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The serum concentration of copper in bipolar disorder

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Summary

Aim. Some scientific reports indicate the changes in the concentration of serum copper in patients with bipolar disorder (BD), however the data are inconclusive. The aim of this study was to assess the concentration of copper in the blood serum of patients in various phases of BD compared to healthy volunteers, taking into consideration the specific clinical features, and the stage of illness.

Methods. The study enrolled 133 patients with a diagnosis of BD (type I, II and NOS), including 61 people in depressive episode, 23 in mania or hypomania and 49 in remission. The control group consisted of 50 people. Atomic absorption spectrometry was used to measure the concentration of copper.

Results. There were no statistically significant differences in the serum copper concentration between patients in various phases of BD (mania/hypomania, depression, remission), sub-types (Type I, Type II + NOS) or stages and healthy volunteers. However, serum copper

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concentrations in patients in stage 1 was significantly higher than in advanced stages (2+3+4), ($\beta = 0.22$; $p = 0.02$). Serum copper concentration was also the higher, the later the age of onset was ($\beta = 0.33$; $p < 0.001$), and the lower, the greater the number of illness episodes ($\beta = -0.23$; $p = 0.02$) (multiple regression model, adj $R2 = 0.19$, $p = 0.0001$).

Conclusions. The dependencies demonstrated above may reflect pathophysiological processes that occur in the course of BD (e.g., inflammatory response and oxidative stress) with a different intensity depending on its stage.

Key words: bipolar disorder, biomarkers, copper

Introduction

Copper is an element that plays an important role in many aspects of action and pathophysiology of central nervous system (CNS), which are associated with the etiology of mental disorders, with particular emphasis on mood disorders [1]. Physiological concentrations of copper, for example, take part in the activation of this element-dependent enzymes involved in the catecholamine transmission, such as dopamine beta-hydroxylase, monoamine oxidase and tyrosine hydroxylase [1–4]. Copper is also a necessary element for the proper functioning of cytochrome C oxidase and Cu/Zn superoxide dismutase. These enzymes are involved in the elimination of reactive oxygen species (ROS) [5, 6]. Pathological changes in the copper concentration can lead to overproduction of ROS with subsequent intensification of oxidative stress – the phenomenon resulting in dysfunction, damage and even death of neurons. More and more data indicate the presence and intensification of oxidative stress in the course of depression and bipolar disorder [7]. Another phenomenon associated with the pathogenesis of affective disorders is glutamate-dependent excitotoxicity and resulting from overstimulation of ionotoropic glutamate NMDA receptors [8, 9]. Studies suggest that copper acts as a noncompetitive antagonist of the NMDA receptor complex and indirectly interfere with binding of glutamate and glycine to the receptor [1, 10, 11]. Copper also affects neurotrophic factors such as brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) [12, 13].

Most of the copper ions are attached to plasma ceruloplasmin, which is one of acute phase proteins involved in the inflammatory process. Results of several studies indicate the activation of the immune system and inflammatory markers in depression and bipolar disorder. It is another element linking copper with the pathophysiology of affective disorders [14, 15].

Pathological changes in copper concentration in the blood and its accumulation in the body are often accompanied by psychopathological symptoms, particularly by mood disorders. For example, mental and behavioral disorders (mainly depression, irritability and suicidal thoughts), are among the most common symptoms of Wilson's disease and, depending on the research, concern from 30 to 100% of patients [16–18]. Furthermore, according to recent studies, patients suffering from Wilson's disease, as compared to healthy population, are characterized by a greater risk (up to 13 times) of diagnosis of bipolar disorder [18, 19]. Disturbances of copper metabolism in the body can be found

in other neurodegenerative disorders associated with mental disorders, such as, Menkes disease, Alzheimer's disease, Parkinson's disease and Huntington's disease [20].

The results of experimental studies may also indicate a relation between copper and mood disorders. Exposing experimental animals to antidepressants and electroconvulsive therapy leads to significant changes in the level of copper in the blood and central nervous system. For example, long-term administration of imipramine or citalopram to rats is associated with a significant decrease in serum copper concentration, and after performing electroconvulsive therapy there is an increase in the copper concentration in the hippocampus and cerebellum with no change in its concentration in the blood [20, 21].

