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# EFFECT OF LITHIUM TREATMENT ON THE CONTENT OF LITHIUM, COPPER, CALCIUM, MAGNESIUM, ZINC AND IRON IN THE HAIR OF PATIENTS WITH BIPOLAR DISORDER*

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## Abstract

The homeostasis of elements is essential for a healthy nervous system, which is crucial for patients with bipolar disorder. Abnormalities related to the supply, absorption, intracellular and extracellular concentrations of micronutrients are considered as factors in the pathogenesis of affective disorders. Lithium carbonate treatment influences levels of elements. The paper presents a study on the influence of lithium carbonate treatment on the content of selected elements (lithium, copper, iron, zinc, magnesium and calcium) in hair sample from bipolar patients. The study group consisted of 57 patients with the diagnosis of bipolar affective disorder. Each patient was in the remission phase. Two groups were distinguished according to the treatment – with or without lithium carbonate. The levels of lithium, copper, iron, zinc, magnesium and calcium were measured based on an analysis of hair samples. Patients taking lithium carbonate are characterized by a higher level of lithium ($p<0.01$), zinc ($p<0.05$) and magnesium ($p<0.05$), and lower iron content ($p<0.05$) in a hair sample. Significant differences were found for magnesium distribution ($p<0.01$) in the group not treated with lithium. In this group, moderate dependence occurred between magnesium and lithium ($r=0.517$, $p=0.034$), magnesium and calcium ($r=0.564$, $p=0.018$), magnesium and iron ($r=0.510$, $p=0.037$), and iron and lithium ($r=0.532$, $p=0.028$). The study shows that BD is associated with specific abnormalities in the level of trace elements in the human body. The balance of elements can be affected by medications. The study shows that lithium carbonate can affect the concentrations of elements.

**Keywords:** hair analysis, Li, Mg, Ca, Cu, Fe, Zn.

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# INTRODUCTION

Bipolar disorder (BD) is a lifelong mental illness that requires psychopharmacotherapy. The frequency of subsequent episodes of mania and depression is strongly dependent on the systematic intake of appropriate mood stabilizers or antidepressants (Cerullo, Strakowski 2013). Lithium is used in BD and to treat schizoaffective disorders, recurrent depressive disorders, or neurodegenerative diseases (Gelfo et al. 2017). In general, it has antimanic, antidepressant, and prophylactic properties; at the same time it causes side effects, e.g. nausea, diarrhea, polyuria, polydipsia (Gitlin 2016). Lithium has a narrow therapeutic index between 0.6 mmol $L^{-1}$ to 1.2 mmol $L^{-1}$ (Gitlin 2016). Checking the lithium concentration in the patient's blood is necessary because small differences in a dose may lead to serious therapeutic failures or adverse drug reactions. This mood stabilizer may interact with other medications. Antidepressants may increase the toxic effects of lithium (SSRIs), or cause mania or rapid phase change, tricyclics with lithium (Szymczyk et al. 2017). Researchers do not exclude its potential impact on the functioning of healthy people with reduced dosage of the element described. Two forms of lithium are available (without prescription) for lithium supplementation: aspartate and orotate (Marshall 2005). Despite many years of use, the biological mechanism of lithium treatment is not fully understood.

Trace elements, both essential and non-essential ones, are parts of enzymatic systems and structural parts of biologically active components. Many factors influence the bioavailability of those components, e.g. food (inhibits or increases assimilability), drugs, health condition, the chemical form of the compound, age or contamination in one's area of residence (Krejpcio et al. 1999, Khalique et al. 2005, Suliburska et al. 2015). There is a synergistic or antagonistic interaction between the non-indigenous elements (metals) present in the human body, which respectively strengthen/raise the level or inhibit/lower the level of particular elements (Łojko et al. 2018). Metals may hinder the absorption of essential elements (Afridi et al. 2006). As a highly reactive element, lithium can affect other important components in the human body (Jakobsson et al. 2017). Abnormalities related to the supply, absorption, intracellular and extracellular concentrations of micronutrients are considered as factors in the pathogenesis of affective disorders (Łojko et al. 2018). Studies indicate that BD is also associated with specific abnormalities in the level of trace elements in the human body (Mustak et al. 2008, González-Estecha et al. 2011, Chowdhury et al. 2017, Santa Cruz et al. 2020). Comparisons of patients with BD and the control group show changes throughout chemical mechanisms, causing disturbances in metabolites, proteins and metalloproteins profiles (Sussulini et al. 2011). Researchers directly indicate that adequate nutrition (also at the trace element level) may lead to a better response to pharmacotherapy (Bourre 2006, Beyer, Payne

