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Effects of antidepressants on serum concentrations of bone metabolism markers and major electrolytes in patients from routine psychiatric practice

Dejstvo antidepresiva na serumske koncentracije markera metabolizma kosti i glavnih elektrolita kod bolesnika u rutinskoj psihijatrijskoj praksi

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Abstract

Background/Aim. Data about effects of antidepressant on calcium, phosphorous and magnesium metabolisms are very scorce. The aim of this study was to investigate effects of antidepressants on serum concentration of bone metabolism markers and main electrolytes in patients from routine psychiatric practice. **Methods.** A prospective, before-and-after, time-series research included 9 males and 24 females, with average 53.3 ± 11.5 years-of-age, suffering from depression (n = 26) and neurotic disorders (n = 7), mostly taking selective serotonin reuptake inhibitors. We measured analytes at baseline, and 4th, 6th and 12th weeks during the treatment and tested the parameter changes from baseline and the trends with appropriate statistics at $p \leq 0.05$ significance level. **Results.** The age above 60 years was a significant factor for appearance of negative cumulative changes (in percent) of 25-hydroxyvitamin D – 25(OH)D concentrations from the baseline (OR = 11.4, 95% CI 1.2–113.1, $p = 0.037$). Serum concentrations of calcium significantly correlated with sodium ($r_s = 0.531$, $p < 0.001$), with chloride (r = 0.496, $p < 0.001$), with magnesium ($r_s = 0.402$, $p < 0.001$) and with osteocalcin (r = 0.240, $p = 0.019$). Significant correlations were among phosphorous with chloride (r = -0.218, $p = 0.035$); magnesium with sodium (r = 0.295, $p = 0.004$) and with potassium, (r = 0.273, $p = 0.009$); osteocalcin with C-telopeptide (r = 0.760, $p < 0.001$) with sodium (r = 0.215, $p = 0.039$) and with chloride (r = 0.209, $p = 0.041$); sodium with chloride (r = 0.722, $p < 0.001$). There were no statistically significant changes between antidepressant treatment and changes of absolute serum concentration of calcium, magnesium, phosphorous, 25(OH)D, osteocalcin, C-telopeptide, sodium, potassium and chloride. There were no statistically significant changes in frequency of disturbances in values of laboratory analytes (below/above lower/upper normal limits), too. **Conclusion.** Antidepressant treatment was not significantly associated with the changes in study analytes but some of them positively correlated with each other, suggesting the need for individual patient approach and further research in the field of bone metabolism in patients with mental disorders.

Key words:
antidepressive agents; bone and bones; metabolism; electrolytes; risk assessment; clinical chemistry tests.

Apstrakt

Uvod/Cilj. Nema dovoljno podataka o uticaju antidepresiva na metabolizam kalcijuma, fosfora i magnezijuma. Cilj rada bio je da se istraže efekti antidepresiva na serumsku koncentraciju markera metabolizma kosti i glavnih elektrolita kod bolesnika u psihijatrijskoj praksi. **Metode.** U prospektivnu studiju, dizajna vremenske serije pre i posle, bio je uključeno 9 muškaraca i 24 žene, prosečne starosti 53,3 ± 11,5 godina, koji su bolovali od depresije n = 26 i neurotičnih poremećaja n = 7 i koji su uglavnom lečeni selektivnim inhibitorima preuzimanja serotonina. Određivani su analiti na početku, u 4, 6. i 12. nedelje lečenja i analizirane promene parametara i trendovi u odnosu na početne vrednosti uz statističku značajnost od p ≤ 0,05. **Rezultati.** Utvrđeno je da starost iznad 60 godina predstavlja značajan faktor rizika od pojave negativnih kumulativnih promena (u procentima) u odnosu na bazalne koncentracije 25-hidroksivitamina D – 25(OH)D (OR = 11,4,

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95% CI 1,2–113,1, p = 0,037). Koncentracije kalcijuma u serumu značajno su korelisale sa koncentracijama natrijuma (r_s = 0,531, p < 0,001), hlorida (r = 0,496, p < 0,001), magnezijuma (r_s = 0,402, p < 0.001 i osteokalcina r = 0,240, p = 0,019. Značajne korelacije nađene su između koncentracija fosfora i hlorida (r = -0,218, p = 0,035); magnezijuma i natrijuma (r = 0,295, p = 0,004) i kalijuma (r = 0,273, p = 0,009); osteokalcina i C-telopeptida (r = 0,760, p < 0,001), natrijuma (r = 0,215, p = 0,039) i hlorida (r = 0,209, p = 0,041); natrijuma i hlorida (r = 0,722, p < 0,001). Nije utvrđena statistički značajna razlika između terapije antidepresivima i promena apsolutnih vrednosti serumskih koncentracija kalcijuma, magnezijuma, fosfora, 25 OH D, osteokalcina, C-telopeptida, natrijuma, kalijuma i hlorida. Takođe nisu utvrđene statistički značajne razlike ni u učestalosti poremećaja i vrednostima laboratorijskih analita (ispod/iznad donje/gornje granice referentnih vrednosti. **Zaključak.** Lečenje antidepresivima nije značajno povezano sa promenama studijskih analita ali neki od njih su međusobno bili u pozitivnoj korelaciji, što sugeriše potrebu individualnog pristupa bolesniku i daljih istraživanja u oblasti kosti metabolizma kod osoba sa mentalnim poremećajima.

