Biokemijski pokazatelji koštane pregradnje u poremećaju koštanog metabolizma bolesnika na kroničnom liječenju dijalizom i onih s presatkom bubrega

Bone markers in metabolic bone disorder in patients on chronic hemodialysis and kidney transplant recipients

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Sažetak

Uvod: Poremećaj koštanog metabolizma je česta pojava u kroničnom bubrežnom zatajivanju koje se liječi kroničnom dijalizom i presađivanjem bubrega. Ovo stanje obilježava ubrzana ili usporena koštana pregradnja s posljedičnim nastankom osteopenije ili osteoporoze i povećanog rizika prijeloma. Mjerenje biokemijskih pokazatelja koštane pregradnje omogućuje neizravan uvid u koštanu pregradnju i njene promjene u tijeku ove bolesti. U ovom smo istraživanju izmjerili pokazatelje koštane izgradnje i razgradnje u bolesnika na kroničnom liječenju dijalizom i u onih s presađenim bubregom, a rezultate analizirali s obzirom na čimbenike rizika ovog stanja, i to: koncentraciju paratireoidnog hormona (PTH), životnu dob, spol, trajanje liječenja dijalizom i razdoblje nakon transplantacije.

Materijali i metode: u 79 bolesnika (50 muškaraca, 29 žena) na kroničnom liječenju dijalizom i 36 bolesnika (20 muškaraca, 16 žena) s presađenim bubregom u krvi je izmjerena koštana alkalna fosfataza (engl. *bone alkaline phosphatase*, BALP) kao pokazatelj izgradnje, C-terminalni telopeptid kolagena tip I (CTX, engl. crosslaps) kao pokazatelj koštane razgradnje i intaktni PTH komercijalnim kompletima reagensa. Klinički podaci su uključili dob, trajanje liječenja dijalizom i razdoblje nakon transplantacije.

Rezultati: U bolesnika na kroničnom liječenju dijalizom je koštana alkalna fosfataza bila statistički značajno niža (18,27 IU/L, p<0,0005), a CTX (1,85 ug/L, p<0,0005) i PTH (21,99 pmol/L, p<0,001) viši u odnosu na bolesnike s presađenim bubregom (CTX 1,18 ug/L, PTH 6,34 pmol/L, BALP 45,78 IU/L). U obje skupine je trajanje dijalize koreliralo značajno i pozitivno s koštanom alkalnom fosfatazom (pacijenti na hemodijalizi r=0,318, p=0,005, pacijenti s bubrežnim presatkom r=0,488, p=0,003) i C-terminalnim telopeptidom (pacijenti na hemodijalizi r=0,338, p=0,002, pacijenti s bubrežnim presatkom

Abstract

Background: Chronic kidney failure treated by chronic dialysis and kidney transplantation is characterised by disorder of bone metabolism and high or low bone turnover, resulting in osteopenia/osteoporosis and increased fracture risk. Measurement of bone markers enables indirect insight into the rate of bone remodeling and its changes in the course of disease. In this study bone formation and bone resorption markers were measured in patients on chronic hemodialysis treatment and in kidney transplant recipients with regard to specific risk factors of this metabolic bone disorder, i.e. parathyroid hormone levels (PTH), age, sex, hemodialysis duration and post-transplant period.

Materials and methods: Blood samples were obtained from 79 patients (50 men, 29 women) on chronic hemodialysis and 36 patients (20 men, 16 women) with kidney transplant for measurement of bone alkaline phosphatase (BALP) as a bone formation marker, C-terminal telopeptide of collagen type I (crosslaps) as a bone resorption marker and intact PTH by commercial kits. Data on age, duration of hemodialysis and post-transplant period were included as risk factors for bone disorder.

Results: Patients on chronic hemodialysis had significantly higher crosslaps (1.85 ug/L, p<0.0005) and PTH (21.99 pmol/L, p<0,001), and lower BALP (18.27 IU/L, p<0.0005) in comparison to kidney transplant recipients (crosslaps 1.18 ug/L, PTH 6.34 pmol/L, BALP 45.78 IU/L). In both patient groups hemodialysis duration correlated significantly and positively with BALP (hemodialysis patients r=0.318, p=0.005, kidney transplat recipients r=0.488, p=0.003) and crosslaps (hemodialysis patients r=0.338, p=0.002, kidney transplant recipients r=0.365, p=0.03), and in hemodialysis patients with PTH (r=0.255, p=0.03). A statistically significant and positive correlation existed for both patient groups between PTH and BALP (hemodialysis patients

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r=0,365, p=003), a u bolesnika na dijalizi i s koncentracijama PTH (r=0,255, p=0,03). Statistički značajna i pozitivna korelacija postojala je u obje skupine između PTH i koštane alkalne fosfataze (pacijenti na hemodijalizi r=0,522, p=0,0005, pacijenti s bubrežnim presatkom r=0,456, p=0,02), koštane alkalne fosfataze i CTX (pacijenti na hemodijalizi r=0,459, p=0,0005, pacijenti s bubrežnim presatkom r=0,593, p=0,0005), a samo u bolesnika na dijalizi između PTH i CTX (r=0,323, p=0,004). U bolesnika s presađenim bubregom je razdoblje nakon transplantacije koreliralo značajno i negativno s CTX (r=-0,479, p=0,004). Bolesnici na dijalizi bili su značajno stariji (61 godina, p=0,0005) i značajno dulje liječeni dijalizom (79 mjeseci, p=0,04) nego oni s presađenim bubregom (dob 46 godina, liječenje dijalizom 55 mjeseci). Nije bilo razlike između spolova za parametre ispitivane u ovom istraživanju.

Zaključak: Koštana pregradnja, a osobito razgradnja, bila je ubrzana u obje skupine bolesnika. Trajanje liječenja dijalizom i koncentracija PTH pokazali su se kao rizični čimbenici ubrzane koštane pregradnje u ovih bolesnika, a poboljšanje je ovisilo o vremenu proteklom nakon transplantacije. Spol i dob nisu bili povezani s poremećajem koštane pregradnje. Mjerenje biokemijskih pokazatelja koštane pregradnje i PTH važne su laboratorijske pretrage u procjeni i praćenju koštanog poremećaja bolesnika na kroničnom liječenju dijalizom i nakon transplantacije bubrega.

Ključne riječi: biokemijski pokazatelji koštane pregradnje, koštani metabolizam, bubrežna osteodistrofija

r=0.522, p=0.0005, kidney transplant recipients r=0.456, p=0.02), BALP and crosslaps (hemodialysis patients r=0.459, p=0.0005, kidney transplant recipients r=0.593, p=0.0005), and in hemodialysis patients also between PTH and crosslaps (r=0.323, p=0.004). In kidney transplant recipients crosslaps correlated negatively with post-transplant period (r=-0.479, p=0.004). Patients on chronic hemodialysis were significantly older (61 years, p=0.0005) and were significantly longer treated by hemodialysis (79 months, p=0.04) as compared to the kidney transplant recipients (age 46 years, dialysis treatment 55 months). No difference in data existed between sexes in both patient groups.