2016, Łojko et al. 2018). The undeniable influence of a diet and nutrition on mental disorders has resulted in a new field of research called nutritional psychiatry (Łojko et al. 2018, Adan et al. 2019). So far, most research in this area has focused on depression (Yoshimura 2018, Adan et al. 2019), while more and more studies are being conducted on patients with bipolar disorder or anxiety disorders (Marx et al. 2017, Łojko et al. 2018).

Researchers focus on one variable that can potentially impact the content of elements in the human body. An analysis of the Polish population shows that Fe is low in women and Ca, Mg, Zn, and Cu are higher (Schlegel-Zawadzka 1992). On the other hand, other researchers noticed no gender differences in the body content of Cu and Fe (Bertazzo et al. 1996, Dunicz-Sokolowska et al. 2007). There are some differences in Ca, Mg, Zn levels in young persons, namely boys tend to have lower concentrations of these elements. However, Krejpcio et al. (1999) did not notice a similar trend. Children with ADHD are characterized by lower levels of zinc, copper and magnesium (Elbaz et al. 2017). Nonetheless, researchers point to age-dependent changes. Cu drops with age (since the age of about 60 years), while Zn reaches its optimal value in about 30-40-year-olds, and then it slowly decreases (Bertazzo et al. 1996). Many studies indicate a decrease in the level of calcium in the body (which may be related to the process of bone demineralization) and a low level of its supply to the body (Stefańska et al. 2011). However, some of the results show other trends. In a female group, calcium and magnesium concentrations increased with age (Suliburska et al. 2015). Heavy metals (Pb, Cd) increase with age in men (Dunicz-Sokolowska et al. 2007). Levels of metals (lead, cadmium) are higher in hair samples of children living in areas with worse air pollution (Krejpcio et al. 1999). It has been verified that the type of a diet (conventional vs. vegetarian) does not affect the content of metals in women's hair (Wójciak et al. 2004). Atomic absorption spectroscopic analysis shows lower concentrations of zinc, calcium, iron, selenium, sodium and potassium in BD patients than in healthy controls (Siwek et al. 2016, Chowdhury et al. 2017, Styczeń et al. 2017). The differences in elemental levels in people with psychiatric illnesses are not fully understood. They are often cited as being related to particular psychopathology. Explanations include a role of oxidative damage or specific lifestyles. Further, researchers showed differences in the concentration of particular toxic elements (González-Estecha et al. 2011). Studies also differentiate the concentration of selected minerals in different mania and depression phases, respectively (Mustak et al. 2008, Beyer, Payne 2016, Siwek et al. 2016, Styczeń et al. 2017). Pharmacotherapy with mood stabilizers could be associated with K, P, Cu, Al and Mn pathways (Santa Cruz et al. 2020).

This study aims to investigate the influence of lithium carbonate on the level of selected elements: lithium (Li), copper (Cu), iron (Fe), zinc (Zn), magnesium (Mg), and calcium (Ca) based on an analysis of hair samples. Elemental analysis of hair, same as analysis of serum, urine or tissue, is a way to assess the mineral nutrition status (Suliburska et al. 2015).

