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The assessment of bone tissue metabolism in alcohol dependent women with the use of biochemical markers of bone turnover – osteocalcin and ctx

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Summary

Aim. The aim of this study was the assessment in alcohol dependent women of metabolism of bone tissue and the influence of liver dysfunction on the process of osteogenesis and bone resorption.

Methods. The studied group consisted of 50 alcohol dependent female patients who were divided in two groups: one with an activity of AST or ALT above referential values and level of bilirubin and the second one with the activity of transaminases and level of bilirubin within referential values. The level of sex hormones and markers of bone turnover such as osteocalcin and collagen cross laps (ctx) were determined.

Results. In the group with an AST, ALT or BIL above referential values, the concentration of FSH in ovulation phase and luteal phase as well as LH in luteal phase was significantly higher, while ctx and osteocalcin was lower compared to the group with AST, ALT or BIL within referential values. The mean concentrations of FSH in follicular phase and luteal phase as well as LH in luteal phase and progesterone in follicular phase were increased in the group of patients with AST, ALT or BIL above referential values. The positive correlation between levels of ctx and osteocalcin was found that suggests a balance between processes of bone formation and bone resorption in whole group while lack of such correlation was observed in patients with AST, ALT or BIL above referential values.

Conclusions. The obtained results indicate the multidirectional and mutual relations between the alcohol abuse, liver function, bone turnover and activity of endocrine system.

Key words: alcohol abuse, alcoholic liver disease, bone turnover, female sex hormones.

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Table of abbreviations:

AST	aspartate transaminase
ALT	alanine aminotransferase
BIL	bilirubin
β-ctx	β-crosslaps
ES	estradiol (1,3,5 (10)-estratrien-3,17 p-diol)
FSH	follicle stimulating hormone
LH	luteinizing hormone
PROG	progesterone

Introduction

Alcohol abuse is one of the factors accelerating the risk of osteoporosis in women. Osteoporosis is a metabolic bone disease associated with loss of bone mass and changes in its quality and structure, which in turn leads to fractures. Bone cell metabolism and bone restructuring process is regulated by a number of factors, which include hormones, local growth factors and physical factors. The hormones are responsible for the regulation of synthesis and activation of the local factors that act directly on the cells which results in the modification or differentiation [1, 2]. Differentiation of stromal precursors in the creation of osteoblasts and osteoclasts is complex and subjected to strict control by local and systemic factors, including hormonal ones [3, 4, 5]. In order to identify the risk of osteoporosis, biochemical studies are carried out to assess the bone tissue metabolism. Drinking alcohol, in addition to damage to the liver, can cause a variety of disorders of the endocrine system [6]. This results in abnormal menstrual cycle, anovulatory cycles, luteal phase dysfunction cycle (luteal insufficiency), secondary amenorrhea, early menopause, increased risk of spontaneous abortion, breast cancer, and increased risk of osteoporosis occurring more frequently in women abusing alcohol [7]. This effect of alcoholic beverages may be the result of impaired synthesis, storage and release of hormones by changes in regulating these processes feedback loop functioning of receptors and postreceptoral effects, as a result of the impact of ethanol on the hypothalamus, pituitary, and gonads. It may also result from ethanol-induced organ dysfunction responsible for the metabolism of the hormones and transporting them protein synthesis [8, 9, 10]. The specific role in these processes is played by liver [11]. Drinking induces hepatic microsomal enzymes which may accelerate metabolism of sex hormones, and liver dysfunction may increase their clearance [7, 12, 13].

Patients and methods

50 women with alcohol dependence, treated for a period of 30 days at the Short Term Therapy and Detoxification Ward for Women in Bydgoszcz. E. Warmiński City Hospital were examined. The inclusion criteria for patients were: (1) premenopausal age, regular menstrual cycle (2) compliance with the criteria for alcohol dependence according to ICD-10, (3) providing written consent to participate in the study, and (4) the period of abstinence not longer than 7 days. From the study excluded persons (1) with dependence of substances other than alcohol and/or nicotine, (2) the liver damage

