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**ORIGINAL PAPER**

# THYROID DYSFUNCTIONS DUE TO LITHIUM TREATMENT IN BIPOLAR DISORDER: CHANGES IN OXIDATIVE STRESS, TRACE ELEMENTS, AND HEMORHEOLOGICAL PARAMETERS

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**Abstract**

Lithium is one of the most widely used medications for the treatment of bipolar disorder (BD). It also has some side effects on thyroid functions. We aimed to investigate the role of oxidative stress, trace elements, and hemorheological parameters on the pathophysiology of thyroid dysfunctions developed by lithium treatment in patients with BD. Patients with BD were divided into three groups: patients that non-lithium-treated, lithium-treated patients for 4-6 weeks, and lithium-treated patients for 40-68 weeks. Blood samples for analysis were taken before and after the treatment period. After analysis, patients were divided into six groups: non-treatment BD group (Group 1); short-term lithium-treatment group that did not develop thyroid dysfunctions (Group 2); short-term lithium-treatment group that developed hyperthyroidism (Group 3); long-term lithium treatment group that developed hypothyroidism (Group 4), long-term lithium-treatment group that developed hyperthyroidism (Group 5), and long-term lithium-treatment group

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that did not develop thyroid dysfunctions (Group 6). Plasma and whole blood viscosity levels were significantly increased in Groups 4 and 6 compared to Groups 1, 2, and 3. Hemoglobin levels were lower in Group 4 than in Groups 1, 2, and 5. Fibrinogen values were higher in Groups 4 and 5 than Group 1. Plasma and erythrocyte malondialdehyde levels were higher in Group 4 than In Groups 1, 2, 3, and 5. Also, they were increased in Group 6 in comparison with Groups 2 and 3. Erythrocyte glutathione levels were lower in Groups 4 and 6 than Groups 1, 2, 3 and 5. Plasma protein carbonyls levels were higher in Group 4 than in Group 1, or in Group 5 than in Groups 1, 2, and 3, as well as in Group 6 than Groups 1, and 2. Serum zinc levels were higher in Groups 2, 3 and 6 than in Group1. Serum copper levels increased in Groups 2, 4 and 6 in comparison with Group1. The results of this study indicate that oxidative stress increased with treatment time in lithium-induced thyroid dysfunctions. Also, whole blood viscosity, plasma viscosity, fibrinogen, zinc, and copper levels were affected by lithium treatment and treatment duration induced thyroid dysfunctions.

**Keywords:** bipolar disorder, lithium treatment, thyroid dysfunction, oxidative stress, trace elements, hemorheology.

# INTRODUCTION

Bipolar disorder (BD), also known as manic-depressive illness, is a psychiatric disease characterized by recurrent depression and episodes of mania or hypomania. Despite the clinical significance of BD, the exact pathophysiology has not been fully elucidated. Genetic, biochemical, psychodynamic, social, and environmental factors have been reported to play a role in disease development (Valvassori et al. 2018). Lithium is considered as an important drug in the treatment of BD (Ahmad et al. 2011). However, lithium has been reported to cause dysfunction in various tissues depending on a dose and duration (Rocha et al. 2015). Lithium may adversely affect organs, such as thyroid, brain, intestine, liver, and lungs (Malhotra, Dhawan 2008, Ahmad et al. 2011). Thyroid dysfunction due to lithium treatment has been shown to vary from mild impairment in thyroid-stimulating hormone (TSH) response to severe myxedema in clinical and experimental studies (Eker, Eker 2010, Toplan et al. 2013, Tsui 2015, Sethy et al. 2016).

Oxidative stress is defined as a disorder that leads to damage by disruption of prooxidant/antioxidant balance (Sies 1991). Increased oxidative stress induces lipid peroxidation, particularly in membranes, proteins, and DNA, which has detrimental consequences on signal transduction, synaptic plasticity, and cellular flexibility (De Sousa et al. 2014). Free radicals and other reactive species are produced continuously by all tissues, during some cellular process such as oxidative phosphorylation in the mitochondrial matrix. Under normal conditions, reactive oxygen species (ROS) are eliminated by cellular enzymatic antioxidants such as catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR) and glutathione-S-transferase (GST), and non-enzymatic antioxidants, such as vitamin C, vitamin E, zinc Zn, taurine, hypotaurine, and glutathione (GSH) (Andreazza et al. 2015).

