographic evaluation of carotid arteries has a diagnostic significance in patients with suspected coronary disease, but also a predictive significance in predicting future risk of acute myocardial infarction [13]. Of ultrasonographic markers of early atherosclerosis in previous investigations most frequently used was IMT, although Jogestrand et al showed that calculation of CSIM area compensates variations in artery distention secondary to changes in blood pressure, which is sometimes a cause for measuring falsely lower IMT values [11]. It has been confirmed recently that CSIM area is a much better predictor of coronary atherosclerosis than IMT [14].

Although without clinical signs of inflammation, our results showed that serum level of CRP was higher in 58% of examined HD patients. This is in line with data from literature in which prevalence of inflammation in HD patients varies considerably in different countries: Zimmermann et al recorded higher levels of CRP in 46% stable HD patients in Germany, Yeun et al established inflammation prevalence of 51% in American HD population, while Zoccali et al came up with a prevalence of 62%

dokazano da PPIM ima i veću prediktivnu vrednost za koronarnu aterosklerozu od DIM [14].

Iako bez kliničkih znakova inflamacije, naši rezultati su pokazali da je serumski nivo CRP bio povišen kod 58% ispitivanih bolesnika na HD. To je u saglasnosti sa podacima iz literature u kojima zastupljenost inflamacije kod bolesnika na HD značajno varira u različitim zemljama: Zimmermann i saradnici su zabeležili povišene nivoe CRP kod 46% stabilnih HD bolesnika u Nemačkoj, Yeun i saradnici su naveli prevalenciju inflamacije od 51% u američkoj populaciji HD bolesnika, dok je Zoccali sa saradnicima objavio prevalenciju od 62% medu bolesnicima na HD u Italiji [15]. Iako se u patofiziologiji hroničnog inflamatornog stanja razmatra potencijalna uloga brojnih egzogenih i endogenih faktora, većina autora se slaže da je uzroke aktivacije reaktanata akutne faze kod ovih bolesnika najčešće teško ili čak nemoguće otkriti. U studiji Panishija i saradnika, iz grupe bolesnika koji su dijalizirani najmanje 6 meseci različitim dijaliznim modalitetima, isključeno je već na početku 18,6% bolesnika zbog verifikovanih patoloških stanja koja su pouzdano udružena sa visokim vrednostima CRP. Od preostalih bolesnika čak 47% je imalo povišene vrednosti CRP, što potvrđuje visoku zastupljenost mikroinflamacije kod prividno stabilnih bolesnika na HD [16].

Analiza nutritivnih parametara je pokazala da bolesnike iz grupe I odlikuju značajno niže vrednosti SGP skora i niže serumske koncentracije albumina u odnosu na bolesnike iz grupe II. S obzirom da nije dokazana značajna razlika u vrednostima ostalih nutritivnih parametara izmedu dve grupe bolesnika, naši rezultati ukazuju na mogućnost da su i SGP i nivo albumina u serumu u značajnoj meri uplivisani aktivnošću inflamatornog odgovora. Iako se termin hipoalbuminemija kod bolesnika sa terminalnom fazom HBI već dugo koristi kao sinonim za malnutriciju, nedavno su Kaysen [17] i Qureshi [18] utvrdili da je aktivnost akutne faze odgovora nezavisan prediktor niskog nivoa serumskih albumina kod bolesnika na HD. Hronična inflamacija redukuje serumsku koncentraciju albumina prvo povećanjem brzine katabolizma, a zatim inhibicijom normalnih homeostatskih mehanizama koji učestvuju u održavanju ravnoteže izmedu sinteze i brzine katabolizma [19]. Može se zaključiti da je inflamacija faktor koji komplikuje upotrebu serumskog albumina kao pojedinačnog indeksa nutritivnog statusa kod bolesnika na HD.

