ROLE OF OXIDATIVE STRESS IN PATHOGENESIS OF BONE DESTRUCTION SYNDROME IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA

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Reduced bone mineral density (BMD), osteopenia, and osteoporosis are slightly more common in patients with chronic lymphocytic leukemia (CLL). The risk of osteoporotic fractures in individuals with CLL is higher, than in healthy individuals of the same age. The mechanism underlying the CLL-associated BMD reduction can be related to decreased antioxidant protection and oxidative stress (OS). The study aimed to assess the relationship between oxidative stress, antioxidant protection, and osteopenia indicators in patients with CLL. Males aged 50–70 years were examined. Group 1 consisted of 14 healthy men, group 2 consisted of 54 patients with CLL having no BMD alterations, and group 3 consisted of 22 patients with CLL having signs of osteopenia. A densitometer was used to estimate BMD, T- and Z-scores of the lumbar vertebrae, proximal femoral neck (PFN), proximal femoral bone in all groups. At the beginning of the study, the levels of lipid peroxidation (LPO) products were determined in blood serum in all groups and bone tissue homogenate in groups 2 and 3; the total antioxidant status (TAS) was also determined. Bone densitometry indicators, serum LPO and TAS were assessed in all groups after 6 months of follow-up. At the beginning of the study osteopenia in PFN based on bone densitometry data was revealed in 29% of patients, while 6 months later osteopenia of all localizations was observed in 55% of patients. At the beginning of the study patients with CLL and osteopenia showed OS and reduced TAS in both blood serum and bone tissue. After 6 months patients with CLL and osteopenia showed signs of OS progression and TAS reduction. In patients with CLL, serum and bone tissue OS indicators are comparable and can be used to predict the onset of osteopenia within 6 months.

Keywords: chronic lymphocytic leukemia, bone mineral density, osteopenia, oxidative stress, lipid peroxidation, redox status

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РОЛЬ ОКИСЛИТЕЛЬНОГО СТРЕССА В ПАТОГЕНЕЗЕ ОСТЕОДЕСТРУКТИВНОГО СИНДРОМА У БОЛЬНЫХ С ХРОНИЧЕСКИМ ЛИМФОЛЕЙКОЗОМ

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У пациентов с хроническим лимфоцитарным лейкозом (ХЛЛ) несколько чаще наблюдаются снижение минеральной плотности кости (МПК), остеопения и остеопороз. Риск остеопоротических переломов при ХЛЛ выше, чем у здоровых лиц того же возраста. Механизм снижения МПК при ХЛЛ может быть связан со снижением антиоксидантной защиты и окислительным стрессом (ОС). Целью работы было исследовать взаимосвязь между показателями окислительного стресса, антиоксидантной защиты и показателями остеопении у пациентов с ХЛЛ. Обследовали мужчин в возрасте 50–70 лет. Группу 1 составили 14 здоровых мужчин, группу 2 — 54 пациента с ХЛЛ без изменений МПК, группу 3 — 22 пациента с ХЛЛ с признаками остеопении. На денситометре оценивали МПК, T- и Z-критерии в поясничных позвонках, шейке бедренной кости (ШПОБК), проксимальном отделе бедренной кости во всех группах. На старте исследования в сыворотке во всех группах и в гомогенате костной ткани в группах 2 и 3 определяли содержание продуктов перекисного окисления липидов (ПОЛ); общий антиоксидантный статус (ОАС). Показатели остеоденситометрии, ПОЛ и ОАС в сыворотке крови во всех группах оценивали через 6 месяцев наблюдения. На старте исследования по данным остеоденситометрии у 29% больных с ХЛЛ выявлена остеопения в ШПОБК, а через 6 месяцев остеопению наблюдали во всех локализациях у 55% больных. На старте исследования у пациентов с ХЛЛ и остеопенией в сыворотке и костной ткани зафиксированы ОС и сниженный ОАС. Через 6 месяцев у пациентов с ХЛЛ и остеопенией выявлены признаки прогрессии ОС и снижения ОАС. У пациентов с ХЛЛ показатели ОС в сыворотке и костной ткани сопоставимы и могут быть использованы для прогноза возникновения остеопении через 6 месяцев.