Conclusions: Bone turnover was increased in both patient groups, in particular bone resorption. Duration of hemodialysis and PTH were recognised as determinants of increased bone turnover in both groups, and normalization of bone resorption was related to post-transplantation period. In this bone disorder sex and age were not established as risk factors for impaired bone turnover. Measurement of bone markers together with PTH is a useful clinical tool in assessment and follow-up of metabolic bone disorders of chronic hemodialysis treatment and kidney transplantation.

Key words: bone markers, bone metabolism, renal osteodystrophy

Uvod

Poremećaj koštanog metabolizma pojavljuje se u većine bolesnika s kroničnim zatajivanjem bubrega liječenih kroničnom dijalizom ponajprije zbog poremećene sinteze kalcitriola te povećanog lučenja paratiroidnog hormona (PTH) (1,2,3). Ostali čimbenici, tj. čimbenici regulacije koštanog metabolizma, također su uključeni, primjerice koštani morfogenetski proteini i osteoprotegerin (4). Tijek bolesti i liječenje djeluju na vrstu poremećaja koštane pregradnje, uz posljedičnu bilo pojačanu ili sniženu koštanu pregradnju (5). Karakteristike pojačane koštane pregradnje su pojačana i ubrzana izgradnja kostiju te vrlo brza koštana razgradnja (6,7). Posljedice na koštani sustav su veći koštani volumen, izgradnja neadekvatne (engl. *woven*) kosti, povećana i intratrabekularna razgradnja, a one dovode do osteopenije ili osteoporoze uz povećan rizik prijeloma (8,9). Osteomalacija ili adinamična kost karakterizirana je niskom koštanom pregradnjom, tj. zaustavljenim obnavljanjem koštanog tkiva i popravkom mikroprijeloma, te posljedičnim povećanim rizikom prijeloma (9). Trajanje zatajivanja bubrega i liječenja dijalize, lučenje PTH te njegova terapijska kontrola važne su odrednice tog koštanog poremećaja.

Nakon uspješnog presađivanja bubrega većina je metaboličkih poremećaja riješena, no normalizacija paratiroidne hipersekrecije te poremećaja koštanog metabolizma može trajati 2-4 godine (2,10,11,12). Povećana pregradnja kostiju u bolesnika s presatkom bubrega povezana je s tra-

Introduction

Metabolic bone disorder occurs in most patients with chronic kidney failure treated by chronic dialysis primarily due to impaired calcitriol synthesis and increased secretion of parathyroid hormone (PTH) (1,2,3). Other factors, i.e. bone regulatory proteins, have also been implicated, e.g. bone morphogenetic proteins and osteoprotegerin (4). The course of the disease and treatment has an effect on the type of bone turnover disorder, resulting in either high or low bone remodeling (5). High bone turnover is characterized by increased and accelerated bone formation, and high bone resorption rate (6,7). The consequences on the skeletal system are greater osteoid volume, formation of woven bone, increased and intratrabecular resorption leading to osteopenia or osteoporosis with greater fracture risk (8,9). Osteomalacia or adynamic bone are characterised by low bone turnover, i.e. arrested renewal of bone tissue and microfracture repair also resulting in increased fracture risk (9). Duration of kidney failure and dialysis treatment, PTH secretion and its therapeutic control are important determinants of this bone disorder. After successful kidney transplantation most metabolic disturbances are resolved, but normalization of parathyroid hypersecretion and disorder of bone metabolism may take 2-4 years (2,10,11,12). Increased bone turnover in kidney transplant recipients is related to previous dialysis duration, PTH levels and the time since transplantation. The main condition is additionally aggravated by immu-

janjem prethodne dijalize, koncentracijama PTH te vremenu od presađivanja. Osnovno je stanje dodatno otežano imunosupresivima i kortikosteroidima, tj. lijekovima koji su štetni za cjelovitost skeleta i koji se primjenjuju u razdoblju nakon presađivanja (npr. 13,14,15,16).

Procjena stanja skeleta i koštanog poremećaja u bolesnika na kroničnoj dijalizi i onih s presatkom bubrega postiže se mjerenjem mineralne gustoće kosti, histološkom/histomorfometrijskom analizom biopsije kosti, te određivanjem biokemijskih pokazatelja koštane pregradnje (17). Produkti koštanih aktivnosti mjere se u serumu i mokraći radi pokazatelja koštane izgradnje i razgradnje. Istraživanje koštanih biljega uglavnom je ukazalo na pojačanu koštanu pregradnju u bolesnika na liječenju dijalizom kao i onih s presatkom bubrega (7,18,19,20). Usporedba koštanih biljega i histoloških nalaza kosti naznačila je da bi koštani poremećaj karakteriziran visokom pregradnjom kostiju mogao biti otkriven koštanim biljezima (21,22,23,24,25). U slučaju snižene koštane pregradnje mjerenje normalnih ili sniženih razina koštanih biljega nije bilo prihvaćeno za dijagnosticiranje tog stanja. Studije o kliničkoj iskoristivosti koštanih biljega u bolesnika na kroničnom liječenju dijalizom i onih s presatkom bubrega razlikuju se ovisno o određenim koštanim biljezima i karakteristikama bolesničke populacije. Kako bismo pridonijeli boljem razumijevanju ovoga koštanog poremećaja, cilj je ove studije bio analizirati biljege koštane izgradnje i razgradnje u dvije odvojene skupine bolesnika, tj. u bolesnika na kroničnom liječenju hemodijalizom i bolesnika nakon presađivanja bubrega, a s obzirom na specifične čimbenike rizika za poremećaj koštanog metabolizma.

Materijali i metode

Uzorci krvi bili su prikupljeni tijekom rutinskog praćenja koštanog poremećaja u 79 bolesnika (50 muškaraca, 29 žena) na kroničnoj hemodijalizi, te 36 bolesnika (20 muškaraca, 16 žena) s postojanim presatkom bubrega. Biopsija kosti nije provedena u tih bolesnika, no klinički podatci nisu ukazali na adinamičnu bolesti kosti. Nijedan bolesnik nije imao šećernu bolest. Uzorci krvi uzeti su nakon 12-satnog gladovanja te prije liječenja hemodijalizom u bolesnika sa zatajivanjem bubrega, serumi su odvojeni i zamrznuti (-20 °C) do analize najdulje jedan mjesec. U ovom su ispitivanju korišteni uzorci seruma preostali nakon rutinskih biokemijskih pretraga za praćenje bolesnika.