These data justify the study of changes in the serum copper concentration as a marker of affective disorders. Few such studies, involving patients with a diagnosis of unipolar depression, did not yield conclusive results. Most of them reported an increase in copper concentration in the blood of patients in depressive episodes, compared to healthy subjects [21–25] and in some cases its reduction after the effective antidepressant therapy [24, 26]. Two other observations indicated no differences in the copper concentration between depressive patients and the control group [26, 27]. Even less is known about the changes in the serum copper concentration in bipolar disorder. Until now, three studies involving small groups of patients with a diagnosis of bipolar disorder and giving conflicting results have been conducted [28–30].

Aim

The aim of this study was to assess the concentration of copper in the blood serum of patients in various phases of bipolar disorder (BD) compared to healthy volunteers, taking into consideration the specific clinical features, severity of symptoms and the stage of bipolar disorder.

Material and methods

Recruitment of participants

The study included patients meeting diagnostic criteria for bipolar disorder according to DSM-IV-TR (regardless of the stage of the illness) and healthy volunteers. All participants gave their informed consent to participate in the study. The study was approved by the Bioethical Committee of the Jagiellonian University. During the study the subjects were receiving monotherapy or combined therapy, with proven efficacy in the treatment of bipolar disorder, adequate to current phase of the illness and its clinical picture. Patients were receiving a variety of pharmacological treatment as monotherapy or as part of a combination therapy. 84 people were treated with olanzapine or quetiapine, 49 patients were treated with valproate, 20 with lithium, 25 with lamotrigine and 5 with carbamazepine. In addition to mood stabilizers therapy, some patients in depressive episodes and/or remission were treated with antidepressants (26 with SSRI, 35 with SNRI and 5 with mirtazapine).

Exclusion criteria were as follows: lack of consent to participate in the study, diagnosis of serious mental disorder other than bipolar disorder (e.g., schizophrenia, schizoaffective psychosis, major depressive episode), disorders associated with substance abuse (excluding nicotine or caffeine addiction), co-existence of chronic inflammatory and autoimmune diseases, acute infections present within a month before a patient was included in the study, severe endocrine disorders, nephrotic syndrome and burns, breastfeeding or pregnancy. A group of healthy volunteers was recruited from people with no present or past history of psychiatric disorders and substance abuse, with no above-mentioned acute and chronic somatic diseases and with no psychiatric disorders in the first-degree relatives. Detailed information concerning the place and rules of recruitment, inclusion and exclusion criteria are included elsewhere [31].

Diagnostic tools

The severity of depressive symptoms in patients was measured using the Hamilton Depression Rating Scale (HDRS) [32] and the Montgomery-Asberg Depression Rating Scale (MADRS) [33]. The severity of manic symptoms was measured using the Young Mania Rating Scale (YMRS) [34].

The criteria proposed by Kapczinski et al. were used to determine the severity of bipolar disorder [35]. The criteria are as follows:

- **stage 1** is characterized by features such as: full remission of symptoms after episodes, with full restoration of premorbid functioning;
- **stage 2** refers to the clinical picture of bipolar disorder in which the symptoms of comorbid psychiatric disorders (dependence or abuse of alcohol or other substances, anxiety disorders, personality disorders) which affect the impairment of functioning are clearly visible between episodes. There is also the possibility of rapid cycling. Cognitive impairment is recognized in neuropsychological tests, however, did not disclose in the examination and the functioning of the patient;
- **stage 3** is characterized by subsyndromal affective symptoms between episodes, overt clinical impairment of cognitive functioning, gradual shortening of the duration of periods of euthymia, increasing number of exacerbations, significant impairment of the family and professional functioning (patient is unable to work or is able to work on a position below the qualifications and skills);
- **stage 4**: intensification of the features typical of stage 3 and progressive disability and deterioration of the patient.