Rezultati našeg istraživanja ukazuju da bolesnici sa povišenim vrednostima CRP imaju značajno veće vrednosti svih ultrazvučnih markera ateroskleroze, kao i češće klinički ispoljenu aterosklerozu u odnosu na bolesnike sa normalnim vrednostima CRP. *Stepwise* multipla regresiona analiza je pokazala da je nivo CRP najvažniji prediktor karotidne ateroskleroze koji sam objašnjava 38,3% opserviranih razlika u PPIM, dok uključivanje još dva faktora rizika (starost i pušenje) u regresioni model objaphysiology of a chronic inflammatory condition a potential role of numerous exogenic and endogenic factors is considered, majority of authors agree that causes of activation of acute phase reactants in these patients is most frequently difficult or even impossible to detect. In study of Panishi et al. from group of patients who had been dialyzed for at least 6 months, at the very beginning some 18.6% patients were excluded for verified pathological condition which was positively associated with high values of CRP. Of the remaining patients, even 47% had higher CRP values which confirms high prevalence of microinflammation in apparently stable HD patients [16].

Analysis of nutritional parameters showed that patients in group I are characterized by significantly lower values of SGA scores and lower serum concentrations of albumin compared to patients in group II. Since no significant difference in values of other nutritional parameters between the two groups was confirmed, our results show a possibility that both SGA and level of albumin in serum are largely affected by the inflammatory response. Although the term hypoalbuminemia in patients with ESRD has been used for a long time as a synonym for malnutrition, Kaysen [17] and Qureshi [18] have recently confirmed that acute response phase is an independent predictor of a low level of serum albumin in HD patients. Chronic inflammation reduces serum concentration of albumin, first by accelerating catabolism and then by inhibition of normal homeostatic mechanisms which participate in preserving balance between the rate of albumin synthesis and albumin fractional catabolic rate [19]. It can be concluded that inflammation is a factor which further complicates the use of serum albumin as a single index of nutritional status in HD patients.

Results of our investigation show that patients with higher values of CRP have significantly higher values of all ultrasonographic markers of atherosclerosis, as well as more frequent clinical atherosclerotic vascular disease in comparison with patients with normal values of CRP. Stepwise multiple regression analysis showed that the level of CRP is the most important predictor of carotid atherosclerosis, which explains 38.3% observed differences in the CSIM area, while inclusion of two more risk factors (age and smoking) in regression model explains the total of 55% variability in the CSIM area. The fact that inflammation plays an important role in all phases of atherosclerosis, was of decisive importance for changing the pathogenic concept of atherosclerosis from degenerative to chronic inflammatory disease [20]. In the last years, numerous studies have confirmed the predictive importance of inflammation for overall and cardiovascular mortality of dialyzed patients, but the relation

šnjava ukupno 55% varijabiliteta PPIM. Činjenica da inflamacija ima važnu ulogu u svim fazama ateroskleroze bila je od presudnog značaja za izmenu patogenetskog koncepta ateroskleroze od degenerativne do hronične inflamatorne bolesti [20]. Poslednjih godina su brojne studije dokazale prediktivni značaj inflamacije za ukupni i kardiovaskularni mortalitet bolesnika na dijalizi, ali je poveza- nost pojedinih inflamatornih markera sa ranim strukturalnim promenama ateroskleroze slabije izučavana.

Cross-sectional studija Stenvinkela i saradnika je pokazala da CRP predstavlja nezavisan faktor rizika za povećanu PPIM već kod bolesnika u predijaliznoj fazi HBI [5]. Nedavno je saopštena značajna korelacija DIM sa logaritmovanim vrednostima CRP i koncentracijama cirkulišućih endotelnih adhezivnih molekula kod bolesnika na HD [21]. Druga studija *cross-sectional* dizajna je označila logaritmovanu vrednost cirkulišućeg IL-6 kao značajan prediktor DIM i PPIM u istoj populaciji bolesnika [22]. Zoccali sa saradnicima je prvo pokazao u *cross-sectional* studiji da je CRP nezavisno povezan sa karotidnom aterosklerozom kod bolesnika na hroničnom dijaliznom tretmanu [23], da bi u prospektivnoj kohortnoj studiji koja je obuhvatila samo bolesnike na HD interakcija između CRP i asimetričnog dimetilarginina postala jedini nezavisni prediktor lezija intime [24]. Očigledno je da još uvek nedostaju prospektivne i dobro kontrolisane studije koje bi sa više preciznosti definisale ulogu i značaj pojednih inflamatornih markera u ubrzanoj aterogenezi vezanoj za HBI i HD.