Bolesnici na hemodijalizi bili su podvrgnuti 4-satnoj terapiji po presađivanju bubrega triput tjedno, a koncentracija kalcija u dijalizatu bila je u prosjeku 1,50 mmol/L (raspon 1,25-1,75 mmol/L). Standardna terapija za te bolesnike uključivala je fosfatna veziva (kalcijev karbonat, Sevelamer hidroklorid) i/ili kalcitriol prema kliničkim zahtjevima i laboratorijskim pretragama.

nosuppressives and corticosteroids, the drugs deleterious to skeletal integrity which are administered in the post-transplant period (e.g. 13,14,15,16).

Assessment of the skeletal status and bone disorder in patients on chronic dialysis and kidney transplant recipients is accomplished by measurement of bone mineral density, histologic/histomorphometric analysis of bone biopsy and determination of biochemical bone markers (17). Products of bone cell activities are measured in serum and urine for indicators of bone formation and bone resorption. Investigation of bone markers has mostly shown increased bone turnover in patients on dialysis and also in kidney transplant patients (7,18,19,20). Comparison of bone markers and bone histology indicated that the bone disorder characterized by high bone turnover could be established by bone markers (21,22,23,24,25). In the case of low bone turnover, measurement of normal or decreased levels of bone markers was not found acceptable for diagnosis of this condition. The reports on clinical utility of bone markers in patients on chronic dialysis treatment and kidney transplant recipients differ depending on bone markers measured and patient population characteristics. In order to contribute to the better understanding of metabolic bone disorder, the aim of this study was analysis of bone formation and bone resorption markers in two separate groups of patients, those on chronic hemodialysis treatment and after kidney transplantation with regard to specific risk factors of this disorder.

Materials and methods

Blood samples were collected in the course of routine monitoring of bone disorder in 79 patients (50 men, 29 women) on chronic hemodialysis and 36 patients (20 men, 16 women) with stable kidney transplant. Bone biopsy was not performed in these patients, but clinical data did not indicate adynamic bone. None of the patients had diabetes. Blood was withdrawn after an overnight fast and before hemodialysis treatment in patients with kidney failure, the sera separated and stored frozen (-20 °C) till assayed within one month of storage. The sera remaining after routine biochemical tests for patient monitoring were used in this investigation.

Hemodialysis patients underwent renal replacement treatment for 4 hours 3 times a week, calcium concentration in dialysis fluid was on average 1.50 mmol/L (range 1.25-1.75 mmol/L). Standard therapy for these patients included phosphate binders (calcium carbonate, Sevelamer hydrochloride) and/or calcitriol according to clinical requirements and laboratory tests.

Immunosuppressive therapy in kidney transplant recipients consisted of Cyclosporin A, prednisone and azatioprine mofetil mycophenolate. The mean serum creatinine concentration for this patient group was 119.7 ± 20.5 µmol/L

Imunosupresivna terapija u bolesnika s presatkom bubrega sastojala se od ciklosporina A, prednizona i azatioprin-mofetil-mikofenolata. Prosječna koncentracija kreatinina u serumu u toj skupini bolesnika bila je 119,7 ± 20,5 µmol/L (raspon 79-168 µmol/L), a klirens kreatinina 70,2 ±13,5 mL/minuti (raspon 51-112 mL/minuti). Podatci o dobi, trajanju hemodijalize i poslijetransplantacijskom periodu za bolesnike uključene u ovu studiju prikazani su u tablici 1. U skupinama bolesnika nije bilo razlika između spolova s obzirom na dob, trajanje hemodijalize ili poslijetransplantacijskog perioda (tablica 1).

Tablica 1. Klinički podatci za skupine bolesnika prikazani deskriptivnom statistikom (aritmetička sredina = X, standardna devijacija = SD, raspon).

Sljedeći su biokemijski parametri mjereni komercijalnim kompletima reagensa prema uputama proizvođača: intaktni PTH (iPTH, IBL, Njemačka, referentni interval 1-6 pmol/L; približno trostruka vrijednost gornje granice referentnog intervala preporuča se za bolesnike na liječenju dijalizom), koštana alkalna fosfataza (Ostase BAP, IDS, Tyne&Wear, V. Britanija, granica referentnog intervala <22 IU/L) kao biljeg koštane izgradnje, te C-terminalni telopeptid kolagena tipa I (CTX) (Crosslaps Serum, Nordic Bioscience Diagnostics, Herlev, Danska, granica referentnog intervala <0,9 ug/L) kao biljeg koštane razgradnje. U slučaju različitih referentnih intervala s obzirom na spol, vrijednosti za žene u postmenopauzi korištene su kao najviši rezultat u zdravih osoba.
Statističke su analize provedene uz uobičajeni statistički paket (Microsoft® Office Excell) koji uključuje opisnu statistiku, procjenu korelacije i regresije, te Studentov t-test (dvosmjerni) za razliku između prosječnih vrijednosti uz razinu značajnosti p<0,05.

Rezultati

Podatci o PTH i koštanim biljezima nisu ukazali na razlike nakon ispitivanja t-testom među spolovima u svakoj skupini bolesnika. Stoga su daljnje statističke analize provedene za čitavu skupinu bolesnika na liječenju hemodijalizom te bolesnike s presatkom bubrega.

(range 79-168 µmol/L) and creatinine clearance was 70.2 ±13.5 mL/minute (range 51-112 mL/minute).
Data on age, duration of hemodialysis and post-transplant period for the patients comprised in this study are presented in Table 1. No difference existed between sexes regarding age, hemodialysis duration or post-transplant period for each group of patients.
The following biochemical parameters were measured by commercial kits according to manufacturers' recommendations: intact PTH (iPTH, IBL, Germany, reference range 1-6 pmol/L; for dialysis patients approximately 3x upper reference limit is recommended), bone alkaline phosphatase (Ostase BAP, IDS, Tyne&Wear, UK, reference limit <22 IU/L) as a bone formation marker, and C-terminal telopeptide of collagen type I (Crosslaps Serum, Nordic Bioscience Diagnostics, Herlev, Denmark, reference limit <0.9 ug/L) as a bone resorption marker. In case of different reference ranges for sexes, the values for postmenopausal women were used as the highest result in healthy individuals.
Statistical analyses were performed by a standard statistical package (Microsoft® Office Excell) including descriptive statistics, correlation and regression assessment, student's t-test (two-tail) for difference between means with significance level p<0.05.

Table 1. Clinical characteristics of the studied patient groups as presented by descriptive statistics (arithmetic mean = X, standard deviation = SD, range).