# METHOD

## Participants

The study group consisted of 57 patients with bipolar disorder treated with particular mood stabilizers. Participants were divided into two groups according to whether patients were taking lithium or not. The lithium-treated group consisted of 40 patients while the untreated group included 17 patients. Adults between the ages of 18 and 50 were selected for the study. Based on inclusion criteria, eligible participants: a) suffered from bipolar disorder as defined in the International Statistical Classification of Diseases and Related Health Problems (ICD10), b) had a medical prescription for a mood stabilizer, c) were in the remission / incomplete remission phase. We excluded participants who: a) were older than 60 years, b) were treated with some medication in a hospital less than a month, c) were on an elimination diet, excluding some products, e.g. vegetarian, vegan, etc. The severity of bipolar disorder symptoms was measured on the Hamilton Depression Rating Scale (HDRS) and Young Mania Rating Scale (YMRS). The results on both scales did not show high severity of symptoms that could imply that the subject is manic or depressed. The study group comprised both hospitalized and outpatient patients. All respondents came from Wielkopolskie Voivodship in Poland and lived in medium or large cities. The subjects taking lithium were regularly checked for blood lithium levels, which were standard for the entire group during the study period. The research was conducted from 2018 to 2019. The Ethical Committee at the Poznan University of Medical Sciences approved the study.

## Procedure

Each hair sample was taken from the occipital scalp from at least six different points, using clean stainless steel scissors. Color-treated and permed hair was excluded from the study. The procedure for obtaining hair element content requires: washing (acetone, non-ionic detergent – Triton 100-X, and rinsed three times with redistilled water), drying at 105°C to constant weight, then weighing. After drying, the samples were mineralized with concentrated nitric acid (65%, supra pure, Merck) using an Ethos Easy microwave mineralizer Milestone Srl (Krejpcio et al. 1999, Wójciak et al. 2004). After proper dilutions, the content of copper (Cu), iron (Fe), zinc (Zn), magnesium (Mg), and calcium (Ca) in mineralized hair samples was marked using flame atomic absorption spectrometry on an iCE3500 spectrophotometer (ThermoFisherScientific). The accuracy of the method was checked using certified reference material (NCS DC 73347 human hair). Lithium content was measured by using graphite furnace atomic absorption spectrophotometry (condensed tenfold). Standards recovery was 99.6-102.9% (Wójciak 2019). The hair samples were prepared according to the International Atomic Energy Authority (IAEA) method designed to assess levels of toxic and essential metals in a human organism (International Atomic Energy Agency 1984). In order to check the test results against population-wide norms, it was decided to use specific reference values. We adopted reference values for the content of selected elements in hair from previous studies and publications: (Ca – 600-1000, Mg – 40-60, Zn – 160-200, Cu – 10-20, Fe – 10-20, μg $g^{-1}$ d.m.) (Radomska et al. 1993, Krejpcio et al. 1997, 1999, Wójciak et al. 2004, Dunicz-Sokolowska et al. 2007, Chojnacka et al. 2010).

### Statistical methods

Data were presented and analyzed according to the type of data obtained for each parameter. Statistical analyses were conducted with nonparametric Mann-Whitney *U* test or parametric Student's *t*-test for quantitative variables and with the Chi-squared test ($\chi^2$) for qualitative variables. The dependences between the variables were checked with the Spearman's rank correlation coefficient. Normality of the distribution of variables was examined using the Shapiro-Wilk test. Having verified normality of the data distribution, an appropriate statistical test, i.e. parametric or non-parametric, was employed. All results were evaluated at a confidence level of 95% and statistical significance $p<0.05$. Statistical analyses were implemented in IBM SPSS Statistics version 26. The results are presented in Tables 1–6.

## RESULTS

Table 1 shows the gender characteristics of the study subjects. The group taking lithium was dominated by women (60%), while the group not taking lithium was dominated by men (58.8%). The significance of differences was tested by $\chi^2$ test. Statistical analysis does not indicate differences between the number of women and men in the analyzed groups.