Zaključak

Težina i rasprostranjenost ranih aterosklerotskih lezija u karotidnim arterijama bolesnika na HD i dugotrajna izloženost brojnim tradicionalnim i netradicionalnim faktorima rizika čine ovu grupu bolesnika visokorizičnom za razvoj kardiovaskularnih komplikacija uznapredovale ateroskleroze. Nedavni ubedljivi dokazi o ulozi inflamacije u svim fazama ateroskleroze pobudili su interesovanje za šira istraživanja prognostičke vrednosti cirkulišućih inflamatornih markera. Ako se ima u vidu prognostička vrednost CRP za ukupni i kardiovaskularni mortalitet bolesnika na HD, određivanje serumske koncentracije CRP bi moglo imati dodatnu ulogu u identifikaciji visokorizičnih bolesnika.

Povišene serumske koncentracije CRP su opisane kod značajnog procenta bolesnika na HD i u našem radu su direktno povezane sa svim ultrazvučnim markerima supkliničke ateroskleroze. Statistički značajna povezanost serumske koncentracije CRP sa PPIM karotidnih arterija ukazuje da sistemska inflamacija niskog stepena može biti značajan doprinoseći faktor ranoj fazi karotidne ateroskleroze. Stoga bi preventivne i terapijske mere usmerene na redukciju hroničnog inflamatornog stanja mogle biti značajne u smanjenju kardiovaskularnog rizika bolesnika na HD.

ship between some inflammatory markers with early structural changes of atherosclerosis has been studied insufficiently.

The cross-sectional study of Stenvinkel et al has shown that CRP poses an independent risk factor for enlarged CSIM area, already in the pre-dialysis phase of ESRD [5]. Recently, a significant correlation of IMT with logarithmic CRP values and concentrations of circulating endothelial cell adhesion molecules in HD patients has been reported [21]. Another study of cross-sectional design marked a logarithmic value of circulating IL-6 as a significant predictor of IMT and CSIM area in the same population of patients [22]. Zoccali et al have first showed in a cross-sectional study that CRP is independently related to carotid atherosclerosis in chronic dialysis patients [23], while in a prospective cohort study which included only HD patients, interaction between CRP and asymmetric dimethylarginine became the only independent predictor of intimal lesions [24]. It is obvious that we still need prospective and well-controlled studies which would, with more precision, define the role and importance of some inflammatory markers in accelerated atherogenesis related to ESRD and HD.

Conclusion

Severity and dissemination of early atherosclerotic lesions in carotid arteries of HD patients and long-term exposure to numerous traditional and non-traditional risk factors, make this group of patients at high risk for development of cardiovascular complications of advanced atherosclerosis. Recent convincing proofs on the role of inflammation in all phases of atherosclerosis, raised interest in wider investigations of circulating inflammatory markers and their prognostic values. If we take into account the prognostic value of CRP for overall and cardiovascular mortality in HD patients, determining serum concentrations of CRP could have an additional role in identification of high risk patients.

Increased serum concentrations of CRP were described in a significant percentage of HD patients and in our work are directly related to all ultrasonographic markers of subclinical atherosclerosis. Statistically significant relationship of serum concentrations of CRP with CSIM area of carotid arteries shows that systemic inflammation of low degree can be a significant contributing factor to early phase of carotid atherosclerosis. Hence, preventive and therapeutic measures directed to reduction of chronic inflammatory conditions could be of significance in reducing cardiovascular risk in HD patients.

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