	Chronic hemodialysis patients X±SD (range)	Kidney transplant recipients X±SD (range)
Age (years)	61±14 (24–83)	46±11(21–66)
Hemodialysis treatment (months)	79±73 (6–341)	55±49 (4–218)
Post-transplant period (months)	/	37±56 (2–224)

Results

Data on PTH and bone markers showed that no difference existed as tested by t-test between sexes in each of the patient groups. Further statistical analyses were thus carried out for the entire group of patients on hemodialysis or kidney transplant recipients.
Based on the two tail test, patients on chronic hemodialysis were significantly older (p=0.0005) and were significantly longer treated by hemodialysis (p=0.04) as compared to kidney transplant recipients. Differences in PTH and bone marker levels were tested by t-test between patient groups (Table 2). Patients on chronic hemodia-

Tablica 2. Rezultati t-testa između podataka bolesnika na kroničnoj dijalizi i s presatkom bubrega prikazani aritmetičkom sredinom i standardnom devijacijom, veličinom skupine (n) i postotkom povišenih rezultata pretraga u zagradi. Važna napomena – gornja gradnica referentnog intervala za PTH u bolesnika na dijalizi iznosi 18 pmol/L, a u onih s transplantiranim bubregom 6 pmol/L.

Table 2. Results of t-test between patients on chronic hemodialysis and kidney transplant recipients. Data are presented as mean and standard deviation, patient population size (n) and percentage of increased values in brackets. Please note that increased PTH for patients on chronic hemodialysis are greater than 18 pmol/L and in kidney transplant recipients greater than 6 pmol/L.

	Hemodialysis patients	Kidney transplant recipients	T-test (p value)
PTH (pmol/L)	21.99±27.32; n=76 (35%)	6.34±4.19; n=26 (42%)	0.001
BALP (IU/L)	18.27±16.23; n=79 (25%)	45.78±43.79; n=36 (67%)	0.0005
CTX (ug/L)	1.85±1.08; n=79 (76%)	1.18±0.71; n=36 (56%)	0.0005

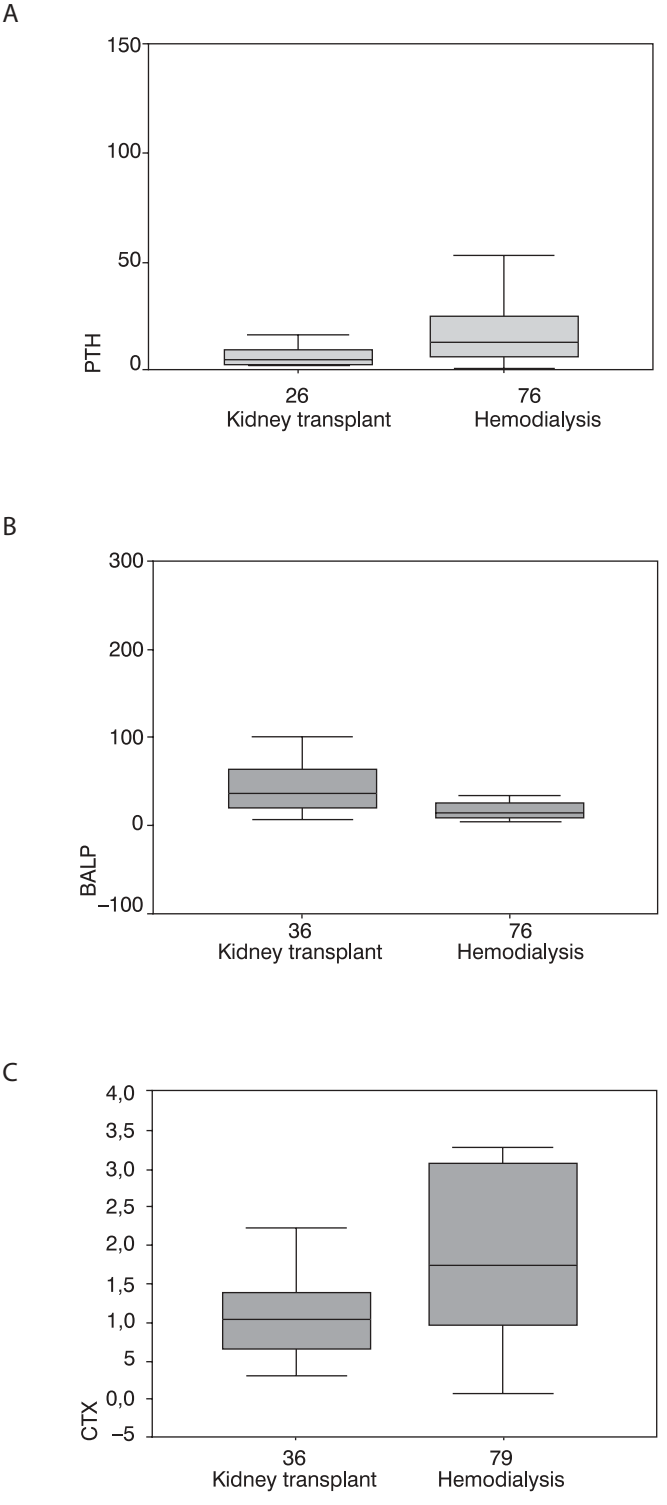
Na temelju dvosmjernog testa (engl. *two tail test*) bolesnici uključeni u kroničnu hemodijalizu bili su značajno stariji (p=0,0005) te značajno dulje podvrgnuti liječenju hemodijalizom (p=0,04) u usporedbi s bolesnicima s presatkom bubrega. Razlike u koncentracijama PTH i koštanih biljega bile su ispitane t-testom između skupina bolesnika (Tablica 2).

Bolesnici uključeni u kroničnu hemodijalizu imali su značajno niži BALP, viši CTX i PTH u usporedbi s bolesnicima s presatkom bubrega (Slika 1).