Ключевые слова: хронический лимфолейкоз, минеральная плотность кости, остеопения, окислительный стресс, перекисное окисление липидов, редокс-статус

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Chronic lymphocytic leukemia (CLL) belonging to the class of malignant lymphomas originates from small B cells. CLL is divided into two clinical variants: lymphoid hyperplasia with clonal lymphocytosis ≥ 5000/μL; lymphoid organ hypertrophy without lymphocytosis. Enlarged lymph nodes, tonsils and the spleen represent a manifestation of small B cell lymphoma that belongs to the same disease entity as CLL [1]. According to epidemiological situation, the annual incidence of CLL in Europe is about 5 cases per 100,000 population, while in Russia it is about 3–4 cases per 100,000 population [2]. CLL is the second most common non-Hodgkin lymphoma. In individuals with CLL, the risk of bone loss reaches 66%, which later leads to fractures of the tubular bones and disability. Reduced bone mineral density (BMD) leads to osteoporosis in 16% of patients and osteopenia in 35% of patients with CLL [3]. In dynamics, skeletal bone destruction associated with CLL begins in the proximal femurs expanding to the spine and other bones; this is most likely to be associated with tumor cell localization in the bone marrow of pelvic bones and interaction with stromal cells [4].

Excess generation of reactive oxygen species (ROS) by leukemic cells, reduced functional activity of the antioxidant system can be the key mechanisms underlying bone loss associated with CLL [5]. The CLL-associated oxidative stress results from the large number of mitochondria in tumor cells, and their survival rate is related to resistance to oxidative stress and associated with high levels of intracellular antioxidants that bind ROS and inhibit apoptosis, which leads to the increase in the number of malignant lymphocytes [6].

The bone resorption process is related to oxidative stress, specifically to the effects of ROS on protein molecules resulting in damage to the surrounding cells in the bone tissue. The lipid peroxidation (LPO) products accumulating in the tissues are used as markers of oxidative stress in many cancers, and their tissue levels suggest functional activity of the antioxidant system in CLL [7]. Pathogenesis of the CLL-associated bone destruction is poorly understood, and the analysis of research on the subject has shown that BMD reduction represents a part of the complex of events involving the immune system, intracellular signaling pathways, and redox status, necessitating further investigation of the mechanism underlying bone loss aimed at improving diagnosis and treatment of such patients.

The study aimed to assess the relationship between the serum and bone tissue levels of lipid peroxidation products and antioxidant status, as well as bone mineral density values in patients with CLL.

METHODS

The study was conducted at the South Ural State Medical University and Chelyabinsk Regional Clinical Hospital. Inclusion criteria: males aged 50–70 years only, who had submitted the informed consent. Exclusion criteria: all diseases and conditions, for which BMD reduction was proven, specifically, end-stage renal disease, genetic disorders (cystic fibrosis, Ehlers–Danlos syndrome, osteogenesis imperfecta, porphyria, etc.), endocrine disorders (type 1 and 2 diabetes mellitus, thyroid diseases, etc.), long-term treatment with glucocorticosteroids, gastrointestinal disorders (Crohn's disease, non-specific ulcerative colitis, primary biliary cirrhosis, celiac disease, malabsorption, etc.), gastrointestinal surgery, oncohematological diseases, except chronic lymphocytic leukemia, rheumatic diseases, human immunodeficiency virus, impossibility of self-care.