Ključne reči:
antidepresivi; kost; metabolizam; elektroliti; rizik, procena; hemija, klinička, testovi.

Introduction

The researches, conducted in recent years, have revealed significant association between depression and fracture risk, mostly based on the presence of prior osteoporosis and often with increased prevalence of vitamin D deficiency in depressive patients [1–3]. Although the researches are still debating about the exact causes and mechanisms of these events, many of them suggested causal contribution of antidepressant drugs. Clinical studies, primarily of observational design, and subsequent meta-analyses reported evidence of significant association between antidepressant use, bone loss and fracture risk [4]. Experimental studies confirmed the presence of the signaling molecules in the bone tissue that, after selective targeting by different antidepressants, measurable changes in bone density were induced [5, 6]. Additional pathways, such as drug-induced disturbances of vitamin D metabolism, could also play the role in bone loss presumably caused by antidepressant medicines [7].

On the other side, disturbances of electrolyte homeostasis, particularly bone minerals, was almost outside the scope of researchers in the field. It is a surprising detail, taking into account crucial role of calcium and phosphate in bone mineralization as well as of magnesium in their regulation. In fact, osteomalacia, a syndrome of bone mineral depletion, represents the underlying risk for development of bone fractures, too [8]. Dietary habits, which directly or indirectly influence the electrolyte content in the body, such as low calcium intake and high-salt nutrition, are the recognized contributing factors for development of osteoporotic fractures [9].

The published studies, which focused on the effects of antidepressant on calcium, phosphorous and magnesium metabolisms (mainly measuring their serum levels), are very scarce, methodologiclly modest and sometimes with controversial results [10, 11]. Recent finding that the use of an antipsychotic with antagonistic action on serotonergic receptors could be associated with significant hypocalcaemia raises a possibility of detecting the influence of antidepressants on homeostasis of bone minerals, at least for some such drugs and in some patients [12]. Indeed, novel basic research has found that antidepressants induced expression of mRNA of intestinal calcium transporter, which stimulated calcium absorption [13]. This or similar biological effects of antidepressant drugs could be, in fact, protective for bone health, which might partially explain the results of some basic and clinical studies that provided evidences against the above-mentioned, harmful associations.

It seems that true nature of antidepressant effects on bone metabolism is a rather complex matter, influenced by many factors including different biological pathways. Therefore, the primary aim of our study was to investigate the effects of antidepressants on serum concentration of calcium proposing its depletion to be one of the possible pathogenetic arms of the observed association between treated depression and fracture risk. We focused on early changes in patients from routine psychiatric practice, during the period of three months, hypothesizing that the altering of the treatment protocol (e.g. dose change) was the trigger for disturbances of calcium homeostatic axis. Serum levels of other bone minerals, their regulator, markers of bone turnover and main electrolytes were secondary outcomes because we believed that they are the proxy measure for both calcium and related bone metabolism processes, which primarily depend on physiologically-maintained mineralization cycles.

Methods

Study design

The clinical study was a before-and-after, time-series trial with prospective data collection aiming to assess the parameters of physical health from the patients with mental disorder who take antidepressants, according to the designs of previous similar studies [14, 15]. We performed the study in Clinical Center "Kragujevac", in Kragujevac, Serbia, at its departments (Psychiatric, Clinical Pharmacology, Clinical Biochemistry, Internal Medicine) during years 2013 and 2014. We included both the hospitalized subjects and outpatients, at the setting of everyday psychiatric practice. Study visit were conducted at baseline and then after 4, 6 and 12 weeks according to experimental data about the temporal changes of calcium homeostasis tracers after the initiation of the disturbing factor [16]. Institutional Ethics Committee approved the study and the patients gave voluntary written informed consent to participate in the research.

Study population and drug treatment

The study participants were adult patients of both genders, 35–85 years old, suffering from mental disorder, which was an indication for starting the antidepressant treatment. The majority of patients had some type of depressive disorder and other neurotic illness, represented as the first episode, chronic stable disease or relapsing episode. In a few study subjects, mental disorders appeared in comorbid pattern, giving the mixed picture of depressive, neurotic and, exceptionally psychotic symptoms, which were the reasons for use of anxiolytics and antipsychotics, too. In all cases of relapsing mental disease, there was a long period of previous clinical stability without antidepressant treatment (three months or more). Psychiatrist screened the patients about the eligibility for study enrollment but he or she made decision about introduction of antidepressant and other psychotropic drugs according to clinical judgment only, independently of the patient's participation in the study. Exclusion criteria were the age outside defined range, pregnancy or lactation and any documented or clinically obvious condition or disease at baseline, which indicated the presence of preexisting, active disorder of bone and mineral homeostasis (e.g. fracture, infection, pancreatitis, rhabdomyolysis, acid-base or electrolyte disorder). Depressive disorders represented the leading mental illness in our study subjects (n = 26, 79%), Some patients suffered from neurotic disorders (n = 7; 21%; either as single clinical entity or as comorbid with another mental illness) which were the reasons for antidepressant use. Consequently, all patients took an antidepressant and many of them an anxiolytic or a hypnotic agent, too. Antidepressants used were: escitalopram (n = 16; 48%), sertraline (n = 7; 21%), paroxetine (n = 4; 12%), venlafaxine (n = 4; 12%), mirtazapine (n = 3; 9%), trazodone (n = 2; 6%), fluoxetine (n = 1; 3%), maprotiline (n = 1; 3%). There were 11 (33.3%) antidepressant-naïve patients taking the drug for the first time. Other 22 (66.7%) study subjects had chronic mental illness (the mean duration of 5.6 years, standard deviation 3.8 years) suffering from the relapsing episode. However, those subjects had prior antidepressant-free period of at least six months before enrollment in the study. Rare subjects with depressive episodes had psychotic symptoms, which were the reasons for prescribing adjunctive antipsychotic drug. Therefore, some patients used combination therapy (in five cases two antidepressants, from different pharmacological classes) in order to augment clinical response. Besides the other psychotropic drugs, the most frequently used were alprazolam [in 8 (24%) patients], diazepam [8 (24%)], zolpidem [7 (21%)], bromazepam [5, (15%)] and risperidone [5 (15%)].