Shematski prikazi također omogućuju zapažanje većih raspona za PTH i CTX u bolesnika na hemodijalizi, dok su varijacije slabije izražene u bolesnika s presatkom bubrega. U dijelu bolesnika iz obje skupine PTH i koštani biljezi bili su iznad gornje granice referentnog intervala, a postotci povišenih rezultata naznačeni su za svaki parametar i svaku skupinu bolesnika u tablici 2. Viši postotci povišenih rezultata utvrđeni su u bolesnika s presatkom bubrega za PTH i BALP, a u bolesnika uključenih u kroničnu hemodijalizu za CTX. Kod vrednovanja rezultata PTH potrebno je uzeti u obzir da su referentne granice bile različite, tj. približno triput više za bolesnike uključene u kroničnu dijalizu. Procjena korelacija je učinjena radi ispitivanja odnosa između biokemijskih parametara i istraživanih kliničkih podataka. U bolesnika na kroničnoj hemodijalizi trajanje liječenja hemodijalizom je značajno i pozitivno koreliralo s PTH, BALP i CTX. Za dob je utvrđeno da negativno korelira s CTX. Statistički značajne i pozitivne korelacije utvrđene su između PTH i BALP te CTX, kao i između BALP i CTX. U bolesnika s presatkom bubrega trajanje prethodne hemodijalize značajno je i pozitivno koreliralo s BALP i CTX, a poslijetransplantacijski period negativno s CTX. Značajna i pozitivna korelacija utvrđena je također između biokemijskih parametara, tj. između PTH i BALP, te BALP i CTX. U tablici 3 prikazane su

lysis had significantly lower BALP, higher crosslaps and PTH in comparison to kidney transplant recipients (Figure 1). Graphic displays also enable observation of greater ranges for PTH and crosslaps in patients on hemodialysis, while variations are less pronounced in kidney transplant recipients. In a proportion of patients in both patients groups, PTH and bone markers were above the reference range, and percentages of increased results were indicated for each parameter and each patient group in Table 2. For PTH and BALP higher percentages of increased results were found in kidney transplant recipients and for crosslaps in chronic hemodialysis patients. When evaluating PTH results, it should be taken into account that reference limits are different, i.e. approximately 3x higher for chronic dialysis patients.

Correlations were assessed in order to examine relationships between biochemical parameters and investigated clinical data. In patients on chronic hemodialysis, the duration of hemodialysis treatment correlated significantly and positively with PTH, BALP and crosslaps. Age was found to correlate negatively with crosslaps. Statistically significant and positive correlations existed for PTH with BALP and crosslaps, and BALP with crosslaps. In kidney transplant recipients previous hemodialysis duration correlated significantly and positively with BALP and crosslaps, post-transplant period negatively with crosslaps. Biochemical parameters were also significantly and positively correlated, PTH with BALP, and BALP with crosslaps. Only statistically significant correlations and linear regression equations for pairs significant in both patients groups are presented in Table 3. Figures 2 and 3 show differences in associations of analyzed parameters between the two patient groups. Relationship of BALP with hemodialysis du-



Slika 1. Podaci za PTH, koštanu alkalnu fosfatazu i β-crosslaps (CTX) statički su se značajno razlikovali između bolesnika s transplantiranim bubregom (*kidney transplant*) i onih na kroničnom liječenju dijalizom (*hemodialysis*) u grafičkom prikazu *box-and-whiskers*.

Figure 1. Data on PTH, BALP and crosslaps were significantly different between kidney transplant recipients (kidney transplant) and patients on chronic dialysis treatment (hemodialysis) as presented by box-and-whiskers plots.

Tablica 3. Statistički značajne korelacije između parova pokazatelja prikazane koeficijentom korelacije (r) i razinom značajnosti (p) su za bolesnike na dijalizi i one s presađenim bubregom. Jednadžba linearne regresije je navedena samo za korelacije koje su bile značajne za obje skupine bolesnika.

Table 3. Statistically significant correlations for pairs of parameters in patients on chronic hemodialysis and kidney transplant recipients presented by correlation coefficient (r) and level of significance (p). Linear regression equations are included only for pairs of variables found to correlate significantly for both patient groups.

Correlating variables	Hemodialysis patients		Kidney transplant recipients	
	r	p	r	p
HD – BALP	0.318	0.005	0.488	0.003
HD – CTX	0.338	0.002	0.365	0.03
HD – PTH	0.255	0.03	/	/
PTH – BALP	0.522	<0.001	0.456	0.02
PTH – CTX	0.323	0.004	/	/
CTX - BALP	0.459	<0.001	0.593	<0.001
CTX - TX	/	/	-0.479	0.004
CTX - age	-0.479	<0.001	/	/

HD – duration of hemodialysis, BALP – bone alkaline phosphatase, CTX – Crosslaps, PTH - parathyroid hormone, TX – duration of post-transplant period

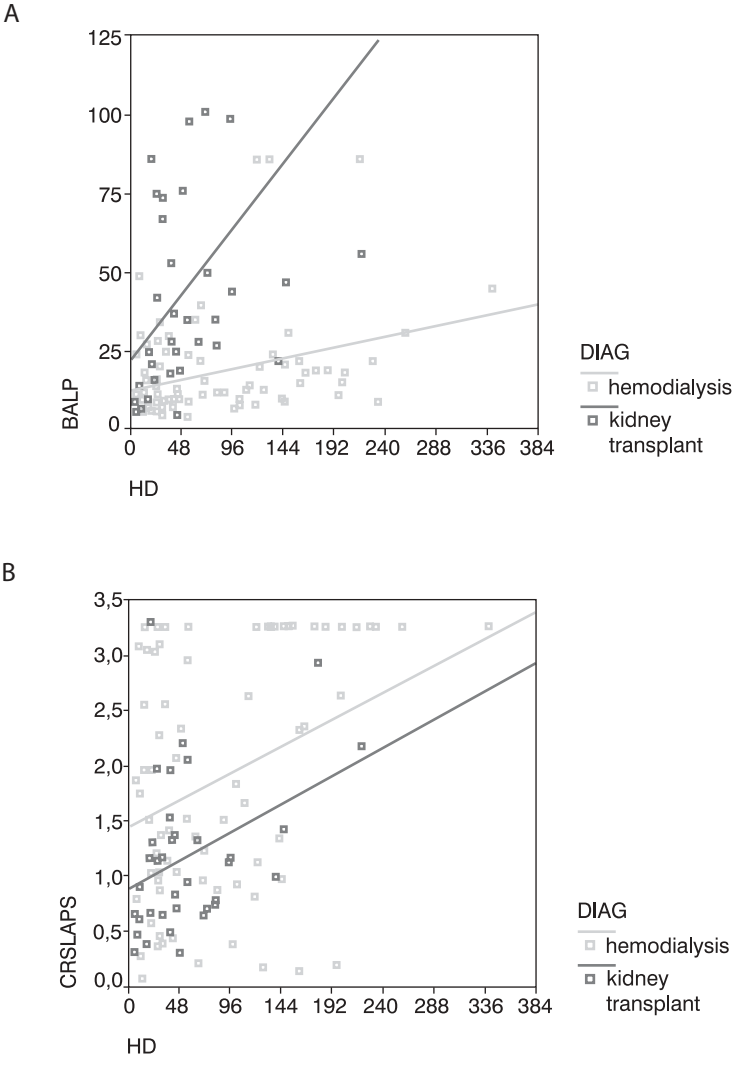
samo statistički značajne korelacije te jednadžbe linearne regresije za parove koji su bili značajni u obje skupine bolesnika. Slike 2 i 3 ukazuju na razlike u povezanosti s analiziranim parametrima između dvije skupine bolesnika. Odnos između BALP i trajanja hemodijalize ukazao je na manji nagib pravca u bolesnika na kroničnoj hemodijalizi te naglo povišenje BALP u odnosu na ranije trajanje hemodijalize u bolesnika s presatkom bubrega. Više koncentracije BALP u bolesnika s presatkom bubrega već su prikazane na slici 1 i u tablici 2. Porast CTX u odnosu na trajanje hemodijalize pokazao se sličnim, no više su vrijednosti zabilježene u bolesnika na kroničnoj hemodijalizi (Slika 2). U odnosima između PTH i BALP te CTX i BALP zapaženi su različiti obrasci povezanosti među bolesnicima na kroničnoj hemodijalizi i onima s presatkom bubrega, uz veće nagibe kod potonjih (Slika 3).