Metal ions are necessary for the function of enzymes as well as many other functions. Copper (Cu), iron (Fe), selenium (Se) and Zn are important for prooxidant/antioxidant balance since they are significant cofactors in the regulation of antioxidant enzyme activity. Fe is the cofactor of the CAT enzyme that catalyzes the decomposition of hydrogen peroxide ($H_2O_2$). It also plays a role in reactive hydroxyl radical production. Fe, and Cu may serve as prooxidants that induce oxidative damage of cell compounds (Negi et al. 2012, Nenkova et al. 2013).

Increased risk of cardiovascular disease in patients with BD is related to behavioral patterns and genetics (Gurok et al. 2019). Thyroid hormones also have some effects on the cardiovascular system since myocardial and vascular endothelial tissues have thyroid hormone receptors (Jabbar et al. 2017). Hemodynamic and hemorheologic factors play an important role in the coronary circulation, since there is a continuous change in blood flow, perfusion pressure and shear rates during each heart cycle. Blood viscosity has been reported to be associated with increased cardiovascular risk (Lee et al. 2019). Also, it is shown that stress causes changes in hemorheological markers, such as hematocrit, hemoglobin, total plasma protein, whole blood, and plasma viscosity (Kalelioglu et al. 2018).

The pathophysiology of BD is not fully understood, but oxidative stress has been reported to be an important factor in disease development (Tsai, Huang 2015). Oxidative stress-induced DNA damage was found to be associated with the severity of depressive symptoms in BD (De Sousa et al. 2014). In the literature, there are studies investigating the effect of lithium treatment on prooxidant/antioxidant (Khairova et al. 2012, De Sousa et al. 2014, Rocha et al. 2015, Mezni et al. 2017). In this study, our aim was to investigate the effect of thyroid dysfunctions triggered by the Li treatment on the oxidative stress intensity, trace element levels, hemorheological parameters in patients with BD.

## MATERIALS AND METHODS

### Patients

The study group consisted of 83 patients (62 females, 21 males; in the age range 35-46 years) who were hospitalized at the Department of Psychiatry, Faculty of Medicine of Namik Kemal University, and diagnosed with BD according to the *Diagnostic and Statistical Manual of Mental Disorders V* (2013). Patients were divided into three groups: 27 patients did not receive lithium treatment, 26 patients received lithium for 4-6 weeks as short-term treatment; 30 patients received lithium treatment for 40-68 weeks as long-term treatment. All patients on lithium treatment were taking multiple dosages of Li carbonate (2 or 3 times a day). None of the patients had a past history of renal disease, cardiovascular disorder, diabetes mellitus, endocrine

diseases or any other systemic diseases. Further exclusion criteria for the groups were abnormal liver functioning and hormone tests, cigarette smoking, alcohol consumption or the use of various antioxidants, vitamin supplements or medication within 3 months before recruitment. At the time of the study, all lithium-treated patients were on lithium alone and lithium-naive patients were drug free at least for 3 weeks. Before enrolment, each subject underwent a physical examination and a laboratory evaluation including the biochemistry analysis and complete blood count and thyroid hormone levels. Patients that had stopped lithium treatment due to being diagnosed with hypothyroidism during their routine thyroid examinations and patients that had started thyroid replacement treatment were excluded. All the test subjects had normal thyroid examinations with palpation and did not have any clinical symptoms of thyroid dysfunctions. The Institutional Ethical Committee approval was granted in accordance with the Declaration of Helsinki (Number: 26379996/01, Date: 29/01/2014). Moreover, informed consent was obtained from each study subject.