The number of identified factors *per* patient, which were initially recognized to bear risk for osteoporosis and consequent bone fractures, ranged from 0 to 7, with the median of 4. The leading risks for our study subjects were female gender, older age, smoking and postmenopause. An internal medicine specialist performed baseline general physical examination of the study subjects. The internist excluded significant symptoms and signs of somatic disorders which could bear additional risk for bone homeostasis disturbances and which required further diagnostics. However, during the study conduct it was revealed that near a half of the subjects had low baseline serum levels of vitamin D, below the deficiency threshold, which itself, represented additional risk factor for bone loss.

Study procedures and biochemical analyses

Patients have been carefully examined and medical records have been retrieved for identification of the array of basal risk factors for osteoporosis, osteomalacia and calcium disturbance and other key parameters [9]. At the study visits, a blood samples (~20 mL) were taken, serum was separated using centrifugation and stored at ~25°C. Shortly after completion of active study phase, clinical biochemist performed serum sample analysis using Beckman Coulter UniCel DxC 800 Synchron Clinical System (Beckman Coulter, Inc., Brea, USA) for calcium (total calcium), magnesium, phosphorous (inorganic phosphorous), sodium, potassium, chloride and albumin. Cobas e411 chemical analyzer (Roche Diagnostics GmbH, Mannheim, Germany) served as the platform for total 25-hydroxyvitamin D (25(OH)D: 25-hydroxyvitamin D_3 and 25-hydroxyvitamin D_2), osteocalcin (N-MID osteocalcin) and C-telopeptide [β-isomerized C-terminal telopeptides (β-CTx)] measurements. Endocrinologist explored bone mineral density (BMD) of the hip and lumbar spine (L1-L4) using dual-energy X-ray absorptiometry (DXA) equipment (Hologic Discovery W, Hologic, Inc. Bedford, USA). Details about exact chemical reactions and methods of measurements are described in the manufacturers' product manuals.

Statistical analysis

Sample size calculation was powered with α and β error 0.05 and 0.2, respectively, considering the decrease of ≥ 5% of serum calcemia (primary outcome variable) in final blood sample from baseline calcemia of 2.40 mmol/L (standard deviation 0.16 mmol/L) as statistically significant, for paired, two-tailed, one-sample analysis. We based calculation on previous research in the topic but concerning antipsychotic treatment as we consider available data of primary endpoint from studies, which investigated antidepressants in the similar setting to be scarce and unreliable [17]. The initial computed sample size was increased for a half, in order to counteract possible non-parametric distribution and missing data, giving the study group of at least 25 subjects. Statistical methods, used to analyze collected data were: descriptions, Student's *t*-test, Wilcoxon signed-rank test, correlation Pearson's r, Spearman's r_s), linear regression, analysis of variance, Friedman's test, χ^2 test, McNemar's test and binary logistic regression, as appropriate. The repeated-measures and paired-sample analysis was used where appropriate, too. The sum of changes from the baseline served as useful outcome variable for logistic regression in order to aggregate small fluctuations of calcemia, based on previous report from another interventional research [18]. A significance threshold was determined at probability of null hypothesis of 5% or less for all statistical calculations, with two-tailed approach.

Results

Characteristics of study patients

The sociodemographic and clinical characteristics of 33 study subjects are summarized in the Table 1. Prevailing features are adult females in the beginning of her sixth life decade, with high school education, satisfactory inhabitance, working, life style and nutrition, being moderate coffee drinker, suffering from depression and taking a selective serotonin reuptake inhibitor. Only eight subjects had a comorbid, somatic disorder, primarily arterial hypertension, which were medically controlled and clinically stable. Majority of females were in postmenopause (frequency difference was not statistically significant), but other reproductive risks were absent (e.g. early menarche, hormonal drug treatment). No patient had previous bone fractures and family history of osteoporotic fractures was identified in only two subjects.