Table 1

Gender distribution of respondents

| Specification | Women | Men | $\chi^2$ | $p$ |
|---|---|---|---|---|
| Lithium treatment (%) | 60 (n=24) | 40 (n=16) | 1.600 | 0.206 |
| Non-lithium treatment (%) | 41.2 (n=7) | 58.8 (n=10) | 0.529 | 0.467 |
| General (%) | 54.4 (n=31) | 45.6 (n=26) | 0.439 | 0.508 |

Table 2. shows the content of the selected elements in the hair of patients with bipolar disorder. We present basic statistical characteristics in each row, including the arithmetic mean, standard deviation, minimum, maximum, and median for lithium, copper, iron, zinc, magnesium and calcium levels. Additionally, respondents are divided into two groups: people treated with lithium (Li) and with other mood stabilizers (Non-Li).

Table 2

The content of selected metals in the hair of adolescent patients with bipolar mood disorder (μg $g^{-1}$ d.m.)

| Parameter | **Li** | | **Cu** | | **Fe** | | **Zn** | | **Mg** | | **Ca** | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Li (n=40) | non Li (n=17) | Li (n=40) | non Li (n=17) | Li (n=40) | non Li (n=17) | Li (n=40) | non Li (n=17) | Li (n=40) | non Li (n=17) | Li (n=40) | non Li (n=17) |
| Arithmetic mean | 21.63 | 10.65 | 11.425 | 12.753 | 16.173 | 19.609 | 217.62 | 178.34 | 51.335 | 43.525 | 1098.35 | 1289.62 |
| Standard deviation | 9.20 | 5.36 | 6.432 | 5.866 | 5.964 | 5.083 | 63.37 | 71.93 | 17.758 | 18.989 | 374.56 | 569.76 |
| Minimum | 6.376 | 2.421 | 2.478 | 6.486 | 4.636 | 11.528 | 109.82 | 46.23 | 14.198 | 21.693 | 350.68 | 312.39 |
| Maximum | 51.442 | 21.538 | 29.578 | 31.822 | 29.947 | 31.191 | 376.98 | 309.80 | 95.452 | 88.922 | 1778.46 | 2438.99 |
| Median | 20.022 | 9.503 | 11.445 | 11.625 | 15.005 | 19.950 | 198.87 | 174.08 | 48.458 | 38.214 | 1124.67 | 1151.67 |

The results indicate that the distribution of the tested variables differs from the normal distribution. For Cu, the mean is about 12 μg g$^{-1}$, Zn 200 μg g$^{-1}$, and Ca 1100 μg g$^{-1}$. The next table presents differences among the means for Mg, Li, and Zn. The most similar median values were observed for Cu (lithium group mean – 11.445 μg g$^{-1}$ vs. non-lithium group mean – 11.625 μg g$^{-1}$) and Ca (lithium group mean – 1124.67 μg g$^{-1}$ vs. non-lithium group mean – 1151.67 μg g$^{-1}$). The minimum and maximum values show quite large ranges, which means that microelements can vary individually.

Comparisons of the groups with and without lithium treatment in terms of the content of specific elements in hair are presented in Table 3.

Table 3

Lithium psychopharmacotherapy and the content of selected elements in hair samples of patients with BD

| Elements | | Treatment | | | | |
|---|---|---|---|---|---|---|
| | | lithium treatment (n=40) | non – lithium treatment (n=17) | Shapiro-Wilk test | test$^{\#!}$ | *p* |
| Lithium (ng g$^{-1}$ d.m.) | arithmetic mean±SEM | 21.63±1.46 | 10.65±1.30 | 0.001 | -4.326$^{\#}$ | **0.000**** |
| | median | 20.02 | 9.50 | | | |
| Copper (μg g$^{-1}$ d.m.) | aritmethic mean±SEM | 11.425±1.02 | 12.753±1.42 | 0.000 | -.820$^{\#}$ | 0.412 |
| | median | 11.46 | 11.63 | | | |
| Iron (μg g$^{-1}$ d.m.) | arithmetic mean±SEM | 16.173±0.94 | 19.609±1.23 | 0.676 | -2.074$^{!}$ | 0.043* |
| | median | 15.01 | 19.95 | | | |
| Zinc (μg g$^{-1}$ d.m.) | arithmetic mean±SEM | 217.62±10.02 | 178.34±14.45 | 0.440 | 2.056$^{!}$ | 0.045* |
| | median | 198.87 | 174.08 | | | |
| Magnesium (μg g$^{-1}$ d.m.) | arithmetic mean±SEM | 51.335±2.81 | 43.525±4.61 | 0.012 | -2.233$^{!}$ | 0.026* |
| | median | 48.458 | 38.214 | | | |
| Calcium (μg g$^{-1}$ d.m.) | Arithmetic mean±SEM | 1098.35±59.22 | 1289.62±138.19 | 0.185 | -1.500$^{\#}$ | 0.129 |
| | median | 1124.67 | 1151.67 | | | |