**Laboratory measurements**

A single fasting morning blood specimen was obtained at 08.00 a.m. from the subjects and 10-12 h after the last Li dose from the patients. Thyroid hormone and Li levels were determined in serum samples. Routine blood count was determined in ethylenediaminetetraacetate (EDTA) anticoagulated blood samples by an electronic counter (Beckman Coulter Inc., CA, USA). The blood samples were also collected in tubes containing EDTA to determine the plasma and blood viscosity, plasma and erythrocyte malondialdehyde (MDA) and erythrocyte glutathione (eGSH) levels. Fibrinogen was collected in vacutainer tubes containing sodium oxalate. Plasma samples were obtained by centrifugation at 1500 x g for 20 min and stored at –80°C. The erythrocytes were prepared by whole blood centrifugation for 10 min at 1500xg and obtained after washing in a 0.9% NaCl solution twice, after which they were removed for measurement. All reagents, unless otherwise noted, were obtained from Sigma Aldrich (St. Louis, MO, USA) and Merck (Darmstadt, Germany).

**Serum thyroid function tests measurement**

The concentrations of total thyroxin (TT4), total triiodo-L-thyronine (TT3), and thyroid stimulating hormone (TSH) in serum were measured using the Abbott I2000 SR immunology assay analyzer (Abbott Laboratories, Abbott Park, IL, USA). For testing thyroid function, TSH is recommended as the first-line screening test. It is a well-known fact that relatively modest changes in TH levels are associated with marked changes in TSH levels. Although, screening exclusively with TSH leads to misdiagnosis of some patients, it enabled us to include subclinical disorders in our research. TSH levels between 0.4 and 4.0 mU $L^{-1}$, TT4 levels between 4.6-12 μg $dl^{-1}$

and TT3 levels between 0.80-1.80 ng $ml^{-1}$ were accepted as normal range according to the manufacturer's product insert.

**MDA measurement**

The formation of thiobarbituric acid reactive substances (TBARS) was used as an indicator of lipoperoxidation. The peroxidation of lipids was estimated by the measurement of compounds reacting with 2-thiobarbituric acid (TBA) (Stocks, Dormandy 1971, Buege, Aust 1978). The absorbance was read at 532 nm. The concentration of TBARS was calculated using a millimolar absorption coefficient for MDA, $\varepsilon = 1.56 \cdot 10^{-5}$ M $cm^{-1}$. The hemoglobin concentration was determined by the Drabkin's method (Drabkin 1946). Absorbance was read at 540 nm. Plasma and erythrocyte MDA levels were expressed respectively as nmol $mL^{-1}$ and pmol $mg^{-1}$ Hb.

**GSH measurement**

The concentrations of GSH in erythrocyte were estimated by the method of Beutler et al. (1963) spectrophotometrically using metaphosphoric acid for protein precipitation and 5, 5'dithiobis 2-nitrobenzoic acid for colour development at 412 nm. GSH levels were expressed as mg $g^{-1}$ Hb.

**NO measurement**

The levels of plasma samples NO were determined by colorimetric assay using a Cayman's Assay Kit (Cayman, Item No. 780001, Ann Arbor, MI, USA). In our study, all determinations were done according to the manufacturer's instructions.

**Protein carbonyl measurement**

Because carbonyl groups (aldehydes and ketones) might be introduced into proteins by ROS and free radicals, quantitation of protein carbonyls was carried out by incubating equal volumes of the plasma samples and 2,4-dinitrophenylhydrazine (3.4 mg per 10 mL 1 mol $L^{-1}$ HCl) at 50ºC for 1 h. After the reaction, the proteins were precipitated with 20% trichloroacetic acid and then centrifuged. The pellet was dissolved in 1 mol $L^{-1}$ NaOH and the absorbance at 450 nm was recorded. The molar absorbance coefficient ($\varepsilon = 25.500$ mol $L^{-1}$ $cm^{-1}$) was used to calculate the carbonyl content (Levine et al. 1990). Protein concentrations were determined by the Lowry method, with bovine serum albumin (BSA) as the standard (Lowry et al. 1951).