Bone minerals, metabolism markers, and other electrolytes

Treatment with antidepressants was not associated with statistically significant changes of absolute values of serum concentration of any measured analyte (Table 2). The calcium concentrations from individual serum samples were placed in the Figure 1 and their range was from 1.87 mmol/L to 2.76 mm/L. Therapy with antidepressants was not associated with statistically significant changes from baseline serum values for any measured analyte except for sodium for which the small fluctuations were statistically significant in analysis of repeated measures (Table 3). Across the study visits, the frequency of serum values which were outside laboratory reference ranges (bellow or upper normal limits) for measured analytes were rather small, except for vitamin D, osteocalcin and chloride (Table 4). The frequency of described disturbances showed some oscillation across the study visits but

Table 1

Characteristics of study subjects

Variable	Value	χ^2; df; *p*
Gender, n (%)		$\chi^2 = 6.8$; df = 1; $p = 0.090$
male	9 (27)	
female	24 (73)	
Age (years), $\bar{x}$ ± SD (min–max)	53.2 ± 11.5 (35–74)	n.a.
≤ 60 years, n (%)	21 (64)	$p > 0.05$
> 60 years, n (%)	12 (36)	
Body mass index, (kg/m^2), $\bar{x}$ ± SD (min–max)	25.0 ± 5.0 (17.3–35.9)	n.a.
Inhabitance, n (%)		
urban	16 (48)	$p > 0.05$
rural	17 (52)	
Education, n (%)		$\chi^2 = 6.5$, df = 2, $p = 0.038$
elementary	11 (33)	
high school	17 (52)	
faculty	5 (15)	
Life style, n (%)		
moderate	15 (45)	$p > 0.05$
comfortable	18 (55)	
Working environment, n (%)		$\chi^2 = 8.8$, df = 1, $p = 0.003$
sedentary	8 (24)	
manual	25 (76)	
Smoking, n (%)		
non-smokers	16 (48)	$p > 0.05$
smokers	17 (52)	
[1]cigarettes per day[2], $\bar{x}$ (min–max)	20 (10–40)	
[1]years of smoking[2], $\bar{x}$ (min–max)	16 (7–50)	
Coffee drinking, n (%)		$\chi^2 = 16$; df = 1; $p < 0.001$
non-drinkers	5 (15)	
drinkers	28 (85)	
cups *per* day[3] for those who drink coffee, $\bar{x}$ (min–max)	2 (1–7)	
Exercise, n (%)		$\chi^2 = 13.4$; df = 1; $p < 0.001$
unwilling	6 (18)	
active	27 (82)	
hours *per* day, $\bar{x}$ (min–max)	4 (1–10)	
Nutrition, n (%)		$\chi^2 = 25.5$; df = 1; $p < 0.001$
inadequate	2 (6)	
satisfactory	31 (94)	
Fracture risks, additional		n.a.
postmenopause females only, n (%)	14 (58)	
t-score, $\bar{x}$ (min–max)	-0.9 ± 1.7 (-4.4 to 1.9)	

n.a. – not applicable; df – degree of freedom. [1] – only for smokers; $\bar{x}$ – mean; SD – standard deviation.