caused by infection with hepatitis B and C, (3) receiving any medications, including hormone treatments, contraceptives and psychotropic drugs. In the course of the analysis patients with alcohol dependence were divided into subgroups with higher activity than the reference value of AST, ALT and bilirubin levels and the activity of AST, ALT and bilirubin levels within the reference values. Demographic and clinical data of the patients with the division into subgroups presents Table 1. The amount of alcohol consumption during the 30 days before the start of the study was determined using the WHO questionnaire Timeline/IDS study. In patients, the concentration of the following hormones was determined: follicle stimulating hormone (FSH), luteinizing hormone (LH), estradiol (ES) and progesterone (PROG): (1) in the follicular phase, between day 5-7 of the cycle, (2) in the peri-ovulatory, between 11-14 day cycle, and (3) in the luteal phase, between day 19-22 of the cycle. At the beginning of the observation also aminotransferase activity, aspartate aminotransferase (AST) and alanine aminotransferase (ALT), bilirubin and osteocalcin level and ctx were determined. The levels of hormones were determined by Roche tests (for each phase the reference values are specified): FSH levels by FSH test – follicle stimulating hormone FSH [mIU/ml]: Follicular phase (FSH1) 3.5 - 12.5 mIU, ovulatory phase (FSH2) 4.7-21, 5mIU, luteal phase (FSH3) 1.7-7.7 mIU, LH by LH luteinizing hormone test LH [mIU/ml]: Follicular phase (LH1) 2.4-12.6 mIU/l, ovulatory phase (LH2) 14.0-95.6 mIU/L, luteal phase (LH3) 1.0-11,4 mI/l, the concentration of progesterone by Progesteron II Progesterone [ng/ml]: follicular phase (PROG1) 0.2-1.5 ng/ml, ovulatory phase (PROG2) 0.8-3.0 ng/ml, luteal phase (PROG3) 1.7-27 ng/ml; estradiol level by Estradiol II test [pg/ml]: follicular phase (ES1) 24 0.5-195 pg/ml, ovulatory phase (ES2) 66.1-411pg/ml, luteal phase (ES3) 40.0-261pg/ml. Level of osteocalcin was assessed with set LUMItest Osteocalcin by BRAHMS, reference values: 0-35 ng/ml. Determining the concentration of β-CrossLaps (β-ctx) was made by set β-CrossLaps/serum Roche, reference values: 0-0.32 ng/ml. The activity of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were also determined. Designations of enzyme activity were performed by Bio Merieux tests. AST reference value range: 4-34 U/l, ALT 2-41 U/l. Bilirubin levels were determined by test of ABBOTT DMSO; reference value <1.1 mg/dl. Statistical analysis was performed using the statistical program STATISTICA 14.0 PL. Results are presented as mean value (x) ± standard deviation (SD). In the analysis of the significance of differences was used: for variables with normal distribution – Student's t-test and for nonparametric distribution of variables U-Mann Whitney test. In regression analysis – Pearson's correlation coefficient was calculated. In all analyzes, as statistically significant the level of significance $p \leq 0.05$ was assumed.

Results

Demographic and clinical data of the studied female patients: age 37.28 ± 7.24, age of onset of dependence 31.81 ± 6.9, duration of dependence 5.47 ± 2.3. The collected data are shown in Table 1 – *next page*.

Table 1. **Demographic and clinical data of the examined women**

Parameter	x	SD
Age	37.28	7.24
Age at the onset of addiction	31.81	6.9
Duration of addiction	5.47	2.3
Number of days of drinking alcohol during the 30 days before the examination	8.73	7.16
Number of standard drinks consumed during the 30 days before the examination	90.66	79.78
FSH1 (mIU/l)	8.77	7.44
FSH2 (mIU/l)	7.73	5.54
FSH3 (mIU/l)	5.97	2.82
LH1 (mIU/l)	4.31	3.16
LH2 (mIU/l)	7.80	6.54
LH3 (mIU/l)	5.98	7.49
PROG1(pg/ml)	1.63	2.62
PROG2 (pg/ml)	1.25	1.75
PROG3 (pg/ml)	3.77	5.23
ES1 (pg/ml)	66.42	46.53
ES2 (pg/ml)	120.10	83.92
ES3 (pg/ml)	125.65	109.69
Ctx (ng/ml)	0.36	0.17
Osteocalcin (ng/ml)	23.59	7.40