**Serum copper, zinc, iron, and lithium concentration measurement**

The Cu, Zn, Fe, and lithium levels of serum samples were measured by a flame atomic absorption spectrophotometer (Shimadzu AAS-6800, Tokyo, Japan). Cu, Zn, Fe and lithium stock standards (of concentration 1000 ppm) were obtained from Merck (Darmstadt, Germany). Results were calculated as µg $dl^{-1}$ in serum samples (for Cu, Zn and Fe). Also, Li results were calculated as mEq $L^{-1}$ in serum.

### Hemorheological parameters measurement

Whole blood and plasma viscosity were determined according to the recommendations of the International Committee for Standardization in Haematology (Harkness 1971) using a Harkness Capillary Viscometer (Coulter Electronics, Ser. No. 6083, Luton, England), and evaluated in relation to distilled water (relative viscosity), where the water bath of which was maintained at 37°C. Plasma fibrinogen levels were measured using a Systex CA-1500 autoanalyzer (Diamond Diagnostics, USA) with a Dade Behring kit. Hemoglobin, hematocrit and erythrocyte values were determined using a CellDYN C1600 (Abbott Pharmacuetical Co., Ltd., Lake Bluff, IL, USA) blood cell counter device.

### Statistical analysis

All results are expressed as mean ± standard deviation. The statistical significance of differences was determined by SPSS version 17.0 for Windows (SPSS, Chicago, IL, USA). The significance level of $P<0.05$ was accepted.

## RESULTS

After the analysis of samples, patients were divided into six groups according to the duration of treatment and/or type of thyroid dysfunction development: Group 1: non-treated BD patients (*n*:27); Group 2: short-term lithium treated patients without thyroid dysfunction (*n*:18); Group 3: short-term lithium treated patients with hyperthyroidism (*n*:8); Group 4: long-term lithium treated patients with hypothyroidism (*n*:9), Group 5: long-term lithium treated patients with hyperthyroidism (*n*:5), and Group 6: long-term lithium treated patients without thyroid dysfunction (*n*:16). The serum lithium levels of all patients were within the therapeutic range of 0.6-1.0 mEq $L^{-1}$.

### Serum thyroid function tests of patient groups

According to thyroid hormone profiles, patients were affected with hyper- or hypothyroidism. If there were not any changes in the thyroid hormone after standard lithium treatment, the case was accepted as "no thyroid dysfunction". Eighteen of 26 (69.2%) patients who received short-term lithium therapy had no thyroid dysfunction (Group 2). Hyperthyroidism was observed in 8 (Group 3) out of 26 patients (30.8%) who received short-term lithium therapy. In 30 patients who received long-term lithium treatment, 9 patients (30%) developed hypothyroidism (Group 4); 5 patients (16.7%) developed hyperthyroidism (Group 5); and 16 patients (53.3%) were free from thyroid dysfunction (Group 6). The TT3 and TT4 values of patients from Groups 4 and 6 were lower than in Groups 1, 2, 3, and 5. But TSH levels in Groups 4 and 6 were higher than in Groups 1, 2, 3 and 5. Also, the TSH values

in Group 4 were higher than in Group 6 (P<0.05). There were no significant differences among other groups (Table 1).

Table 1

The levels of some thyroid function parameters in all groups

| Groups | TT3 (ng ml$^{-1}$) | TT4 (μg dl$^{-1}$) | TSH (mIU ml$^{-1}$) |
|---|---|---|---|
| Group 1 (*n*=27) | 1.16 ± 0.18 | 9.00 ± 0.92 | 1.30 ± 0.31 |
| Group 2 (*n*=18) | 1.37 ± 0.32 | 10.13 ± 1.56 | 1.17 ± 0.19 |
| Group 3 (*n*=8) | 1.43 ± 0.32 | 9.20 ± 0.73 | 0.47 ± 0.10 |
| Group 4 (*n*=9) | **0.64 ± 0.13**$^{a,c,e,g}$ | **3.50 ± 0.60**$^{a,c,e,g}$ | **6.10 ± 1.64**$^{a,c,e,g}$ |
| Group 5 (*n*=5) | 1.52 ± 0.33 | 10.72 ± 1.52 | 0.38 ± 0.17 |
| Group 6 (*n*=16) | **0.86 ± 0.39**$^{b,d,f,i}$ | **4.66 ± 1.48**$^{b,d,f,i}$ | **2.83 ± 0.85**$^{b,d,f,h,i}$ |