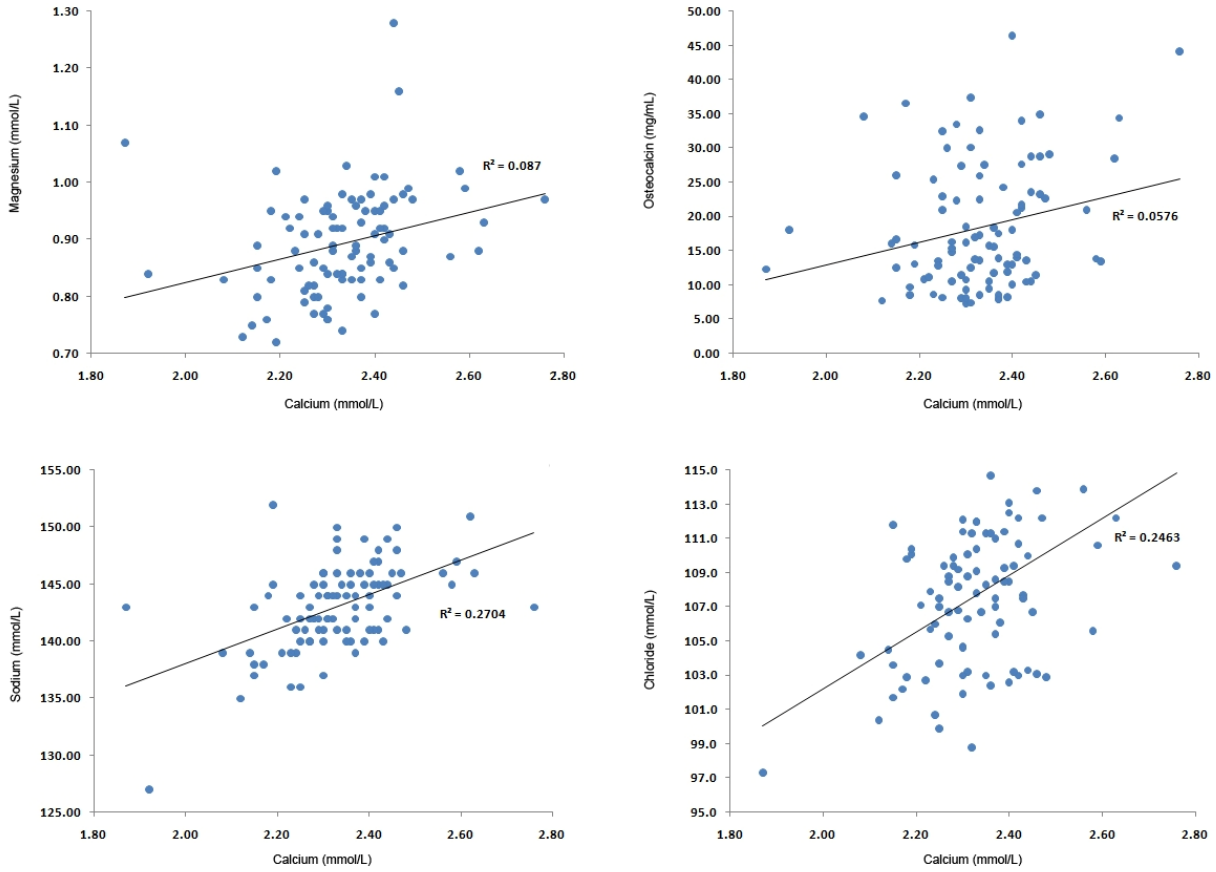

Fig. 1 – Correlations of serum concentrations of calcium with those of magnesium, sodium, osteocalcin and chloride, using data from all visits.

Table 2

Absolute values of serum concentrations of bone metabolism markers and major electrolytes in the study patients

Analyte	Baseline (1st vist)	2nd visit	3rd visit	4th visit	*p-values*
Calcium (mmol/L)	2.35 ± 0.14	2.35 ± 0.14	2.32 ± 0.14	2.32 ± 0.14	0.192
Magnesium (mmol/L)	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	0.573
Phosphorous (mmol/L)	1.2 ± 0.2	1.2 ± 0.2	1.2 ± 0.1	1.2 ± 0.3	0.119
25(OH)D (ng/mL)	22.9 ± 10.9	23.6 ± 10.9	21.8 ± 10.0	22.9 ± 9.0	0.412
Osteocalcin (ng/mL)	18.7 ± 10	18.8 ± 9.3	18.4 ± 8.3	19.0 ± 10.8	0.674
C-telopeptide (ng/mL)	0.3 ± 0.3	0.3 ± 0.2	0.3 ± 0.2	0.3 ± 0.2	0.268
Sodium (mmol/L)	142.7 ± 4.4	143.5 ± 3.9	142.3 ± 3.7	143.5 ± 3.2	0.347
Potassium (mmol/L)	4.6 ± 0.4	4.7 ± 1.1	4.6 ± 0.5	4.8 ± 1.7	0.724
Chloride (mmol/L)	107.7 ± 4.8	107.6 ± 4.3	107.8 ± 4.0	107.5 ± 4.9	0.996

Values represent mean ± standard deviation; 25(OH)D – 25-hydroxyvitamin D.

Table 3

Changes from baseline of serum concentrations of bone metabolism markers and major electrolytes in the study patients

Analyte	2nd visit	3rd visit	4th visit	*p-values*
Calcium	0.1 ± 6.3	-0.8 ± 7.7	-1.2 ± 7.8	0.326
Magnesium	-0.8 ± 9.0	-2.8 ± 9.6	-1.9 ± 9.6	0.682
Phosphorous	5.3 ± 19.7	9.4 ± 18.0	10.6 ± 22.4	0.150
25(OH)D	8.8 ± 29.9	4.6 ± 36.9	8.2 ± 50.3	0.186
Osteocalcin	6.5 ± 33	5.9 ± 30.5	10.0 ± 43.4	0.873
C-telopeptide	15.2 ± 53.3	7.5 ± 55.8	-5.6 ± 58.7	0.293
Sodium	0.6 ± 3.7	-0.2 ± 3.3	1.0 ± 3.4	0.049
Potassium	3.4 ± 23.5	0.3 ± 9.5	6.6 ± 40.9	0.776
Chloride	0.1 ± 4.0	0.2 ± 3.3	0.3 ± 4.9	0.867

Values in percent (%), represent mean ± standard deviation; 25(OH)D – 25-hydroxyvitamin D.

Table 4

The frequency of patients' serum samples in which laboratory values of study analytes were below lower normal limits (LNL) or above upper normal limits (UNL)

Analyte	Below LNL	*p-values**	Above UNL	*p-values***
Calcium	7.7–19.2	0.543	0–3.8	0.392
Magnesium	none	n.a.	0–3.8	0.392
Phosphorous	0–3.8	0.392	none	n.a.
25(OH)D	40.9–50.0	0.860	0–11.5	0.494
Osteocalcin	26.9–33.3	0.934	n.a.	n.a.
C-telopeptide	n.a.	n.a.	none	n.a.
Sodium	0–7.7	0.494	3.8–15.4	0.585
Potassium	0–5.0	0.392	0–5.0	0.572
Chloride	0–4.8	0.572	66.7–73.1	0.927

Values represent range (min–max) of % across the visits; n.a.– not applicable; *p* – probability of change in frequency across the study visits for below LNL and above UNL.

such changes were not statistically significant during antidepressant treatment for any analyte.

The risk factors, examined by means of univariate binary logistic regressions analyses were: female gender, age, age > 60 years, sedentary work, body mass index, obesity, smoking, coffee drinking ≥ 4 cups *per* day, exercise ≤ 4 h *per* day, unhealthy dietary habits (inadequate intake of food reach in calcium and fish product), family history of bone fractures until 75 years of life, postmenopause, dual-energy x-ray absorptiometry (DXA) T-score, number of risk factors for each patient and individual antidepressant drugs. However, none of the examined factors was significantly associated with negative, cumulative changes from baseline serum concentrations of calcium, magnesium, phosphorous, osteocalcin, C-telopeptide, sodium, potassium and chloride. The age of 60 or more was significant risk factor for appearance of negative cumulative changes of vitamin D concentrations from baseline (OR = 11.4, 95% CI 1.2–113.1, p = 0.037) but the other examined factors were not.