* $p<0.05$, ** $p<0.01$

$^{\#}$ Mann-Whitney *U* test, $^{!}$ Student's *t*-test

Results of the Mann-Whitney *U* test and Student's *t*-test show that statistically significant differences are found in the levels of lithium ($p<0.01$), iron ($p<0.05$), magnesium ($p<0.05$) and zinc ($p<0.05$). Patients taking lithium are characterized by higher lithium, zinc and magnesium amounts in a hair sample. At the same time, a lower iron content was noted in this group. The mean values for copper, zinc and calcium also differ, but the differences are not statistically significant. Both Ca and Cu were higher in the non-treatment group. When analyzing the standard error of the mean, it can be observed that the group not taking lithium is less homogenous. The values obtained by the respondents in this group are more diverse than in the lithium treatment group. The analysis also examined whether men and women in the two groups (treated and untreated with lithium) differed in the elemental content of their hair. A statistical difference was shown only for Ca in the lithium-treated group ($t$=3.790, $p$<0.001). Women obtained significantly higher hair Ca levels.

Table 4 shows the distribution of the hair content of elements in two groups. Significant differences were found in the magnesium distribution ($p$<0.01). Most of the values of magnesium in hair (52.5%) were contained in the RVs in the lithium treatment group, 23.5% were below and 25% were above RVs. For the non-lithium treatment, the highest percentage of observations (about 65%) were below RVs, then 23.5% were above, and the least (about 12%) were within RVs. We did not notice significant differences regarding the other elements; however, some observation are worth noting. About 65% of non-lithium and 50% of lithium treatment patients were within reference values for copper distribution. For both groups, more observations were below the reference values than above. For iron, there were no results below RV among the non-lithium treatment group. For the lithium treatment group, there were 17.5% observations below RV. In the non-lithium treatment group, there were almost twice as many observations above RV than in the lithium treatment group (47% to 25%). Zinc was relatively

Table 4

Precentage of patients under lithium treatment (Li) and without lithium treatment (non-Li) according to the level of metals in the hair and its statistical evaluation

| Specification | Cu Li (n=40) | Cu non-Li (n=17) | Fe Li (n=40) | Fe non-Li (n=17) | Zn Li (n=40) | Zn non-Li (n=17) | Mg Li (n=40) | Mg non-Li (n=17) | Ca Li (n=40) | Ca non-Li (n=17) |
|---|---|---|---|---|---|---|---|---|---|---|
| <RVs (%) | 16<br>40.0 | 5<br>29.4 | 7<br>17.5 | 0<br>0.0 | 6<br>15.0 | 4<br>23.5 | 9<br>22.5 | 11<br>64.7 | 4<br>10.0 | 1<br>5.9 |
| RVs (%) | 20<br>50.0 | 11<br>64.7 | 23<br>57.5 | 9<br>52.9 | 15<br>37.5 | 7<br>41.25 | 21<br>52.5 | 2<br>11.8 | 13<br>32.5 | 5<br>29.4 |
| >RVs (%) | 4<br>10.0 | 1<br>5.9 | 10<br>25.0 | 8<br>47.1 | 19<br>47.5 | 6<br>35.3 | 10<br>25.0 | 4<br>23.5 | 23<br>57.5 | 11<br>64.7 |
| $x^2$ | 1.068 | | 4.857 | | 0.942 | | 10.973 | | 0.370 | |
| $p$ | 0.586 | | 0.088 | | 0.624 | | 0.004** | | 0.831 | |