1 – determination of the hormone in the follicular phase, 2 – determination of the hormone in ovulatory phase, 3 – determination of the hormone in the luteal phase

Subjects were divided into subgroups: one with high (that go over the reference value) values of transaminases activity and bilirubin levels, and the second of transaminases activity and bilirubin levels falling within the limits of the reference values. In next part of the study levels of hormones, ctx and osteocalcin between female patients in both groups were compared. Patients with high enzymatic markers of liver damage, bilirubin concentration in comparison to the group of women with normal transaminases activities and bilirubin concentration in the 30 days before the test, drank a similar amount of alcohol (Table 2 – *next page*). In the first ones significantly higher levels of FSH2, FSH3 and LH3 were found, marginally lower concentration of ES2 and significantly lower levels of ctx and osteocalcin (Table 3 – *next page*). The evaluation of the correlation of biochemical markers of bone metabolism in the whole group of the surveyed women was performed and demonstrated that the concentration of ctx and osteocalcin are significantly mutually positively correlated ($p = 0.001$, $r = 0.554$) (Table 4 – *next page*).

Table 2. **Clinical data and the comparison of their value in female patients with high and normal liver damage enzyme markers**

Parameter	Females with elevated activity of AST, ALT and elevated level of bilirubin n = 11	Females with activity of AST, ALT and level of bilirubin within the limits of the reference values n = 39	p
Age	42.4 ± 6.6	36 ± 6.6	0.032
Age at the onset of addiction (years)	37.6 ± 5.3	30.23 ± 6.5	0.012
Duration of addiction (years)	4.89 ± 3.0	5.73 ± 3.0	0.513
Number of days of drinking alcohol during the 30 days before the examination	7.14 ± 2.5	11.0 ± 8.2	0.238
Number of standard drinks consumed during the 30 days before the examination	94.14 ± 73.7	108.7 ± 88.3	0.697

Table 3. **Ctx, osteocalcin and sex hormones values in women with elevated and normal liver damage enzyme markers**

Parameter	Females with elevated activity of AST, ALT and elevated level of bilirubin n = 11	Females with activity of AST, ALT and level of bilirubin within the limits of the reference valuesn = 39	p
FSH1 (mIU/l)	12.8 ± 9.0	7.6 ± 7.1	0.126
FSH2 (mIU/l)	12.1 ± 9.9	6.3 ± 2.6	0.017
FSH3 (mIU/l)	8.8 ± 3.3	5.4 ± 2.2	0.004
LH1 (mIU/l)	5.7 ± 4.5	3.8 ± 2.6	0.174
LH2 (mIU/l)	10.1 ± 5.6	7.2 ± 7.2	0.328
LH3 (mIU/l)	12.3 ± 13.4	4.5 ± 3.6	0.018
PROG1(pg/ml)	2.4 ± 2.5	1.5 ± 2.8	0.466
PROG2 (pg/ml)	0.5 ± 0.4	1.4 ± 2.0	0.228
PROG3 (pg/ml)	1.5 ± 2.6	4.2 ± 6.0	0.262
ES1 (pg/ml)	88.6 ± 55.3	61.7 ± 43.2	0.191
ES2 (pg/ml)	68.9 ± 45.1	138.5 ± 92.2	0.067
ES3 (pg/ml)	100.2 ± 65.2	135.0 ± 126.8	0.495
ctx (ng/ml)	0.25 ± 0.1	0.41 ± 0.2	0.021
Osteocalcin (ng/ml)	17.2 ± 7.3	25.3 ± 6.2	0.007

1 – determination of the hormone in the follicular phase, 2 – determination of the hormone in ovulatory phase, 3 – determination of the hormone in the luteal phase

Table 4. **Correlation of ctx and osteocalcin in the group of examined women**

Parameter	Ctx
Osteocalcin	p = 0.001 r = 0.554

In the later stage of the analysis the impact of liver damage on the balance of the processes of bone resorption and bone formation in examined women with alcohol dependence was assessed. For this purpose an assessment of the correlation of values of ctx and osteocalcin in women with high values of activity of enzymatic markers of liver damage and bilirubin in women with low values of these indicators was performed. The results are shown in Table 5.