Values are presented as the mean ± the standard deviation. Group 1 – non-treatment BD group, Group 2 – short-term lithium-treatment group without thyroid dysfunctions, Group 3 – short--term lithium-treatment group that developed hyperthyroidism, Group 4 – long-term lithium--treatment group that developed hypothyroidism, Group 5 – long-term lithium-treatment group that developed hyperthyroidism, and Group 6 – long-term lithium-treatment group that did not develop thyroid dysfunction, [a] Group 1 vs.Group 4, [b] Group 1 vs. Group 6, [c] Group 2 vs. Group 4, [d] Group 2 vs. Group 6, [e] Group 3 vs. Group 4, [f] Group 3 vs. Group 6, [g] Group 4 vs. Group 5, [h] Group 4 vs. Group 6, [i] Group 5 vs. Group 6, Bold type: $P$<0.05.

## Hemorheological parameter levels of patient groups

The blood hemorheological parameters are shown in Table 2. Plasma viscosity and whole blood viscosity levels were significantly increased

Table 2

The levels of some hemoreological parameters in all groups

| Groups | Fibrinogen (mg dl$^{-1}$) | Plasma viscosity (mPas) | Whole blood viscosity (mPas) | Hematocrit (%) | Hemoglobin (g dl$^{-1}$) | Erythrocyte (x 10$^6$ mm$^{-3}$) |
|---|---|---|---|---|---|---|
| Group 1 (*n*=27) | 194.18 ± 35.61 | 1.18 ± 0.11 | 3.03 ± 0.19 | 40.95 ± 3.05 | 13.18 ± 0.90 | 4.30 ± 0.22 |
| Group 2 (*n*=18) | 213.13 ± 35.31 | 1.11 ± 0.08 | 3.20 ± 0.30 | 39.93 ± 2.84 | 12.93 ± 0.80 | 4.60 ± 0.40 |
| Group 3 (*n*=8) | 212.86 ± 25.66 | 1.14 ± 0.13 | 3.22 ± 0.29 | 40.14 ± 3.76 | 12.57 ± 0.98 | 4.30 ± 0.42 |
| Group 4 (*n*=9) | **248.29 ± 13.44**$^{a}$ | **1.45 ± 0.08**$^{a,d,g,i}$ | **4.09 ± 0.18**$^{a,d,g,i}$ | 40.00 ± 2.58 | **11.29 ± 0.49**$^{a,d,i}$ | 4.68 ± 0.35 |
| Group 5 (*n*=5) | **260.50 ± 43.40**$^{b}$ | **1.32 ± 0.10**$^{e}$ | **3.42 ± 0.28**$^{k}$ | 41.25 ± 3.30 | 13.00 ± 0.82 | 4.40 ± 0.39 |
| Group 6 (*n*=16) | 215.46 ± 30.95 | **1.43 ± 0.15**$^{c,f,h}$ | **3.89 ± 0.30**$^{c,f,h}$ | 39.92 ± 2.53 | **12.85 ± 0.80**$^{j}$ | 4.49 ± 0.46 |

Values are presented as the mean ± the standard deviation. Group 1 – non-treatment BD group, Group 2 – short-term lithium-treatment group without thyroid dysfunctions, Group 3 – short-term lithium-treatment group that developed hyperthyroidism, Group 4 – long-term lithium-treatment group that developed hypothyroidism, Group 5 – long-term lithium-treatment group that developed hyperthyroidism, and Group 6 – long-term lithium-treatment group that did not develop thyroid dysfunction, [a] Group1 vs. Group 4, [b] Group 1 vs. Group 5, [c] Group 1 vs. Group 6, [d] Group 2 vs. Group4, [e] Group 2 vs. Group5, [f] Group 2 vs. Group 6, [g] Group 3 vs. Group 4, [h] Group 3 vs. Group 6, [i] Group 4 vs. Group 5, [j] Group 4 vs. Group 6, [k] Group 5 vs. Group 6, Bold type: $P$ < 0.05