Collection, preparation and processing of blood samples – determination of concentration of copper in the blood serum

According to the study protocol, from each patient and healthy volunteer no more than 9.8 ml of venous blood was obtained using Monovette closed blood-collection system. After the formation of the clot, the samples were centrifuged for 30 minutes

at 1,800 RPM. The obtained serum was stored at – 80°C until the scheduled start of the analysis.

After thawing and mixing thoroughly, the quantitative analysis of the samples was performed using electrothermal atomic absorption spectrometry (ET AAS). The authors used PerkinElmer AA spectrometer Model 3110 (USA) equipped with the Perkin-Elmer HGA-600 graphite furnace. Pre-treatment temperature was 950°C and the temperature of atomization process was 2,300°C. Copper was measured at a wavelength of 324.8 nm and 0.7 nm slit.

Before starting the series of measurements, analytical procedure had been optimized (Method Development procedure) in order to obtain high sensitivity. Besides dilution of samples, no other procedures had been used prior the measurement of concentrations. Depending on the total volume of the sample measurements were performed in triplicate. The accuracy was tested by means of recovery analysis, which for copper was in the range of 96–103%.

Statistical methods

The χ^2 test was used to analyze the differences between the quality variables. The Shapiro-Wilk test as well as the analysis of skewness and kurtosis was performed in order to evaluate the normal distribution of quantitative data. Due to the lack of normal distribution of quantitative data the Kruskal-Wallis ANOVA or the Mann-Whitney U test was used. Due to the lack of normal distribution, correlations between quantitative variables were analyzed using the Spearman's rank correlation. In order to examine the relationship between the serum copper concentration (as a dependent variable) and selected clinical and demographic parameters (as independent variables) a multiple regression model was built. The variables included in the model were selected on the basis of literature data and the results of basic statistical analysis obtained earlier and presented in this paper (analysis of variance, correlations).

Results

The study included a total of 133 patients (84 women and 49 men) diagnosed with bipolar disorder type I (n = 69), bipolar disorder type II (n = 60) or bipolar disorder NOS (n = 4); 61 people were in the current depressive episode, 23 people in the current manic or hypomanic episode and 49 patients were in remission. At the moment of inclusion to the study, 21 patients presented features of rapid cycling. The average number of previous episodes of illness was 11.51 ± 9.61, and the average total duration of illness: 12.63 ± 8.06 years.

The control group consisted of 50 people (including 14 men and 36 women). The group of patients and a group of healthy volunteers did not differ in terms of age of the participants (43.33 ± 12.85 vs. 45.82 ± 12.43; Z = – 0.49; p = 0.62; Mann-Whitney U test), and the percentage of men and women (W: 63.2% vs. 72%) (χ^2 test = 1.26; p = 0.26).

Concentrations of copper in the control group and in various phases, sub-types and stages of bipolar disorders are shown in Table 1.

Table 1. **Serum copper concentrations in the control group and in various phases, sub-types and stages of bipolar disorder [ug/ml]**

Healthy volunteers Median (lower/upper quartile)	N = 50 0.82 (0.68/1.04)			
Patients	Episodes			
	Mania/hypomania	Depression	Remission	All phases
Bipolar disorder (total) Median (lower/upper quartile)	N = 23 0.72 (0.52/0.89)	N = 61 0.79 (0.64/1.00)	N = 49 0.83 (0.66/1.00)	N = 133 0.80 (0.61/1.00)
sub-types of bipolar disorder				
Bipolar disorder type I Median (lower/upper quartile)	N =18 0.81 (0.46/0.92)	N = 23 0.84 (0.65/1.07)	N = 28 0.81 (0.61/0.95)	N = 69 0.81 (0.61/0.99)
bipolar disorder type II + NOS Median (lower/upper quartile)	N = 5 0.64 (0.58/0.76)	N = 38 0.77 (0.60/0.96)	N = 21 0.85 (0.75/1.07)	N = 64 0.79 (0.64/1.04)
Stages of bipolar disorder				
Stage 1 Median (lower/upper quartile)	N = 1 0.72 (0.72/0.72)	N = 11 0.92 (0.75/1.11)	N = 13 1.01 (0.75/1.34)	N = 25 0.92 (0.75/1.17)
Stage 2 Median (lower/upper quartile)	N = 6 0.76 (0.64/0.89)	N = 11 0.81 (0.40/1.10)	N = 11 0.80 (0.71/0.90)	N = 28 0.80 (0.64/0.91)
Stage 3 Median (lower/upper quartile)	N = 12 0.81 (0.28/1.00)	N = 24 0.82 (0.63/1.02)	N = 14 0.86 (0.46/1.00)	N = 50 0.84 (0.52/1.00)
Stage 4 Median (lower/upper quartile)	N = 4 0.70 (0.67/1.08)	N = 15 0.73 (0.46/0.92)	N = 11 0.80 (0.58/0.86)	N = 30 0.74 (0.56/0.87)