Table 5. **Correlation of ctx, osteocalcin and sex hormones levels in women with elevated and normal liver damage enzyme markers**

Parameter	Females with elevated activity of transaminases and bilirubin level n = 11		Females with activity of transaminases and bilirubin level within the reference values n = 39	
Osteocalcin	ctx		ctx	
	r	p	r	p
	-0.041	0.917	0.685	0.000

In patients with low activity of transaminases and bilirubin a statistically significant correlation between concentrations of ctx and osteocalcin was indicated. In women with liver dysfunction lack of correlation has been shown between values of ctx and osteocalcin concentrations. Then evaluating correlations of bone turnover markers, hormones, age of subjects, time of dependence, age at onset of addiction – a statistically significant negative correlation between levels of osteocalcin and LH3 concentration was found – in both studied groups (Tables 6 and 7).

Table 6. **Correlations of ctx and osteocalcin levels with the values of hormone concentrations determined in the group of the examined women with values of AST, ALT and bilirubin concentrations exceeding the reference range**

The examined parameter	ctx		osteocalcin	
	r	p	r	p
FSH1	-0.412	0.271	0.822	0.007
FSH2	-0.117	0.765	-0.156	0.689
FSH3	-0.290	0.449	0.520	0.151
PROG1	0.244	0.527	-0.432	0.245
PROG2	0.025	0.949	0.480	0.191
PROG3	-0.375	0.320	-0.195	0.615
LH1	0.311	0.415	-0.222	0.566
LH2	-0.355	0.348	-0.452	0.222
LH3	0.243	0.528	-0.811	0.008
ES1	-0.246	0.524	0.366	0.332
ES2	-0.248	0.520	-0.057	0.883

table continued on next page

ES3	0.123	0.753	-0.176	0.651
TEST1	-0.126	0.746	-0.488	0.182
TEST2	-0.242	0.531	-0.385	0.307
TEST3	-0.052	0.895	-0.001	0.998
Prol1	0.568	0.110	-0.334	0.380
Prol2	0.473	0.198	-0.202	0.603
Prol3	0.478	0.193	-0.367	0.331
Age	-0.513	0.158	0.608	0.082
Duration of addiction	-0.315	0.410	0.027	0.944
Age at the onset of addiction	-0.451	0.224	0.621	0.074

Table 7. **Correlations of ctx and osteocalcin with the values of hormone concentrations determined in the group of the examined women with values of AST, ALT and bilirubin concentrations within the reference range**

The examined parameter	ctx		osteocalcin	
	r	p	r	p
FSH1	0.089	0.686	0.047	0.831
FSH2	-0.121	0.582	-0.253	0.244
FSH3	-0.350	0.102	-0.481	0.020
PROG1	0.379	0.075	0.058	0.794
PROG2	0.100	0.649	0.148	0.500
PROG3	0.059	0.789	0.339	0.114
LH1	-0.076	0.731	0.037	0.867
LH2	-0.203	0.352	-0.168	0.444
LH3	-0.446	0.033	-0.470	0.024
ES1	0.024	0.915	-0.118	0.593
ES2	0.090	0.684	0.340	0.113
ES3	-0.032	0.885	0.124	0.572
TEST1	-0.201	0.357	-0.321	0.135
TEST2	-0.344	0.108	-0.173	0.429
TEST3	-0.238	0.275	-0.060	0.785
Prol1	-0.294	0.174	-0.320	0.137
Prol2	-0.106	0.629	-0.114	0.604
Prol3	-0.199	0.363	-0.166	0.499
Age	0.051	0.816	-0.189	0.387
Duration of addiction	0.122	0.581	-0.018	0.953
Age at the onset of addiction	-0.003	0.989	-0.190	0.386