in Group 4 compared to Groups 1, 2, 3, and 5. Also, plasma viscosity and whole blood viscosity levels were significantly increased in Group 6 compared to Groups 1, 2, and 3. Plasma viscosity was higher in Group 5 than in Group 2, also whole blood viscosity was lower in Group 5 than in Group 6. Hemoglobin levels were lower in Group 4 than in Groups 1, 2, and 5. Fibrinogen values were higher in Groups 4 and 5 than in Group 1 ($P < 0.05$ for all). There were no significant differences in these markers between the other groups. Also, there were no significant changes in hematocrit and erythrocyte counts among all the groups ($P > 0.05$).

**Oxidative stress parameters of patient groups**

Blood prooxidant and antioxidant parameters are shown in Table 3. Plasma MDA (pMDA) and erythrocyte MDA (eMDA) levels were higher

Table 3

The levels of prooxidant and antioxidant parameters in all groups

| Groups | Plasma NO ($\mu M\ L^{-1}$) | Plasma MDA ($nmol\ mL^{-1}$) | Erythrocyte MDA ($pmol\ mg^{-1}$ Hb) | Erythrocyte GSH ($mmol\ g^{-1}$ Hb) | Plasma Protein Carbonyls ($nmol\ mg^{-1}$ prot) |
|---|---|---|---|---|---|
| Group 1 ($n$=27) | 78.18 ± 9.65 | 4.90 ± 0.89 | 2.64 ± 0.22 | 18.27 ± 1.95 | 0.80 ± 0.08 |
| Group 2 ($n$=18) | 80.00 ± 9.27 | 4.56 ± 0.57 | 2.38 ± 0.27 | 13.77 ± 1.73 | **0.93 ± 0.11**$^{h}$ |
| Group 3 ($n$=8) | 85.43 ± 8.18 | 4.23 ± 0.80 | 2.43 ± 0.49 | 14.30 ± 1.81 | **0.95 ± 0.14**$^{i}$ |
| Group 4 ($n$=9) | 84.43 ± 9.17 | **6.14 ± 0.84**$^{a,b,d,f}$ | **3.60 ± 0.28**$^{a,b,d,f}$ | **13.12 ± 2.05**$^{a,b,d,f}$ | **1.02 ± 0.14**$^{a}$ |
| Group 5 ($n$=5) | 74.00 ± 5.04 | 4.48 ± 1.06 | 2.49 ± 0.33 | **12.64 ± 1.04**$^{j,l,m}$ | **1.19 ± 0.07**$^{j,l,m}$ |
| Group 6 ($n$=16) | 83.69 ± 8.90 | **5.74 ± 0.97**$^{c,e}$ | **3.32 ± 0.54**$^{c,e,g,h}$ | **12.66 ± 1.97**$^{c,e,g,h}$ | **1.08 ± 0.14**$^{c,g}$ |

Values are presented as the mean ± the standard deviation. Group 1 – non-treatment BD group, Group 2 – short-term lithium-treatment group without thyroid dysfunctions, Group 3 – short--term lithium-treatment group that developed hyperthyroidism, Group 4 – long-term lithium--treatment group that developed hypothyroidism, Group 5 – long-term lithium-treatment group that developed hyperthyroidism, and Group 6 – long-term lithium-treatment group that did not develop thyroid dysfunction, [a] Group 1 vs. Group 4, [b] Group 2 vs. Group 4, [c] Group 2 vs. Group 6, [d] Group 3 vs. Group 4, [e] Group 3 vs. Group 6, [f] Group 4 vs. Group 5, [g] Group 1 vs. Group 6, [h] Group 5 vs. Group 6, [i] Group 1 vs. Group 3, [j] Group 1 vs. Group 5, [k] Group 1 vs. Group 2, [l] Group2 vs. Group 5, [m] Group 3 vs. Group 5, Hemoglobin: Hb, Bold type: $P < 0.05$