Rasprava

U ovom je istraživanju koštani metabolizam uspoređen u bolesnika na kroničnom liječenju hemodijalizom i onih s presatkom bubrega procjenom PTH i koštanih biljega, te u odnosu na dob, spol, trajanje hemodijalize i razdoblja nakon presađivanja bubrega. Pregradnja kosti je bila povišena u obje skupine bolesnika, osobito koštana razgradnja. Trajanje hemodijalize i PTH prepoznati su kao odrednice povećane pregradnje kosti u obje skupine, a smanjena koštana razgradnja bila je povezana s poslijetransplantacijskim periodom. U tom koštanom poremećaration demonstrated lesser slope in patients on chronic hemodialysis and a steep increase of BALP with previous hemodialysis duration in kidney transplant recipients. Higher BALP levels in kidney transplant recipients were already reported in Figure 1 and Table 2. Increase of crosslaps in relationship to hemodialysis duration showed a parallel pattern, but higher values in patients on chronic hemodialysis (Figure 2). In relationships of PTH with BALP and crosslaps with BALP, different patterns of associations between patients on chronic hemodialysis and kidney transplant recipients were observed, with steeper slopes for kidney transplant recipients (Figure 3).

Discussion

In this investigation bone metabolism was compared in patients on chronic hemodialysis treatment and kidney transplant recipients as assessed by PTH and bone markers, and also with regard to age, sex, hemodialysis duration and post-transplant period. Bone turnover was increased in both patient groups, in particular bone resorption. Duration of hemodialysis and PTH were recognized as determinants of increased bone turnover in both groups, and decrease in bone resorption was related to post-transplantation period. In this bone disorder, sex and age were not established as risk factors for impaired bone turnover.
The measured biochemical parameters PTH and crosslaps were significantly higher in patients on chronic hemodialysis in comparison to kidney transplant recipients, and



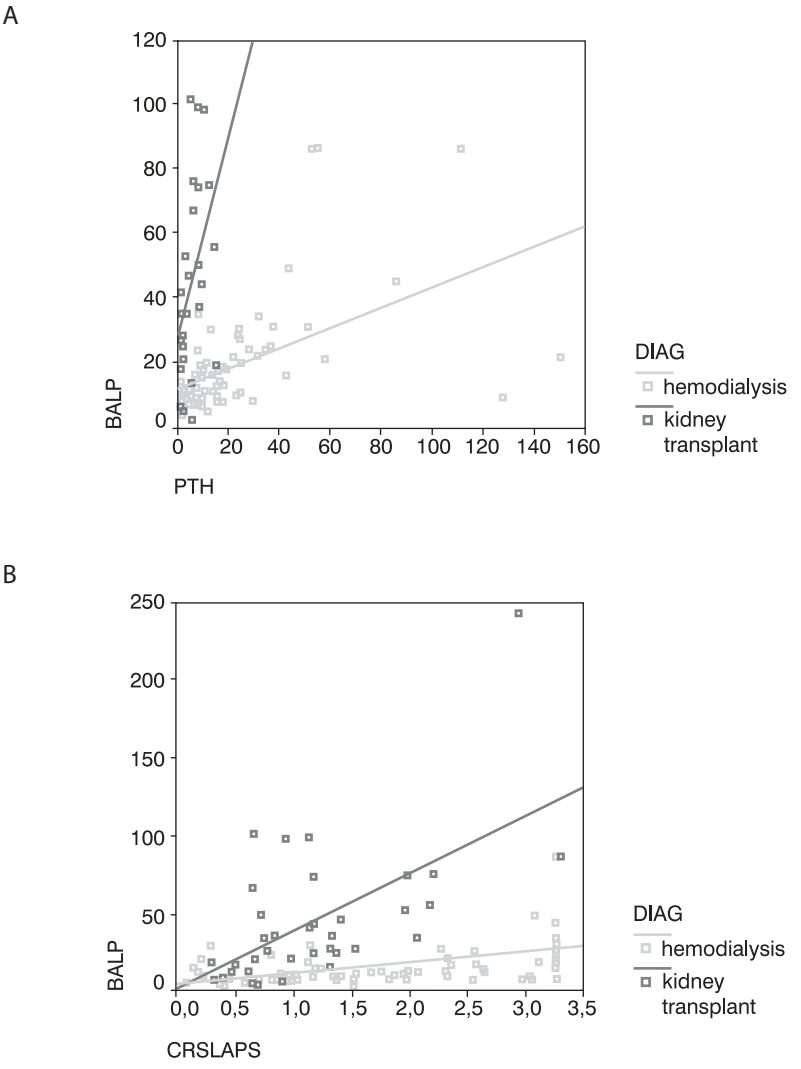
Slika 2. Grafički prikaz odnosa koštane alkalne fosfataze (BALP) i crosslapsa (CRSLAPS) s trajanjem dijalize (HD) u bolesnika na dijalizi (hemodialysis) i onih s presađenim bubregom (kidney transplant). Linearna regresija ukazuje na razliku između skupina bolesnika u povezanosti parova parametara.

Figure 2 Plots of BALP and crosslaps (CRSLAPS) in relationship to duration of dialysis treatment (HD) for patients on chronic dialysis (hemodialysis) and kidney transplant recipients (kidney transplant) with linear regression indicate different associations of analyzed parameters for patient groups.

ju spol i dob nisu bili ustanovljeni kao čimbenici rizika za oslabljenu pregradnju kosti.