In contrast, women addicted to alcohol with high values of activity of enzymatic markers for liver damage and high concentration of bilirubin, were characterized by a positive correlation of the concentration of osteocalcin and level of FSH1 (Table 6), while those with low values of transaminases activity presented a significant positive correlation with the concentration of FSH3 (Table 7) . In the next stage of the analysis in the whole group of examined women evaluated the correlation of concentrations of ctx and osteocalcin with the marked hormones and clinical data: age, duration of addiction, addiction beginning age. There was a statistically significant negative correlation between concentrations of ctx and osteocalcin and the level of LH3 and significant negative of osteocalcin and TEST1 (Table 8). However, no significant correlation of the level of ctx and osteocalcin with duration of dependence and age at the onset of dependence in the studied women was found (Table 8).

Table 8. **Correlations of ctx and osteocalcin with the values of hormone concentrations determined in the group of the examined women**

The examined parameter	ctx		osteocalcin	
	r	p	r	p
FSH1	0.039	0.832	0.112	0.540
FSH2	-0.119	0.516	-0.240	0.185
FSH3	-0.332	0.064	-0.269	0.137
PROG1	0.341	0.056	0.016	0.929
PROG2	0.087	0.637	0.206	0.257
PROG3	-0.016	0.93	0.244	0.179
LH1	-0.022	0.904	0.001	0.994
LH2	-0.238	0.189	-0.232	0.202
LH3	-0.379	0.032	-0.471	0.006*
ES1	-0.005	0.976*	-0.074	0.686*
ES2	-0.019	0.919	0.215	0.237
ES3	-0.011	0.953	0.082	0.657
TEST1	-0.178	0.330	-0.361	0.042*
TEST2	-0.317	0.077	-0.221	0.225
TEST3	-0.203	0.264	-0.059	0.750
Age	-0.072	0.696	-0.023	0.900
Duration of addiction	0.064	0.727	-0.028	0.878

Discussion

Osteoporosis is a systemic skeletal disease characterized by low bone mass, impaired bone microarchitecture and consequently its increased fragility and the susceptibility to fracture [4]. The metabolism of bone cells and bone reformulation is regulated by

a number of factors, which include hormones, growth factors, and local physical factors [2, 14]. Bone metabolism is characterized by constant coexistence of processes of bone formation and bone resorption. The dynamics of these processes can be assessed by the release from bone tissue specific indicators which are possible to be marked in serum or urine. For the diagnosis of risk of osteoporosis is performed: 1) assessment of biochemical bone tissue metabolism (determination of protein fragments of bone structural elements or their degradation products, the determination of enzymes and proteins produced by osteoblasts or osteoclasts activity), 2) densitometric examination showing bone density [15, 16]. Biochemical bone turnover markers are fragments of bone protein structural elements or their degradation products, and enzymes and proteins released into the circulation during the metabolic activity of osteogenic cells – osteoblasts and osteoclasts. In the assessment of bone metabolism the greatest diagnostic strength has ctx – a marker of bone resorption and osteocalcin - a marker of bone formation. Osteocalcin (BGP) – a marker of bone formation – is a non-collagenous bone matrix protein, synthesized only by osteoblasts, made up of 49 amino acids, which undergoes rapid proteolysis to numerous fragments used for the determination of BGP.

Markers of bone resorption – pyridinoline (PYD) and deoxypyridinoline (DPD) are amino acids forming the binding connection stabilizing collagen molecules in bone matrix. During the distribution of osteoid by osteoclasts these compounds are released into the body fluids in the form of free PYD and DPD and crosslinked with N-or C-terminal peptides of collagen (CTX and NTX, respectively) [17]. Within the whole group of examined women a positive correlation between concentrations of ctx and the osteocalcin was found, which demonstrates the creation and sustainability of bone resorption. However, women with liver dysfunction were characterized by the lack of the correlation between values of concentrations of ctx and osteocalcin. This may indicate a disturbed balance in the process of bone formation in patients with liver dysfunction. Moreover, it was found that patients with liver dysfunction compared with women with normal liver enzymes had statistically significantly higher levels of FSH2, FSH3 and LH3 and – on the borderline of variability – lower ES2. It was also shown that patients with high transaminase values had quite different, and even reversed course of LH concentration changes in the menstrual cycle, because it increased in the luteal phase, while physiologically and in the subgroup of patients with normal aminotransferases activity was observed its decline. It seems that the direction of changes in concentrations of LH in the early period of alcohol abstinence could define the individual susceptibility to alcoholic liver damage.