A separate analysis was done for calcium values adjusted for serum concentrations of albumin in the respective blood samples. The mean albumin concentration in all serum samples was 43.7 g/L with standard deviation of 3 g/L (range 35–50 g/L). In general, such results, in essence, follow those related to the uncorrected calcium values. Therefore, the details of this part of analysis are omitted for the sake of clarity.

Correlation analysis

Across the study days, statistically significant trend was not observed with serum concentrations of calcium (r_s = -0.083, p = 0.426), magnesium (r_s = -0.062, p = 0.554), vitamin D (r = 0.001, p = 0.993), osteocalcin (r_s = 0.024, p = 0.817), C-telopeptide (r_s = -0.225, p = 0.059), sodium (r = 0.041, p = 0.697), potassium (r_s = -0.034, p = 0.786) and chloride (r = -0.033, p = 0.747). On the other side, during the study course, fluctuations of serum concentrations of several parameters correlated with each other, in the same direction for almost all pairs (Table 5). Taking into account the strength of association, calcium serum concentrations significantly correlated firstly with sodium and chloride concentrations, then with magnesium concentrations and finally with osteocalcin concentrations (Figure 1). Calcium albumin-adjusted values, in general, showed almost identical pat-

Table 5

Correlation between serum concentrations of study variables, which includes data from all blood samples

Variable	Calcium	A	B	C	D	E	F	G	H
A – Calcium, adj.[1]	r_s = 0.848*								
	p < 0.001								
B – Vitamin D	r = 0.118	r_s = -0.043							
	p = 0.265	p = 0.683							
C – Phosphorous	r_s = -0.075	r_s = -0.09	r = 0.046						
	p = 0.473	p = 0.388	p = 0.668						
D – Osteocalcin	r = 0.240*	r_s = 0.203*	r = 0.021	r = 0.139					
	p = 0.019	p = 0.047	p = 0.838	p = 0.181					
E – Magnesium	r_s = 0.402*	r_s = 0.207*	r = 0.087	r = 0.020	r = 0.106				
	p < 0.001	p = 0.045	p = 0.412	p = 0.849	p = 0.310				
F – C-telopeptide	r_s = -0.014	r_s = 0.042	r = 0.161	r = 0.106	r = 0.760*	r = -0.058			
	p = 0.890	p = 0.686	p = 0.123	p = 0.311	p < 0.001	p = 0.581			
G – Sodium	r_s = 0.531*	r_s = 0.418*	r = -0.016	r = -0.069	r = 0.215*	r = 0.295*	r_s = -0.096		
	p < 0.001	p < 0.001	p = 0.884	p = 0.514	p = 0.039	p = 0.004	p = 0.360		
H – Potassium	r = -0.197	r_s = 0.153	r = 0.202	r = 0.029	r = -0.002	r = 0.273*	r_s = -0.002	r = 0.124	
	p = 0.059	p = 0.142	p = 0.058	p = 0.787	p = 0.987	p = 0.009	p = 0.983	p = 0.238	
I – Chloride	r = 0.496*	r_s = 0.380*	r_s = 0.035	r = -0.218*	r = 0.209*	r = -0.050	r_s = -0.002	r = 0.722*	r_s = 0.114
	p < 0.001	p < 0.001	p = 0.738	p = 0.035	p = 0.041	p = 0.629	p = 0.982	p < 0.001	p = 0.276

[1]adjusted for serum albumin concentration in the same blood sample; *considered statistically significant.

tern of correlations with the other parameters as uncorrected calcium concentrations. Concentrations of osteocalcin and C-telopeptide showed strong, significant correlation with each other, too. Interestingly, sodium, chloride and, in one case potassium, correlated significantly with some parameters of bone homeostasis like osteocalcin, magnesium and phosphorous. On the other side, no parameter was statistically correlated with concentrations of vitamin D in serum samples.

Correlation analysis, with the data which were clustered according to the visit samples, showed dynamic relationship between the study variables during the antidepressant treatment course. Correlations between calcium and magnesium serum concentrations were statistically significant in blood samples from the visit 1 ($r_s = 0.389$, $p = 0.050$), the visit 2 ($r = 0.449$, $p = 0.036$) and the visit 3 ($r = 0.497$, $p = 0.015$) but not from the visit 4 ($r_s = 0.075$, $p = 0.747$). Correlations between calcium and osteocalcin serum concentrations were statistically significant in blood samples only from the visit 4 ($r_s = 0.496$, $p = 0.022$), between calcium and sodium, from the visit 1 ($r = 0.705$, $p < 0.001$) and the visit 3 ($r = 0.734$, $p < 0.001$) (Figure 2) and between calcium and chloride, from the visit 1 ($r = 0.601$, $p = 0.001$) and visit 3 ($r = 0.563$, $p = 0.003$). Albumin-adjusted values of calcium, as in the case of aggregate data, showed comparable pattern of correlations with other parameters like that of uncorrected calcium concentrations when analysis included only data from particular visits.