RVs – reference values; * $p$<0.05, ** $p$<0.01

evenly distributed among the respondents in the non-lithium treatment group. As for the patients treated with lithium, almost 50% of observations were above the RVs. Most of the measurements for zinc (about 60%) were above RVs, while 30% of the observations were within the RVs. For calcium, most observations were above the reference values (57.5% and 64.5%, respectively).

Table 5 and Table 6 present results of the analysis of the Spearman correlations.

Table 5

Correlations between elements for the group under lithium treatment

| Elements | Li | Ca | Mg | Zn | Cu |
|---|---|---|---|---|---|
| Ca | $r=-0.131$<br>$p=0.422$ | | | | |
| Mg | $r=0.122$<br>$p=0.453$ | $r=-0.125$<br>$p=0.442$ | | | |
| Zn | $r=0.167$<br>$p=0.304$ | $r=-0.105$<br>$p=0.520$ | $r=0.272$<br>$p=0.089$ | | |
| Cu | $r= 0.032$<br>$p= 0.842$ | $r=-0.008$<br>$p=0.962$ | $r=0.101$<br>$p=0.536$ | $r=-0.272$<br>$p=0.089$ | |
| Fe | $r=0.198$<br>$p=0.220$ | $r=-0.140$<br>$p=0.390$ | $r=-0.062$<br>$p=0.703$ | $r=0.056$<br>$p=0.733$ | $r=0.242$<br>$p=0.132$ |

Table 6

Correlations between elements for the group without lithium treatment

| Elements | Li | Ca | Mg | Zn | Cu |
|---|---|---|---|---|---|
| Ca | $r=0.400$<br>$p=0.112$ | | | | |
| Mg | **$r=0.517$*<br>$p=0.034$** | **$r=0.564$*<br>$p=0.018$** | | | |
| Zn | $r=-0.422$<br>$p=0.092$ | $r=-0.120$<br>$p=0.646$ | $r=-0.206$<br>$p=0.428$ | | |
| Cu | $r=0.159$<br>$p=0.541$ | $r=0.140$<br>$p=0.593$ | $r=0.145$<br>$p=0.580$ | $r=0.316$<br>$p=0.216$ | |
| Fe | **$r=0.532$*<br>$p=0.028$** | $r=0.355$<br>$p=0.162$ | **$r=0.510$*<br>$p=0.037$** | $r=-0.316$<br>$p=0.216$ | $r=0.375$<br>$p=0.138$ |

* $p<0.05$

There were no statistically significant correlations for the lithium treatment group (Table 5). The analysis identified several dependencies in the non-lithium treatment group (Table 6). Moderate dependence occurred between magnesium and lithium ($r=0.517$, $p=0.034$), magnesium and calcium ($r=0.564$, $p=0.018$), magnesium and iron ($r=0.510$, $p=0.037$), and iron and lithium ($r$=0.532, $p$=0.028). All significant correlations are positive, hence when one of the elements increases, the other increases as well. Most correlations were found for magnesium. When analyzing all the data without division into the two groups, a statistically significant correlation was shown only for lithium and magnesium ($r$=0.350, $p$=0.008). The higher the lithium concentration, the higher the magnesium concentration.

## DISCUSSION

The main aim of the study was to estimate the level of essential trace elements in bipolar patients treated with different mood stabilizers (with or without lithium carbonate). We assumed that a highly reactive element like lithium could have an impact on particular elements because its biological mechanisms are multifactorial and complex (SCHRAUZER 2002). The consequence of these interactions can be pathological states in processes regulated by these elements (KREJPCIO et al. 1999). There are not many studies in the literature considering lithium as an active factor influencing the homeostasis of other elements. This balance is necessary for a healthy nervous system, crucial for patients with imbalanced neuronal processes, as in bipolar disorder.