The impact of estrogen on bone tissue metabolism has not yet been fully understood. Both osteoblasts and osteoclasts possess nuclear receptors for estrogen, but their expression is weakly intensified. Estrogens inhibit the proliferation of osteoclast precursor cells, and decrease the activity of mature osteoclasts [12, 18]. Physiologically - low estradiol levels correspond to high levels of LH and FSH, while Tivis in his work shows that in women with cirrhosis of the liver caused by alcohol, there was no such relationship between ES, LH and FSH [18]. Just as we have shown in our study.

Demonstrated in the work relationship between the concentrations of sex hormones and liver damage is partly consistent with data from the literature. Alcoholic

liver disease contributes to higher concentrations of steroid hormones (progesterone, estradiol), mainly due to inhibition of hepatic metabolism [7, 13, 20, 21]. In postmenopausal women and in men the degree of liver damage correlated positively with the concentration of sex hormones, also pituitary, although in some of the works, there was observed no impact of liver function on the level of gonadotropins [12, 22-25]. Shown in our work, higher levels of FSH and LH in patients with liver damage at similar values of steroid hormones in both groups can be explained according to La-Paglia as impaired negative feedback in the pituitary-gonadal axis, e.g. due to a defect of synthesis of inhibin, which is produced by the granulosa cells of ovarian follicles, and responsible for the feedback inhibition of FSH secretion [8, 26, 27].

The observed in the own work hormonal imbalance de Koning explains by inter alia impaired synthesis of regulatory substances in the liver and lower levels of free sex hormones due to their binding to carrier proteins circulating in high concentrations in patients with alcoholic liver disease [28]. Some researchers, including Itturiaga, recognize that the effect of ethanol is a partial resistance of ovaries to the action of gonadotropin or direct damage to the gonads by alcohol and their primary failure [11, 26, 29]. Regardless of these potential mechanisms that may explain the pathophysiological substrate of the obtained results , the observed hormonal imbalance may have certain clinical implications in patients with alcohol dependence. They may be manifested in, inter alia, changes in sexual phenotype, altered menstrual cycle, fertility, and impaired bone metabolism and increased incidence of cancer [7, 30, 31]. It appears that the observed in the own work relationship between the concentration of sex hormones and liver damage translates into bone metabolism processes. Evidence of this is: 1) significantly lower levels of ctx and oseokalcin in women with liver dysfunction, accompanied by ctx and oseokalcin correlation with the concentration of LH3, a hormone which levels are significantly lower in women with damaged liver, 2) the lack of correlation between the concentrations of ctx and osteocalcin in group of women with impaired liver signaling imbalance in processes of bone formation and bone resorption.

Abuse of alcohol, which may result in liver damage, is accompanied by various comorbidities. It also contributes to the development of certain disorders. These may include hormonal dysfunction, bone metabolism. The above results of the work suggest multidirectional mutually overlapping relationship between alcohol, liver function, bone metabolism and hormonal axis hypothalamus-pituitary-gonad. Because of this numerous biochemical parameters should be dealt with, more numerous should be also the studied group of women, as without that the studies are limited. Mechanisms influencing the development and course of the disorder coexist in many areas with clinical results. Getting to know them seems to be important for the treatment of patients. Our stydy fit into this current of research.

Conclusions

1. Patients with values of enzymatic activity markers of liver damage and bilirubin levels above the reference range, compared with women with normal values of these parameters had lower levels of of ctx and osteocalcin, which in the absence of correlation of ctx and osteocalcin with age implies a relationship of bone metabolism disorders with liver dysfunction.

2. The women addicted to alcohol with properly functioning liver metabolism were characterized by balanced bone turnover.
3. In the group of women with liver dysfunction the imbalance of processes of bone formation and resorption has been demonstrated.
4. The observed in the own work relationship between the concentration of sex hormones and liver damage results in bone metabolism processes.

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