The Kruskal-Wallis Rank ANOVA showed no statistically significant differences in serum copper concentrations between various phases of bipolar disorder (mania/hypomania, depression, remission), and healthy volunteers ($H = 2.64$; $p = 0.45$). There were also no differences between episodes and between episodes and the control group in subgroups of patients with bipolar disorder type I ($H = 1.59$; $p = 0.66$) and bipolar disorder type II + bipolar disorder NOS ($H = 3.03$; $p = 0, 22$) (Kruskal-Wallis Rank ANOVA).

Analysis of variance of serum copper concentration with regard to the division into the stages of the illness showed no statistically significant differences between various stages and above-mentioned stages and healthy volunteers ($H = 6.76$, $p = 0.15$). It has been found, however, that copper concentration in the serum of patients in stage 1 of BD was significantly higher compared to other stages of the illness treated jointly (2+3+4), ($z = – 2.25$; $p = 0.024$; Mann-Whitney U test) (Figure 1).

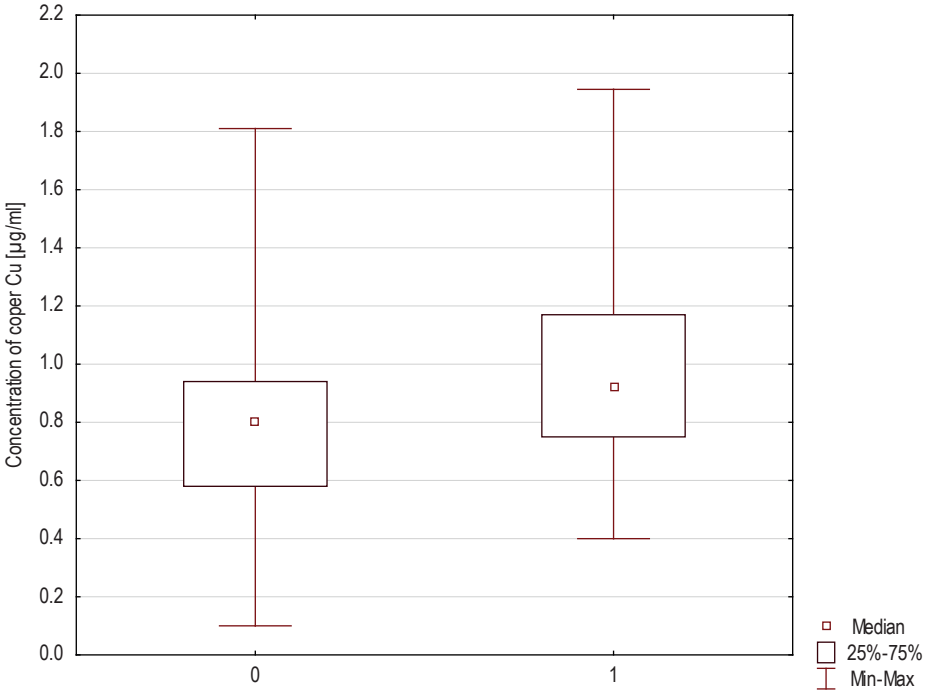

Figure 1. **Comparison of the serum copper concentration between patients in the first stage of bipolar disorder (1) and other patients representing the later, more advanced stages of bipolar disorder (0)**

There were also no differences between particular episodes and between episodes and the control group in the subgroups of patients in stage 1 (H = 1.55; p = 0.46), 2 (H = 0.30; p = 0.86), 3 (H = 0.79; p = 0.67) and 4 of bipolar disorder (H = 0.46; p = 0.79) (Kruskal-Wallis Rank ANOVA).