The elements selected for our analysis significantly affect the functioning of a human body. Because of their role in enzymatic mechanisms that protect against free radicals, toxic derivatives of oxygen (BOURRE 2006), any imbalance in components such as copper, zinc and manganese may contribute to poor childhood development. Besides, copper is involved in the etiology of mental disorders, for example by participating in the conversion of dopamine to norepinephrine (SIWEK et al. 2017). Its deficiency can influence neurodegenerative diseases, e.g. Alzheimer's disease (BOURRE 2006). Zinc, whose highest concentration is found in the hippocampus, is essential for synthesizing GABA, the main neurotransmitter with inhibitory effects (TAKEDA, TAMANO 2009). Studies indicate that its deficiency is associated with anxiety and depressive states (BOURRE 2006, TAKEDA, TAMANO 2009, GRØNLI et al. 2013, SIWEK et al. 2016). The proper functioning of cognitive processes is associated with normal iron levels (KHALIQUE et al. 2005). Its deficits are observed in depression episodes (iron deficiency anemia) or in children with ADHD (BOURRE 2006, WÓJCIAK et al. 2014). It takes part in the synthesis of neurotransmitters and production of energy in the cerebral parenchyma (BOURRE 2006). As one of the essential intracellular cations, magnesium plays a role in oxidation-reduction and ionic regulation processes. Deficits in this element can also cause lower mood episodes or exhaustion (BOURRE 2006). Calcium is the primary building material of bones and teeth, but it is also an essential activator of many enzymes involved in blood clotting. Moreover, it regulates the nervous system, muscular activity and the secretion of hormones.

Researchers have noted that patients treated with lithium have lower Se concentrations than those treated with other mood stabilizers (Santa Cruz et al. 2020). An opposite effect was observed for Br and Al. Lithium treatment resulted in significantly higher levels of Al and Br in the subjects (Harvey et al. 1992). Moreover, lithium could interact with sodium, potassium, magnesium, calcium, aluminum, manganese or vanadium (Schrauzer 2002). Our study supports the assumption of an active effect of lithium on other micronutrients. The group of patients treated with lithium salts showed significantly higher lithium levels in the hair samples. The detected trend is confirmed by patients' blood tests (Sussulini 2011). We also determined a significant difference in the magnesium content, which was significantly higher in those treated with lithium (lithium group mean – 51.335, non-lithium group mean – 44.525). Other authors have also reported some correlations between lithium and magnesium (Birch 1999, Santa Cruz et al. 2020). Lithium treatment has been shown to affect magnesium metabolism, e.g. it is suggested that urinary magnesium excretion increases when a patient is on lithium therapy (Birch 1999). Lithium is not metabolized but is almost completely excreted by the kidneys. Researchers note that lithium can compete at the biological level with sodium and magnesium, which as ions are most similar to lithium in charge and size (Jakobsson et al. 2017, Erdemir, Gucer 2018). We observed a significant difference in the iron level. The group not treated with lithium had higher Fe levels than the group with lithium treatment. Cruz et al. proved a higher level in BD than in healthy controls. They hypothesized that it could be *associated with oxidative stress* (Santa Cruz et al. 2020). The association of lithium and iron is also noted by Lei et al., who worked on Parkinson's disease. Low-dose lithium treatment induces reversible hippocampal T2 relaxation time changes in substantia nigra consistent with increased iron levels (Lei et al. 2017). We showed no significant statistical differences for Ca, Cu, Zn. Studies on changes in Zn concentrations as a consequence of lithium therapy are inconclusive. The results of some studies support our observation (Santa Cruz et al. 2020); on the other hand, some data indicate that Zn was at a higher level in BD patients treated with Li than in BD patients not treated with this drug (Sussulini et al. 2011). Kuo and Hess (1993) noticed that lithium could inhibit calcium because of its ability to enter calcium channels. Our study does not indicate a significant difference but shows that the mean Ca level in the two groups is not the same. The lithium group had lower micronutrient levels in hair samples (lithium group mean – 1098.35, non-lithium group mean – 1289.62). Women taking lithium had higher calcium levels than men taking the drug. This is the only element whose levels differed by gender. It is possible that testing more people could present a statistically significant effect. We can conclude that lithium will compete with some of the elements, including calcium.