The control group (group 1) consisted of 14 conditionally healthy males; group 2 consisted of 54 patients with CLL with normal bone system indicators; group 3 consisted of 22 patients with CLL showing significant BMD reduction. All groups were matched by age ($p > 0.05$): group 1 — 59.0 [55.7; 63.5] years, group 2 — 62.0 [59.7; 65.3] years, group 3 — 65.0 [59.0; 66.1] years. The diagnosis of CLL was verified using the BD FACSCanto II flow cytofluorometer (BD Biosciences; USA) by immunophenotyping of peripheral blood lymphocytes of the following clonality: $CD5^+$, $CD19^+$, $CD20^+$, $CD23^+$, $CD22^+$, $CD23^+$, $CD43^+$, $CD200^+$, immunoglobulin kappa or lambda light chains. Patients were distributed based on the stage (Binet staging system): stage A — 28 patients (37%), stage B — 36 patients (47%), stage C — 12 patients (16%) [1]. The average disease duration was 10.5 months, and the duration of the study was 6 months. At the beginning of the study we assessed BMD, T- and Z-scores of the lumbar spine (LS), proximal femoral neck (PFN), proximal femoral bone (PHB) using the DEXXUM 3 bone densitometer (OsteoSys Co; South Korea). The 10-year fracture risk was calculated using the internationally accepted Fracture Risk Assessment Tool (FRAX) [8]. All patients with CLL underwent trephine biopsy from the the posterior superior iliac spine for confirmation of the diagnosis. The bone tissue biopsy specimen was homogenized for 3 min at the temperature of 4 °C (1 : 10) with added 0.9% sodium chloride solution. The levels of LPO products were assessed in blood serum in all groups and in bone tissue homogenate in patients with CLL by the extraction spectrophotometric method using the SF-56 spectrophotometer (LOMO-Spectrum; Russia) [9]. Optical density of LPO products was determined in the heptane and isopropanol phases of the lipid extract vs. appropriate control at 220 nm (levels of products with isolated double bonds), 232 nm, 278 nm, 400 nm. The results were measured in the oxidative index units (OIU): E232/E220 — relative content of diene conjugates (DC), E278/E220 — relative content of ketodienes and conjugated trienes (KD and CT), and E400/E220 — relative content of Schiff bases (SB). The total antioxidant status (TAS) was assessed using the Chem Well 2910 Combi automated analyzer (Awereness Technology; USA) and the B-7501 Total Antioxidant Status test system (Vector-Best; Russia); the results were expressed as total antioxidant levels measured in mmol/L.

At the second point of the study, 6 months later, we assessed bone densitometry indicators, serum LPO and TAS indicators. Three mathematical models predicting BMD alterations based on bone tissue and serum LPO and TAS values were constructed based on the results obtained at two points of the study.

The results were processed using IBM SPSS Statistics v. 23 (SPSS: An IBM Company; USA) and presented as median and interquartile range (Me [Q_1; Q_3]). Significance of differences was assessed using the Mann–Whitney U test. The medians of several samples were compared using the Kruskal–Wallis test. The differences were considered significant at $p \leq 0.05$. Linear regression analysis was used to reveal statistical relationships between indicators. The quality of models was estimated based on the R^2 determination coefficient, residual distribution type. Correlation analysis was performed using Spearman's rank correlation coefficient (R).

RESULTS

At the beginning of the study, 29% of patients with CLL (group 3) showed a significant decrease in T-score of the lumbar spine (4.8-fold based on median relative to group 1), Z-score — by 100% based on median, BMD — by 10% based on median; decrease in proximal femoral neck T-score — 8-fold based on

Table 1. Bone mineral density indicators (Me [Q_1; Q_3])

Indicators	Group 1 (*n* = 14)	Group 2		Group 3	
		Beginning (*n* = 54)	6 months (*n* = 18)	Beginning (*n* = 22)	6 months (*n* = 12)
FRAX, %	28.50 [25.60; 30.00]	28.00 [26.80; 30.50]	27.10 [24.20; 28.70]	28.70 [23.00; 30.10]	28.55 [26.20; 31.10]
T-score of LS, SD	1.15 [0.70; 3.00]	1.30 [–0.10; 2.50]	0.20 [–0.50; 0.80]$	–0.30 [–0.60; 1.00]*#	–1.10 [–1.80; –1.00]*#&
Z-score of LS, SD	1.30 [0.80; 3.00]	1.60 [0.40; 2.90]	1.20 [0.00; 2.10]	0.00 [–1.10; 1.40]*#	–1.05 [–1.20; –0.55]*#&
BMD of LS, g/cm^2	1.31 [1.06; 1.36]	1.37 [1.21; 1.52]	1.24 [1.16; –1.35]$	1.18 [1.06; 1.34]#	1.15 [1.11; 1.21]#
T-score of PFN, SD	0.20 [–0.20; 0.50]	–0.20 [–0.60; 0.10]	–0.80 [–1.20; –0.40]$	–1.40 [–2.00; –1.10]*#	–2.10 [–2.30; –1.60]*#&
Z-score of PFN, SD	0.90 [0.10; 1.10]	0.70 [0.40; 1.00]	0.00 [–0.40; 0.50]&	–0.60 [–1.00; 0.00]*#	–1.00 [–1.30; –0.60]*#&
BMD of PFN, g/cm^2	1.05 [0.98; 1.10]	1.04 [0.99; 1.08]	0.96 [0.91; 1.02]&	0.88 [0.80; 0.93]*#	0.89 [0.85; 0.92]*#
T-score of PFB, SD	0.25 [–0.10; 0.50]	–0.10 [–0.30; 0.30]	–0.60 [–0.80; 0.20]&	–0.80 [–2.00; –0.60]*#	–1.65 [–2.50; –1.20]*#&
Z-score of PFB, SD	0.07 [0.88; 2.40]	0.70 [0.30; 1.10]	–0.10 [–0.30; 0.30]&	–0.20 [–1.30; –0.10]*#	–1.85 [–2.20; –1.50]*#&
BMD of PFB, g/cm^2	1.07 [0.88; 1.12]	1.09 [1.05; 1.14]	1.01 [0.98; 1.05]&	0.93 [0.83; 1.01]#	0.94 [0.92; 0.97]*#