Osteocalcin and C-telopeptide serum concentrations, which indicate bone deposition and absorption, showed statistically significant and strong correlations not only for aggregate sample data but also for each separate subgroup including the visit 1 ($r = 0.771$, $p < 0.001$), the visit 2 ($r = 0.710$, $p < 0.001$), the visit 3 ($r = 0.780$ $p < 0.001$) and the visit 4 ($r = 0.732$, $p < 0.001$) (Figure 3). Although values for both osteocalcin and C-telopeptide, significantly correlated

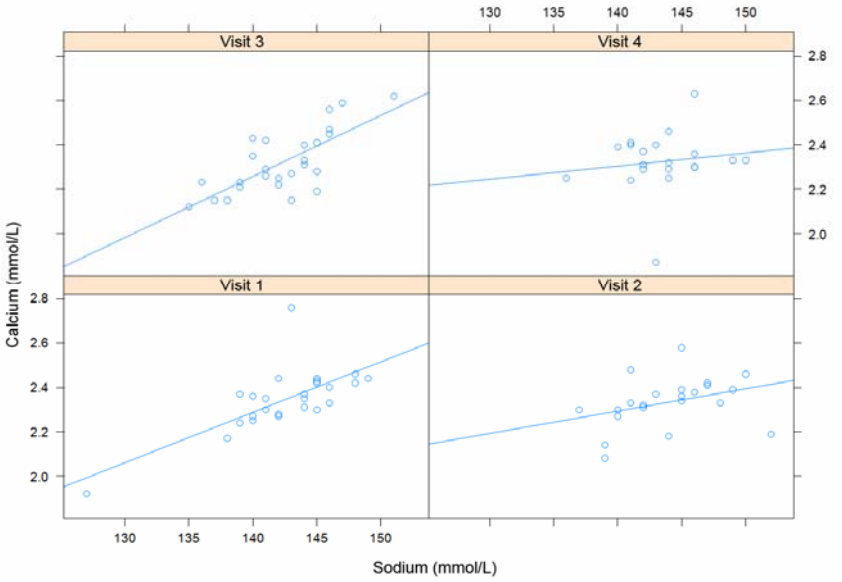

Fig. 2 – Correlations between calcium and sodium serum concentrations from samples taken at separate study visits.

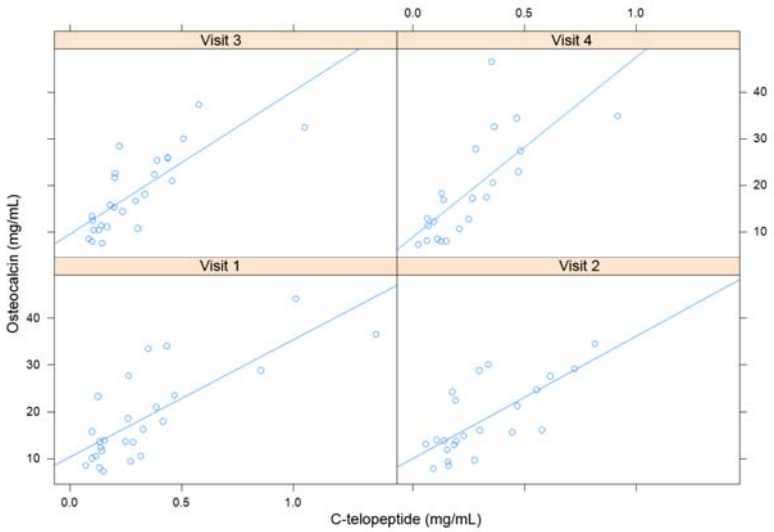

Fig. 3 – Correlations between osteocalcin and C-telopeptide serum concentrations from samples taken at separate study visits.

with sodium concentrations when aggregated data have been analyzed (blood samples from all visits), such correlation was not observed when data sets have been divided into the four visit subgroups ($p > 0.05$). Similarly, weak but statistically significant correlations between the aggregated values of magnesium and sodium, magnesium and potassium, together with phosphorous and chloride were not confirmed throughout analysis of separate visits. Significant correlations have been observed only for magnesium and potassium values from the visit 1 ($r_s = 0.595$, $p = 0.002$) as well as for phosphorous and chloride values from the visit 4 ($r = -0.526$, $p = 0.017$).

Discussion

The results of our study showed that antidepressant treatment did not significantly change the values of primary study variable, serum concentrations of calcium, during three months in the setting of routine psychiatric practice. In addition, other bone minerals, vitamin D and markers of bone deposition and absorption were unaffected, too. However, changes of some parameters connected with bone metabolism positively correlated with each other during the study, suggesting existence of active bone turnover in our patients. In addition, older age of our study subjects was significantly associated with vitamin D depletion. Therefore, the evidence presented in our study favored importance of underlying patient's characteristics relative to antidepressant medication for bone metabolism. Final result of the interplay of different risk and protective factors could be either bone health or bone disease in the subject who suffer from a mental disorder which needs particular antidepressant drug.

Previous studies reported significant association among antidepressants, osteoporosis and bone fractures [4, 19, 20] but some recent clinical studies found that pharmacotherapy of depression either suppressed or unaffected markers of bone absorption [11, 21]. In addition, results of laboratory studies told us that antidepressants were capable to induce bone formation in experimental animals [6, 22]. Even in the studies with "positive" findings, including the most recent ones, the risk for fracture that could be attributed to antidepressants was rather small and very variable between different societies, and might diminish during the treatment course [4, 19, 23]. All these experiences are in well agreement with the findings presented in our study which contributed to the knowledge about relative safety of antidepressant for calcium homeostasis, at least in short term, the topic rarely aimed in previous studies as the primary research focus.