Researchers have mostly focused on determining micronutrient levels for BD compared to healthy controls. Our study compares the values obtained in the two groups of patients during the remission period with the reference values. We found a marked difference in the distribution of the hair magnesium content between the groups of patients. 52.5% of observations in the lithium treatment group were within RVs whereas for the non-lithium group, this percentage was as low as 12%. About 60% of non-lithium group observations were below standard. The results indicate that those taking lithium carbonate have a distribution of magnesium values more similar to norms than those not taking the drug. Other authors show that the serum magnesium concentration of patients with BD during periods of depression, mania and hypomania is significantly higher than in healthy subjects. During the remission period, this parameter normalized and did not differ from the control (SIWEK et al. 2015). For copper, most observations in both groups were at or below average. Studies indicate that copper in patients with BD is generally within the normal range (regardless of the disease phase). The copper concentration showed a relationship with the number of affective episodes as it decreased with the growing number of outbreaks of the disease (SIWEK et al. 2017). The smallest number of observations for Fe was within the post-baseline values. Our data are not consistent with the statement that decreases in Fe levels are characteristic for BD patients (MUSTAK et al. 2008). For zinc, most of the measurements were above RVs or within RVs. This finding agrees with the results of other scientists (MUSTAK et al. 2008). Additionally, it is known that in the depressive phase, lower blood Zn levels are reported to return to normal during mania/hypomania (SIWEK et al. 2016). Some researchers have suggested that lithium influences calcium ion levels by affecting the rate of transmission of nerve signals (SZYMCZYK et al. 2017). In our case, no statistical intergroup differences were observed. Most measurements (with no statistical difference) were below the reference level.

The study also checked the relationship (correlation) between elements. Researchers distinguishing between antagonistic and synergistic interactions note different trends. Some authors have found a synergistic interaction between Ca-Mg, Ca-Zn, Ca-Cu, Mg-Zn, Mg-Cu, Zn-Cu, and Zn-Fe (DUNICZ-SOKOLOWSKA et al. 2007). Our analysis of the research data indicates statistically significant, moderate relationships for magnesium and lithium. The correlation is present for subjects not treated with lithium ($r$=0.517, $p$=0.034) and for all subjects, without grouping ($r$=0.350, $p$=0.008). The higher the lithium concentration, the higher the magnesium concentration. Other researchers also confirm this relationship (RADOMSKA et al. 1993, DUNICZ-SOKOLOWSKA et al. 2007). Furthermore, our study indicated magnesium-calcium and magnesium-iron relationships. Other researchers have detected a correlation between Fe and Zn (DUNICZ-SOKOLOWSKA et al. 2007). However, our study did not confirm this. The group taking lithium did not show any statistically significant association. Lithium may help regulate magnesium levels. The hypothesis requires more in-depth research.

Future studies may be conducted on larger number of samples. This will allow additional statistical analyses. Future studies could involve analyses of more elements. Furthermore, we could extend the research model to include healthy individuals matched on selected characteristics. In the present study, we do not consider changes in concentrations of elements in each patient. It would be interesting to perform the research at the moment of making a diagnosis, as well as before and after treatment, and at every bipolar disorder phase. Additionally, we plan to include an analysis of diets of the subjects in subsequent studies. Hair is only to a certain extent a valuable biomarker of imbalances in the range of metals studied. Future research could expand measurement methods to include blood serum, urine, or saliva.

In conclusion, the present study demonstrates that lithium carbonate can influence selected elements in bipolar patients. The research confirms that patients with this disorder struggle not only with mood instability but also with the dysregulation of essential minerals in their bodies. The study presented in this paper makes a step towards understanding the impact of lithium carbonate psychopharmacotherapy on patients' health.

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