Note: * — significant ($p < 0.05$) differences from group 1 based on the Kruskal–Wallis test, # — from group 2; $ — differences in group 2; & — differences in group 3.

median relative to group 1, Z-score — 3-fold based on median, BMD — by 16% based on median; in the proximal femur bone, T-score decreased 4.2-fold based on median, Z-score — 1.3-fold, BMD — by 13% based on median (Table 1). According to the National Guidelines, the decrease in the PFN T-score in group 3 can be considered as osteopenia. When compared with group 2, patients with CLL and osteopenia showed a significant decrease in the LS indicators: T-score — 6-fold based on median, Z-score — by 100%, BMD — by 13.5%; PFN indicators: T-score — 7-fold based on median, Z-score — 2.1-fold, BMD — by 15% based on median, and PFB indicators: T-score — 8-fold based on median, Z-score — 4.5-fold based on median, BMD — by 14% based on median. The 10-year skeletal bone fracture risk calculated using the FRAX algorithm and including BMD values in all groups showed no significant differences ($p > 0.05$). In group 2 followed up for 6 months, significant changes were reported for LS: T-score decreased 6.5-fold based on median, BMD — by 9% based on median, and for PFN: T-score decreased 4-fold based on median. However, the bone densitometry characteristics, including T-scores of LS and PFN, were normal based on the parameters specified in the National Guidelines. After 6 months patients with CLL and osteopenia showed the following LS indicators that were significantly decreased compared to group 2: T-score — 3.7-fold based on median, Z-score — 1.05-fold, BMD — by 2% based on median; PFN: T-score — 1.5-fold based on median, Z-score — 1.7-fold, BMD — by 13% based on median; PFB: T-score — 22-fold based on median, Z-score — 1.2-fold, BMD — by 12% based on median. The in-depth analysis of the dynamic changes in bone densitometry indicators after 6 months in group 3 showed that BMD indicators were reduced to osteopenia values in all the studied localizations: in LS, the T-score was reduced 4-fold based on median, Z-score — 0.1-fold; in PFN: T-score — 1.5-fold based on median, Z-score — 1.2-fold; in PFB: T-score — 2.1-fold based on median, Z-score — 9.3-fold.

Oxidative stress in bone tissue of patients with CLL was assessed based on the levels of oxidized lipid molecule residues and total antioxidant status. At the beginning of the study group 3 showed a significant increase in the levels of ketodienes and conjugated trienes (by 5.5% based on median relative to group 2) and Schiff bases (24-fold based on median) in the heptane phase of the lipid extract (Table 2).