It was well established that many cases of bone fractures in patients with depression were, in fact, caused by falls, particularly in older people, and the importance of confounders was pointed out [24]. Furthermore, deficiency of vitamin D might not be associated with presence of depression in some populations of adults, so treatment with vitamin D would unlikely improve depressive symptoms, and in the same time different antidepressants might variously influence metabolic pathways of the vitamin [7, 25, 26]. Therefore, prediction of the risk for disturbances of bone metabolism and consequent fractures in a patient taking a depressant seems to be much more complex task than simple connection with the drug class. Our study indicates that, if antidepressants do increase fracture risk, disturbances of calcium and, possible, other bone minerals seem to play no or little role in this process. In this regard, further research in the field, focused on well-characterized underlying risks and, particularly, on their interactions either with each other as well as with individual antidepressants is needed.

Statistically significant, moderate-to-strong and rather consistent link between serum concentration of bone minerals and major electrolytes, during the study visits, is very interesting and, in some regard, novel finding, which our study presents. It is reasonably assumed that maintaining physiological electrolyte homeostasis represent important health issue for the patients who take antidepressants. Excessive sodium dietary intake is a recognized risk factor for development of osteoporosis [27]. In addition, hypomagnesaemia could be associated with hypokalemia, due to increased calciuria and consequent potassium tissue redistribution, sometimes requiring potassium and magnesium supplementation [28]. However, as far as we know, prospective clinical studies, which examined relationships between serum levels of bone minerals and sodium, chloride and potassium during the antidepressant treatment and their consequences on major clinical outcomes were not conducted previously. For example, the newest systematic evidence strongly put in question widespread, indiscriminate use of dietary calcium supplements as they had minimal (if at all) impact on bone mineral density and fracture risk [29,30]. Therefore, individual approach to patients and future, focused researches are necessary in order to provide more data about both risks and preventive and treatment strategies which have the most relevant to everyday clinical practice.

Frequency of important disturbances of serum parameters in our patients (mostly below the lower normal limits) did not change significantly from baseline to the end of the study. Antidepressant-induced hyponatremia is a well-recognized clinical entity, with syndrome of inappropriate secretion of antidiuretic hormone being major mechanism [29]. In our study, we detected minimal, but statistically significant relative changes of sodium from baseline serum levels. There are case-reports directly connecting use of some antidepressants with disturbances of potassium and chloride serum levels, sometimes with concomitant depletions of other major serum parameters [30–32]. Therefore, the possibility that antidepressants in an individual patient induced subtle fluctuations of some parameters within the reference range, which further disturbed homeostasis of other counterparts, could not be excluded in our research. It remains unknown which biological mechanisms connect the observed effects in our study and what are consequences for bone health, so further focused research is necessary.

Our results have to be considered taking into account several limitations of the study such as relatively small sample-size, patients of heterogeneous characteristics, limited time-period, absence of control group and lack of information about some serum markers important for bone metabo-

lism. However, we believe that improvement of these methodological shortcomings would not significantly change the main finding in our study, that antidepressants, as a drug-class, do not cause *per se* disturbances of serum calcium and, likely, bone minerals during short-to-medium time period. For example, our sample-size was sufficiently powered to detected small but notable fluctuations of primary variable and the researchers have already used self-controlled design (without control-group) as a valid approach in order to examine the somatic risks in the patients who has taken the psychotropic drugs [15]. Due to logistic constraints, the serum level of parathyroid hormone was not measured, but it has been reported that, if its baseline levels were increased in patients with depression the antidepressants rather normalized than disturbed them; alternatively, finding of increased parathyroid hormone could be confounded with other regulators, as the vitamin D [11, 33].

Many patients in our study took benzodiazepines and some antipsychotics but, as we are aware, no protective effects of these drugs on bone metabolism were reported. Instead, sedatives increase fracture risk mainly due to gait disturbances and antipsychotics induce hyperprolactinemia and hypogonadism predisposing patients to osteoporosis. In fact, the fracture risk in people taking antipsychotic drugs had much more magnitude than in patients taking antidepressants [34–36]; moreover, they could significantly disturb calcemia in the notable number of patients, manly toward low levels [17]. Therefore, if anxiolytics and antipsychotics used by patients in our study did influence bone mineral homeostasis and their serum levels, the aggregate resultant would either facilitate or counteract presumed action of antidepressants. In both cases the oscillation of their serum calcemia would escape the pre-defined level (change of 5% from baseline), the event which had to be powerfully detected within our sample size. In addition, the mean serum levels of other bone minerals, markers of bone turnover and vitamin D remained within reference ranges and frequencies of their baseline disturbances did not change significantly throughout our study course. It decreases possibility of major influence of factors other than antidepressants for study outcomes followed in the patients, who were enrolled in our research.

Conclusion

Results of our study demonstrated that antidepressants do not disturb significantly serum levels of calcium and other bone minerals and vitamin D homeostasis during acute treatment phase within the settings of routine psychiatric practice. Further research should prospectively examine the effects of long-lasting treatment course, beyond three month of therapy of individual antidepressant drugs in different subgroups of patients with particular risk factors for disorders of bone and electrolyte metabolisms.

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Declaration of interest

There is no conflict of interest for any author.

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