Izmjereni biokemijski parametri PTH i CTX bili su značajno viši u bolesnika na kroničnoj hemodijalizi u usporedbi s bolesnicima s presatkom bubrega te su stoga postotci povišenih CTX bili viši. Udio povišenih rezultata PTH bio je niži u bolesnika na kroničnom liječenju hemodijalizom, što je moguće objasniti višom granicom referentnog intervala nego u ispitanika s normalnom bubrežnom funkcijom (tj. približno triput viši od normale). Činjenica da je povišeni PTH utvrđen u samo 35% bolesnika uključenih u

accordingly the percentages of increased crosslaps were higher. The proportion of increased PTH results was lower in chronic hemodialysis patients, which can be explained by higher reference limit than in subjects with normal renal function (i.e. approximately >3x normal). The fact that increased PTH was found in only 35% of chronic hemodialysis patients also suggested efficient clinical control of PTH secretion. Increased bone turnover in patients on chronic hemodialysis can be explained by the metabolic bone disorder that developed already in the course of deterioration of kidney function (26). The main determi-



Slika 3. Grafički prikaz odnosa koštane alkalne fosfataze (BALP) i PTH, te koštane alkalne fosfataze (BALP) i crosslapsa (CRSLAPS) u bolesnika na dijalizi (hemodialysis) i onih s presađenim bubregom (kidney transplant). Linearna regresija ukazuje na razliku između skupina bolesnika u povezanosti parova parametara.

Figure 3. Plots of BALP with PTH and BALP with crosslaps (CRSLAPS) for patients on chronic dialysis (hemodialysis) and kidney transplant recipients (kidney transplant) with linear regression indicate different associations of analyzed parameters for patient groups.

kroničnu hemodijalizu također je ukazao na djelotvornu kliničku kontrolu lučenja PTH. Povišena pregradnja kosti u bolesnika na kroničnoj hemodijalizi može se objasniti poremećajem koštanog metabolizma koji se razvio već tijekom pogoršanja bubrežne funkcije (26). Glavna odrednica ubrzane pregradnje kosti je hipersekrecija PTH, dok su povišeni koštani biljezi posljedica kako povišene aktivnosti koštanih stanica, tako i nakupljanja njihovih produkata zbog oštećenog klirensa bubrega. Neočekivani nalaz ove studije bio je veći postotak povišene aktivnosti koštane

nant of accelerated bone turnover is PTH hypersecretion, while increased bone markers are a consequence of both increased, bone cell activity and accumulation of their products due to impaired kidney clearance. An unexpected finding in this study was higher, and in greater percentage increased, bone alkaline phosphatase in kidney transplant recipients than in patients on chronic hemodialysis. This was in contrast with PTH and crosslaps results for the two patient groups. Alkaline phosphatase is not cleared by the kidney and thus not increased in subjects

alkalne fosfataze u bolesnika s presatkom bubrega nego u bolesnika na kroničnoj hemodijalizi. Taj je nalaz bio u suprotnosti s rezultatima za PTH i CTX u obje skupine bolesnika. Alkalna fosfataza se ne eliminira kroz bubreg te stoga nije povišena u ispitanika s oštećenom bubrežnom funkcijom. Zbog toga se mjerenje koštane alkalne fosfataze smatra adekvatnim biljegom za procjenu koštanog metabolizma u bolesnika na kroničnoj dijalizi (7,27,28). Mogući uzrok povišene BALP u bolesnika s presatkom bubrega mogao bi biti učinak imunosupresivnih lijekova (ciklosporina A) primijenjenih nakon presađivanja bubrega koji mogu potaknuti porast alkalne fosfataze (29,30). U ranom poslijetransplantacijskom razdoblju prolazna je stimulacija koštane pregradnje bila povezana s ciklosporinom A te smanjenim dozama prednizona (31). Zapažene više vrijednosti BALP u bolesnika s presatkom bubrega mogu se pripisati imunosupresivnoj terapiji, no mogle bi također biti specifična obilježja poremećaja koštanog metabolizma u toj populaciji bolesnika. Poznata je činjenica da su bubrežna osteodistrofija i koštani poremećaj u bolesnika na kroničnoj dijalizi i onih s presatkom bubrega karakterizirani velikom promjenljivošću kliničkih oblika, što je uzrokom dijagnostičkih i terapijskih problema (17).

Pozitivna povezanost izgradnje kosti i biljega razgradnje u obje populacije bolesnika ukazala je na vezu između staničnih procesa u kosti u poremećaju koštanog metabolizma (7,32,33). Na razlike između dvije skupine bolesnika u odnosima između trajanja hemodijalize, PTH te CTX i BALP snažno je utjecala viša BALP u bolesnika s presatkom bubrega koja je rezultirala višim nagibom u povezanosti u usporedbi s podatcima za bolesnike na hemodijalizi.

Utvrđene korelacije između biokemijskih parametara i ostalih podataka o bolesniku ukazale su na sličnosti i razlike u karakteristikama kroničnog liječenja hemodijalizom i poslijetransplantacijskog poremećaja. Trajanje hemodijalize i lučenje PTH su odrednice pojačane koštane pregradnje u bolesnika na kroničnom liječenju hemodijalizom, a također u onih s presatkom bubrega (6,20,34,35), što je zapaženo kroz značajne pozitivne korelacije u ovoj studiji. U bolesnika s presatkom bubrega prethodno liječenje hemodijalizom korelira s ispitivanim koštanim biljezima, no ne i s PTH. Uzrok tome mogle bi biti umjereno povišene koncentracije PTH u ovoj skupini bolesnika, uz naglašenu važnost dobre kontrole paratiroidne funkcije tijekom bolesti (36). Nadalje, PTH je korelirao samo s BALP, a ne s CTX kao biljegom koštane razgradnje. Trajanje prijašnje hemodijalize je čimbenik rizika za povećanu koštanu pregradnju nakon presađivanja bubrega, tj. predtransplantacijski koštani poremećaj se nastavlja nakon presađivanja bubrega (13,14,37). Korelacije između trajanja hemodijalize i CTX za obje skupine bolesnika ukazale su da trajanje zatajivanja bubrega ima štetno djelovanje na povećanu koštanu razgradnju (6) koja je bila izrazitija u bolesnika na kroničnoj hemodijalizi nego onih s presatkom bubrega.

with impaired kidney function. Measurement of bone alkaline phosphatase is thus considered an appropriate marker for assessment of bone metabolism in patients on chronic dialysis (7,27,28). Possible cause of higher BALP in kidney transplant recipients could be the effect of immunosuppressive drugs (cyclosporine A) administered after kidney transplantation which may induce a rise in alkaline phosphatase (29,30). In the early post-transplant period a transient stimulation of bone remodeling was associated to cyclosporine A and decreasing prednisone doses (31). The observed higher BALP values in kidney transplant recipients could be attributed to immunosuppresive therapy but might also be a specific trait of bone metabolism disorder in this patient population. It has been is well established that renal osteodystrophy and bone disorder in patients on chronic dialysis and with kidney transplant are characterized by great variability in clinical forms, giving rise to a diagnostic and therapeutic problem (17).