In the isopropanol phase of the lipid extract, there was a significant increase in Schiff base levels (by 41% based on median relative to group 2). In patients with CLL and osteopenia, no significant changes in the diene conjugate levels were found in the heptane and isopropanol phases of the bone tissue lipid

Table 2. Bone tissue oxidative stress indicators in patients with CLL (Me [Q_1; Q_3])

Indicators	Group 2 (*n* = 54)	Group 3 (*n* = 22)
DC (h), OIU	0.609 [0.602; 0.617]	0.612 [0.608; 0.626]
(KD and CT (h), OIU	0.073 [0.056; 0.077]	0.077 [0.076; 0.081]#
SB (h), OIU	0.003 [0.001; 0.007]	0.076 [0.038; 0.078]#
DC (i), OIU	0.504 [0.485; 0.526]	0.506 [0.489; 0.507]
(KD and CT (i), OIU	0.104 [0.097; 0.121]	0.100 [0.095; 0.109]
SB (i), OIU	0.061 [0.047; 0.065]	0.086 [0.084; 0.094]#
TAS, mmol/L	0.88 [0.81; 1.04]	0.61 [0.49; 0.62]#

Note: # — significant ($p < 0.05$) differences from group 2 based on the Mann–Whitney U test; (h) — heptane phase of the bone tissue lipid extract; (i) — isopropanol phase.

Table 3. Serum oxidative stress inficators (Me [Q_1; Q_3])

Indicators	Group 1 (n = 14)	Group 2		Group 3	
		Beginning (n = 54)	6 months (n = 18)	Beginning (n = 22)	6 months (n = 12)
DC (h), OIU	0.014 [0.013; 0.014]	0.582 [0.561; 0.613]*	0.800 [0.776; 0.804]*$	0.679 [0.534; 0.680]*#	1.126 [1.087; 1.309]*#&
KD and CT (h), OIU	0.001 [0.001; 0.002]	0.088 [0.077; 0.093]*	0.114 [0.109; 0.139]*$	0.097 [0.092; 0.103]*#	0.161 [0.137; 0.193]*#&
SB (h), OIU	0.003 [0.002; 0.004]	0.058 [0.040; 0.062]*	0.061 [0.026; 0.096]*	0.075 [0.048; 0.075]*	0.089 [0.043; 0.135]*#
DC (i), OIU	0.049 [0.047; 0.051]	0.518 [0.500; 0.527]*	0.932 [0.912; 0.942]*$	0.611 [0.608; 0.624]*#	1.312 [1.286; 1.547]*#&
KD and CT (i), OIU	0.025 [0.023; 0.027]	0.267 [0.235; 0.281]*	0.847 [0.829; 0.856]*$	0.305 [0.293; 0.317]*#	1.193 [1.169; 1.407]*#&
SB (i), OIU	0.012 [0.011; 0.016]	0.039 [0.033; 0.047]*	0.062 [0.041; 0.074]*$	0.031 [0.026; 0.046]*#	0.087 [0.042; 0.105]*#&
TAS, mmol/L	1.97 [1.89; 2.27]	1.73 [1.54; 2.01]*	2.03 [1.78; 2.35]*$	0.91 [0.85; 0.94]#	0.53 [0.43; 0.54]*#&

Note: * — significant (p < 0.05) differences from group 1 based on the Kruskal–Wallis test, # — from group 2; $ — differences in group 2; & — differences in group 3; (h) — heptane phase of the bone tissue lipid extract; (i) — isopropanol phase.

extract, as well as in the ketodiene and conjugated triene levels in the isopropanol phase. The bone tissue total antioxidant status was significantly reduced in group 3 (by 30% based on median relative to group 2).

Serum oxidative stress indicators for all groups are provided in Table 3.

It was found that at the beginning of the study patients with CLL and reduced BMD showed a significant increase in the diene conjugate levels (by 14% based on median), ketodiene and conjugated triene levels (by 9% based on median) in the heptane phase of the lipid extract relative to group 2. In the isopropanol phase, significantly increased serum levels of diene conjugates (by 15% based on median), ketodienes and conjugated trienes (by 13% based on median) were reported, however, SB levels were reduced by 20% based on median. At the beginning of the study group 3 showed a significantly reduced serum total antioxidant status (by 47% based on median) relative to group 2.

Group 3 showed a significant increase in the levels of diene conjugates — by 28%, ketodienes and conjugated trienes — by 29%, and Schiff bases — by 31% based on median in the heptane phase of the serum lipid extract, and diene conjugates, ketodienes and conjugated trienes — by 29% and Schiff bases — by 28% based on median in the isopropanol phase relative to group 2 after 6 months of follow-up. TAS of group 3 was significantly lower (by 74% based on median) relative to group 2 after 6 months of follow-up.