No statistically significant differences in serum copper concentration were observed between patients with rapid cycling, patients without such a feature and the control group (H = 1.04; p = 0.60) (Kruskal-Wallis Rank ANOVA).

In the entire group of patients with bipolar disorder, currently in depressive episode there were no statistically significant differences in the concentrations of copper between: atypical depression (n = 17) and depression without atypical symptoms (n = 44), (Z = 0.032; p = 0.97; Mann-Whitney U test); psychotic depression (n = 8) and depression without psychotic symptoms (n = 53) (Z = 0.64; p = 0.52; Mann-Whitney U test), as well as between depression with melancholic syndrome (n = 28) and depression that do not meet criteria for melancholic syndrome (n = 32) (Z = 1.02; p = 0.30; Mann-Whitney U test) (Table 2).

Table 2. **Serum copper concentrations in subgroups of patients with various clinical features of depressive episode**

clinical feature of depression	Concentration of copper [ug/ml] Median (lower/upper quartile)
With atypical features	0.76 (0.52/1.06)
Without atypical features	0.79 (0.64/0.97)
With melancholic syndrome	0.73 (0.56/1.05)
Without melancholic features	0.85 (0.66/0.97)
Psychotic	0.91 (0.62/1.01)
Without psychotic features	0.77 (0.62/0.99)

Table 3. **Correlation between the serum copper concentration and selected quantitative features of the course and the picture of bipolar disorder (Speraman's correlation)**

	Cu			
	Remission	Depression	Mania	Entire group
Age	0.09	0.03	0.60*	0.14
Number of episodes	0.05	-0.36*	0.23	-0.09
Number of relapses in the last year	0.06	-0.15	-0.09	-0.05
Age at onset of bipolar disorder	0.05	0.28*	0.44*	0.24*
Duration of bipolar disorder in years	0.06	-0.31*	0.19	-0.11
Duration of episode/remission	-0.04	0.20	-0.17	-

* statistical significance $p < 0.05$

Analysis of relationships between the serum copper concentration and the variety of clinical parameters (Table 3) showed a positive correlation with age at onset, both in the entire group of patients (mania/hypomania + depression + remission) ($r = 0.24$; $p = 0.006$) as well as in the phase of mania/hypomania ($r = 0.44$; $p = 0.045$) and depression ($r = 0.28$; $p = 0.03$) (Spearman's correlation). In the manic phase, the copper concentration was correlated positively also with the age of patients ($r = 0.60$; $p = 0.004$), and in depression was correlated negatively with the number of episodes in lifetime ($r = – 0.36$; $p = 0.009$) and duration of bipolar disorders in years ($r = – 0.31$; $p = 0.014$). There was no statistically significant correlation between the serum copper concentration and severity of manic symptoms measured by YMRS ($r = – 0.007$; $p = 0.98$) or depressive symptoms measured by MADRS ($r = 0.09$; $p = 0.48$) or HDRS ($r = 0.10$; $p = 0.45$).

Regression model covering the entire group (explaining 19% of variation of the variable Cu) confirmed the effect of: stage 1 of bipolar disorder ($\beta = 0.22$; $p = 0.02$,

higher concentration in patients in stage 1 compared to the other stages), and age at onset ($\beta = 0.33$; $p < 0.001$; the older the age, the higher the concentration of copper) on copper concentration. In addition, an effect of: sex ($\beta = -0.19$; $p = 0.04$, higher concentrations in females) and the number of previous episodes of ($\beta = -0.23$; $p = 0.02$, the more episodes, the lower the concentration of copper) was observed (Table 4).