Positive association of bone formation and resorption markers in both patient populations indicated the coupling of the bone cell processes in metabolic bone disorder (7,32,33). Differences between the two patient groups in relationships of hemodialysis duration, PTH and crosslaps with BALP were strongly influenced by higher BALP in kidney transplant recipients resulting in greater slope in associations as compared to the data for patients on hemodialysis.

The correlations found between biochemical parameters and other patient data indicated similarities and differences in characteristics of chronic hemodialysis treatment and post-transplant disorder. Duration of hemodialysis and PTH secretion are determinants of increased bone turnover in patients on chronic hemodialysis treatment and also in kidney transplant recipients (6,20,34,35), as observed in significant positive correlations in this study. In kidney transplant recipients, previous treatment by hemodialysis correlated with the investigated bone markers, but not with PTH. This could be due to moderately increased PTH levels in this patient group, stressing the importance of good control of parathyroid function in the course of the disease (36). In turn, PTH correlated only with BALP, and not with the bone resorption marker crosslaps. Previous hemodialysis duration is a risk factor for increased bone turnover after kidney transplantation, i.e. the pre-transplant bone disorder continues after kidney transplantation (13,14,37). Correlations of hemodialysis duration with crosslaps for both patient groups demonstrated that duration of kidney failure was correspondingly detrimental for increased bone resorption (6), which was more pronounced in chronic hemodialysis patients than in kidney transplant recipients. As previously stated, this was primarily due to higher PTH levels found in patients treated by chronic hemodialysis. Similar to patients treated by chronic hemodialysis, PTH level is also an

Kao što je već ranije navedeno, to se dogodilo ponajprije zbog povišenih koncentracija PTH utvrđenih u bolesnika koji su liječeni kroničnom hemodijalizom. Slično kao kod tih bolesnika, razina PTH je također važna odrednica pojačane koštane pregradnje nakon presađivanja bubrega (2,13,14). To, međutim, nije potvrđeno u ovoj studiji, što je vjerojatno posljedica specifičnosti i veličine uključene skupine bolesnika. Nakon uspješnog presađivanja bubrega koštana se pregradnja normalizira u poslijetransplantacijskom periodu (13), što je i u ovom istraživanju zapaženo kroz negativnu korelaciju s biljegom koštane razgradnje, a o tome je izvještavano u prijašnjim studijama (37,38).

Činjenicu da su bolesnici uključeni u kroničnu hemodijalizu bili značajno stariji i liječeni hemodijalizom tijekom duljega razdoblja nego populacija bolesnika s presatkom bubrega ne bi trebalo zanemariti. Ona je uglavnom odražena u tome što su bolesnici koji nisu ispunjavali kriterije za presađivanje bili stariji i stoga dulje liječeni kroničnom hemodijalizom, što pak predstavlja sinergistične čimbenike poremećaja koštanog metabolizma.

Analiza biokemijskih parametara u dvije skupine bolesnika nije ukazala na razlike s obzirom na spol. U normalnoj se populaciji koštani biljezi razlikuju s obzirom na spol kako u odraslih tako i u djece. Negativan utjecaj spola kod žena na koštanu cjelovitost također je zapažen u postmenopauzi i priznat kao čimbenik rizika za osteoporozu. Svi istraživači nisu ustanovili razliku između spolova u bolesnika na kroničnom liječenju hemodijalizom ili onih s presatkom bubrega (39), čime je implicirana uključenost drugih čimbenika rizika za koštano oboljenje nasuprot opće prihvaćenim čimbenicima rizika za osteoporozu.

Glavnina odnosa između koštanih biljega, PTH i trajanja hemodijalize je uobičajena i slična onima kod bolesnika na kroničnoj hemodijalizi i onih s presatkom bubrega (10). Ta se činjenica odrazila i na koeficijente korelacije koji su bili istoga smjera i predstavljali slične veličine u obje skupine bolesnika.

Poseban nalaz za bolesnike na kroničnoj hemodijalizi bio je negativna korelacija dobi i CTX kao biljega koštane razgradnje. Starija dob jest čimbenik rizika za osteoporozu, premda općenito nije potvrđeno da je od značenja u bolesnika uključenih u kroničnu hemodijalizu. U ovoj je studiji ustanovljeno da je koštana razgradnja bila manje izražena u starijih bolesnika. Za koncentracije PTH nije utvrđena slična povezanost.

Mjerenje koštanih biljega zajedno s PTH može se smatrati korisnim kliničkim pomagalom u procjeni i praćenju poremećaja koštanog metabolizma kod kroničnog liječenja hemodijalizom i presađivanja bubrega (40). Ono također omogućuje pregled čimbenika rizika tog koštanog poremećaja i istraživanje njegovih promjenljivih kliničkih očitovanja.

important determinant of increased bone turnover after kidney transplantation (2,13,14). This was not confirmed in this study and was probably a consequence of the specific patient group and its size. After successful kidney transplantation, bone turnover was normalized in the post-transplant period (13), as observed in negative correlation with bone resorption marker in this investigation and also reported in our previous studies (37,38).

The fact that chronic hemodialysis patients were significantly older and were treated by hemodialysis for longer period than the kidney transplant patient population should not be disregarded. This mostly reflects that patients not fulfilling criteria for transplantation were older and thus longer treated by chronic hemodialysis, which are synergistic factors for disorder of bone metabolism.

Analysis of biochemical parameters in the two patient groups showed no difference between sexes. Bone markers in normal population differ between sexes both in adults and children. The negative influence of female sex on skeletal integrity is also observed in the postmenopause and is recognized as risk factor for osteoporosis. Difference between sexes in patients on chronic hemodialysis or kidney transplant recipients was not found by all investigators (39), thus implying an involvement of other risk factors of bone disease as opposed to generally accepted risk factors for osteoporosis.

Most of the relationships between bone markers, PTH and hemodialysis duration are common and similar to patients on chronic hemodialysis and kidney transplant recipients (10). This was reflected in correlation coefficients, which were of identical direction and similar magnitude in both patient groups.

A particular finding for patients on chronic hemodialysis was the negative correlation of age and the bone resorption marker crosslaps. Older age is a risk factor for osteoporosis, although not generally confirmed to be of significance in patients on chronic hemodialysis. In this study, bone resorption was found to be less pronounced in older patients. Similar association was not found for PTH levels.

Measurement of bone markers together with PTH can be considered a useful clinical tool in assessment and follow-up of metabolic bone disorders related to chronic hemodialysis treatment and kidney transplantation (40). It also enables insight into risk factors of this bone disorder and investigation into variations of its clinical presentations.

Ovaj članak je zaprimljen na engleskom jeziku. Za prijevod članka na hrvatski jezik odgovorno je Uredništvo Časopisa.

This manuscript has been submitted in English. Croatian translation is the responsibility of the Editorial.

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