At the beginning of the study group 3 showed significantly increased levels of diene conjugates, ketodienes and conjugated trienes — by 98%, Schiff bases — by 95% in the heptane phase of the serum lipid extract, and diene conjugates, ketodienes and conjugated trienes — by 92%, Schiff bases — by 62% based on median in the isopropanol phase relative to group 1. Group 3 showed a significantly decreased TAS (by 53% based on median) relative to group 1.

After 6 months, patients with CLL and osteopenia showed significantly increased serum levels of DC — by 98.7%, KD and CT — by 99%, and SB — by 96% based on median in the heptane phase of the lipid extract, and DC — by 96%, KD and CT — by 98%, and SB — by 86% based on median in the isopropanol phase relative to group 1. The serum TAS of group 3 significantly decreased (by 73%) relative to group 1 after 6 months.

Group 3 showed a significant increase in the serum levels of DC, KD and CT — by 40% based on median in the heptane phase, and DC — by 53% based on median, KD and CT — by 74.5%, SB — by 64% in the isopropanol phase of the extract after 6 months. Furthermore, group 3 showed a serum TAS decrease (by 43% based on median) during the 6-month follow-up.

The use of mathematical modeling based on the Spearman's rank correlation revealed a significant negative correlation between the femoral neck bone mineral density and the diene conjugate levels in the heptane phase of the serum lipid extract at the beginning of the study (Table 4). The model can be described by the following equation:

$$x = 1.396 - 0.62 \times \text{DC (h)},$$

where x is the PFN BMD value, DC (h) are diene conjugates in the heptane phase.

The linear regression analysis method allowed us to compare the bone tissue oxidative lipid destruction values and the serum LPO values at the beginning of follow-up using mathematical modeling, thereby making it possible to stand down from re-assessment of the dynamic changes in the bone tissue redox status and exclude extra medical traumatization of patients resulting from trephine biopsy from the posterior superior iliac spine. The model can be described by the following equation:

$$x = 0.5 - 0.41 \times \text{KD and CT (h)} + 0.66 \times \text{SB (h)},$$

where x is the bone tissue concentration of diene conjugates in the isopropanol phase, KD and CT (h) are ketodienes and conjugated trienes in the heptane phase, SB (h) are Schiff bases in the heptane phase.

According to another model, serum levels of diene conjugates in the heptane phase and the levels of Schiff bases in the isopropanol phase of the extract showed a significant negative correlation with the PFB BMD 6 months after the beginning of follow-up. The model can be described by the following equation:

$$x = 2.39 - 1.99 \times \text{DC (h)} - 3.12 \times \text{SB (i)} + 0.27 \times \text{osteopenia},$$

where x is the likelihood of the PFB BMD (g/cm^2) reduction, DC (h) are diene conjugates in the heptane phase, SB (i) are Schiff bases in the isopropanol phase. The trait of having osteopenia was included in the model, since it improved the prediction

Table 4. Regression models

Models	Traits	Coefficients	*p*
Model of the relationship between PFN BMD and serum LPO at the beginning adjusted $R^2 = 0.34$; $p = 0.001$	Intercept	1.396	< 0.001*
	DC (h)	–0.620	0.00156*
Model of the relationship between bone LPO and serum LPO at the beginning adjusted $R^2 = 0.36$; $p = 0.003$	Intercept	0.49817	< 0.001*
	KD and CT (h)	–0.40958	0.03754*
	SB (h)	0.66482	0.00107*
Model to determine PFB BMD after 6 months based on the serum LPO values adjusted $R^2 = 0.71$; $p = 0.002$	Intercept	2.39	< 0.001*
	DC (h)	–1.99	0.024*
	SB (i)	–3.12	0.017*
Group 3		0.27	0.128

Note: * — significant ($p < 0.05$) differences.

quality, but it showed no statistical significance (1 — present, 0 — absent).

DISCUSSION

T-score is considered to be a standardized indicator when performing bone densitometry; it represents the number of standard deviations from the maximum BMD and is used in postmenopausal women and men over the age of 50. Z-score represents the number of standard deviations from the average BMD value in individuals of the same age group [8].

In CLL, the femoral neck is considered to be the most common localization of reduced BMD. However, in individuals with severe disease, alterations of BMD indicators can be found in other parts of the skeleton, including the spine [4].