Table 4. **Multiple regression model. The relationship between the serum concentration of copper (as the dependent variable) and selected clinical features of bipolar disorder (as independent variables)**

Cu							
Model	R^2	adj R^2	F	p	β	t	p
	0.24	0.19	5.15	0.0001			
Sex (Men)					-0.19	-2.11	0.04
Total number of episodes of bipolar disorder in lifetime					-0.23	-2.29	0.02
Age at onset					0.33	3.68	< 0.001
duration of bipolar disorder					0.07	0.62	0.54
Stage 1 of bipolar disorder					0.22	2.30	0.02
Smoking					0.16	1.83	0.07

R – coefficient of determination; R^2 adj – adjusted coefficient of determination R^2; F – F-statistics; p – level of statistical significance; β – partial regression coefficient, t – t-statistics.

Discussion

There were no statistically significant differences in the serum copper concentration in patients in various phases of bipolar disorder (mania/hypomania, depression, remission), with regard to the division into its subtypes, and the healthy volunteers. In addition, in the majority of people involved in the study, the copper concentration was within the normal range (0.7–1.4 ug/ml).

Similar results were obtained by González-Estecha et al. [29]. Similarly to the present study, blood samples were collected from patients receiving different pharmacological treatment in the course of bipolar disorder. Finally, the study included 25 people, 19 of which were in the manic phase, and 6 in the depressive phase. The comparison with a group of 29 healthy subjects showed no significant difference in the serum copper concentration. Observation conducted by Mustak et al. [28] gave different results. It covered 40 patients in mania in the course of bipolar disorder type I, 50 patients with a diagnosis of bipolar disorder type II (25 in hypomanic episode and 25 in depressive episode), 25 people with a diagnosis of bipolar disorder type V (depressive phase). The concentration of copper in patients with manic episode (BD type I) and depressive episode (BD type II and V) proved to be significantly higher

compared to the control group of 25 people. Dissimilarity of the obtained results may result from methodological differences. The most important difference is the fact that the blood samples in the case of the last survey had been taken before the implementation of pharmacotherapy.

In this study, serum copper concentrations did not differ between subpopulations of depressive patients who met and did not meet the criteria for depression with atypical symptoms, melancholic syndrome or psychotic depression. There were also no significant differences in concentration of copper between patients with rapid cycling and those without such a feature. Moreover, the concentration of copper was not dependent on the severity of depression and mania/hypomania. We have shown, however, that it was positively correlated with age at onset and decreased with the number of completed episodes. In addition, after taking into consideration the division into stages of bipolar disorder (staging) the concentration of copper in patients in stage 1 was significantly higher compared to other patients in more advanced stages of bipolar disorder. Other two studies did not analyze the abovementioned clinical parameters. It can be hypothesized that these relationships, demonstrated in this study, are associated with or reflect pathophysiological processes that occur in the course of bipolar disorder (such as e.g., inflammatory response and oxidative stress) and reach different intensity depending on its stage [7, 35, 36]. Elevated concentrations of copper may thus be a marker of early-stage BD, as well as increased activity of MMP-9, demonstrated in other study [37]. The verification of this hypothesis, however, requires further research in this area. In the context of copper-zinc homeostasis, increased serum level of copper may, however, correspond to lower concentrations of serum zinc, but this is more pronounced in the later stages of the illness [38].

Limitations of this study include: lack of a prospective model examining the dynamics of changes in the concentration of copper in the same patients, significant variation of the treatment and the inability to estimate its impact on the obtained results as well as a small number of patients in subgroups presenting specific clinical features, phases and stages of bipolar disorder. It should be noted, however, that the study was conducted on a large group of patients with bipolar disorder and the largest control group so far; it also took into consideration the largest number of variables characterizing detailed clinical picture of bipolar disorder, its course and stage. The obtained data can be an important source of knowledge about the role of copper in the pathophysiology of bipolar disorder. Due to the small amount of data and their inconsistency, there is a need for further research in this area.

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