The mechanism underlying the development of osteopenia of different localization is likely to be associated with the negative effect of the large number of free radical synthesized by tumor cells on protein and lipid molecules, as well as with generation of products of their oxidative destruction [10, 11].

As is well-known, diene conjugates, ketodienes and conjugated trienes are early stage lipid peroxidation products. Their levels reflect activity of the LPO processes and oxidative stress intensity. The non-metabolizable Schiff bases are markers of dystrophic processes in cells and tissues. Today, it is believed that the polyunsaturated fatty acid peroxide-derived carbonyl residues of oxidized lipids, such as 4-hydroxynonenal or malondialdehyde, are biomarkers of oxidative stress in tissues associated with CLL [7]. Survival of clonal B cells is associated with the oxidative phosphorylation (ROS conversion to less harmful forms), increased antioxidant protection, specifically activation of the superoxide dismutase isoforms (SOD, SOD1 (Cu/Zn SOD), SOD2 (Mn-SOD)), thioredoxin system, and the enzyme cascade inducing glutathione biosynthesis and recirculation [12]. In CLL, ROS are synthesized mainly in mitochondria, in contrast to other tumors, in which this function is performed by NADPH oxidases [12]. ROS are buffered under exposure to the antioxidant factors, the expression of which is controlled by the transcription factors regulated by oxidation and phosphorylation [12]. Oxidative stress is considered to be the main mechanism underlying bone loss [13–15]. Oxidative stress indirectly, via activation of signaling pathways (FGF23, Nrf-2, JNK, ERK1/2, NF-κB, RANKL/OPG), stimulates differentiation of osteoclasts, affects proliferation and life span, reduces activity of osteoblasts and takes part in their apoptosis [15]. In particular, excess Nrf-2 expression, activation of heme oxygenase-1 (HO-1) and RUNX2-dependent transcription activity in CLL are associated with the decrease in osteoblast differentiation [16, 17]. Furthermore, the mechanism of bone tissue mineralization reduction under conditions of oxidative stress is associated with increased FGF23 expression in the bone tissue due to apopotosis of osteocytes and osteoblasts, activation of mitogen-activated protein kinases (MAPK) [18, 19].

As a consequence, the tumor-induced oxidative stress results in the fact that mature osteoclasts resorb bone matrix allowing tumor cells to grow and migrate in the tissues. The antioxidant protection dysfunction associated with CLL contributes to apoptosis of osteoblasts and osteocytes, causing further decrease in BMD leading to osteopenia and osteoporosis [20].

A software tool “Modeling Changes in Bone Mineral Density Depending on Redox Status in Patients with Chronic Lymphocytic Leukemia” allowing one to predict osteopenia or osteoporosis in patients with CLL within 6 months based on the serum LPO indicators was created based on the data obtained. According to our results, testing of this model made it possible to demonstrate high sensitivity and specificity of the tool created and had high predictive value. Practical use of the tool by healthcare specialists will make it possible to determine the subgroup of patients with CLL at risk of developing osteopenia.

CONCLUSIONS

In patients with CLL, the rate of determining osteopenia based on the proximal neck of the femur bone densitometry is 29%. During the 6-month follow-up the signs of osteopenia are found in 55% of patients in all instrumental assessment localizations. Bone tissue of patients with CLL and osteopenia shows signs of oxidative stress: accumulation of secondary and end products in the heptane phase of the lipid extract, accumulation of lipid oxidation end products in the isopropanol phase, reduced total antioxidant status. Furthermore, systemic oxidative stress associated with the increased levels of primary and secondary products of oxidative lipid destruction in the heptane phase, levels of primary, secondary and end products in the isopropanol phase, reduced serum total antioxidant status has been discovered in patients with CLL and osteopenia. After 6 months, progression of oxidative stress in blood serum relative to the values reported at the beginning of the study is observed: the levels of primary, secondary and end products of oxidative lipid destruction in the heptane and isopropanol phases are increased, the total antioxidant status is decreased. The serum and bone tissue redox status indicators are comparable, and serum oxidative stress indicators can be used to predict the development of osteopenia. The study can have an impact on the choice of patient management tactics, preparation of treatment and preventive measures, such as prescription of antioxidant therapy or bisphosphonate therapy.

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