in Group 4 than In Groups 1, 2, 3, and 5. pMDA and eMDA levels were higher in Group 6 than in Groups 2 and 3. Also, eMDA levels were higher in Group 6 than in Groups 1 and 5. Erythrocyte GSH (eGSH) levels were lower in Groups 4 and 6 than in Groups 1, 2, 3 and 5. Also, eGSH levels were decreased in Group 5 in comparison with Groups 1, 2 and 3. Plasma Protein Carbonyl levels were increased in Groups 2 and 3 relative to Group 1,

in Group 4 relative to Groups 1, 2 and 3, in Group 5 relative to Groups 1, 2 and 3, and in Group 6 relative to Groups 1 and 2 ($P$<0.05 for all). There were no significant differences in these markers between other groups. Also, plasma NO levels were not significantly changed among all groups ($P$ > 0.05).

## Serum trace element levels of different patient groups

Serum Zn, Cu and Fe levels were shown in Figures 1, 2 and 3. Serum Zn levels were higher in Groups 2, 3, and 6 than in Group 1. Serum Cu levels

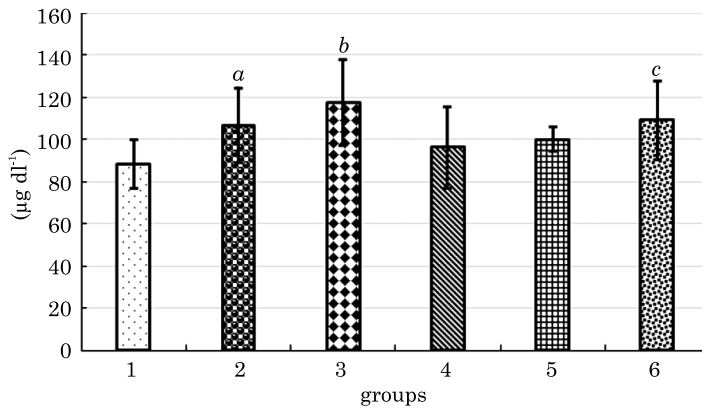


Fig. 1. The levels of zinc in all study groups. Values are presented as the mean ± the standard deviation. Group 1 – non-treatment BD group, Group 2 – short-term lithium-treatment group without thyroid dysfunctions, Group 3 – short-term lithium-treatment group that developed hyperthyroidism, Group 4 – long-term lithium-treatment group that developed hypothyroidism, Group 5 – long-term lithium-treatment group that developed hyperthyroidism, and Group 6 – long-term lithium-treatment group that did not develop thyroid dysfunction, $P$ < 0.05, [a] Group 1 vs. Group 2, [b] Group 1 vs. Group 3; [c] Group 1 vs. Group 6

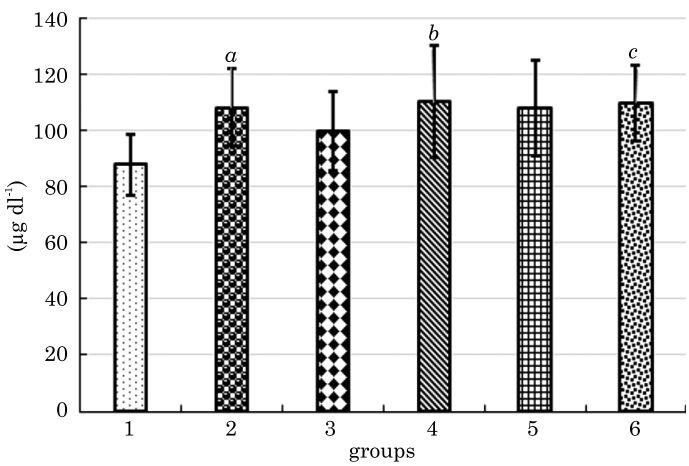


Fig. 2. The levels of copper in all study groups. Values are presented as the mean ± the standard deviation. Group 1 – non-treatment BD group, Group 2 – short-term lithium-treatment group without thyroid dysfunctions, Group 3 – short-term lithium-treatment group that developed hyperthyroidism, Group 4 – long-term lithium-treatment group that developed hypothyroidism, Group 5 – long-term lithium-treatment group that developed hyperthyroidism, and Group 6 – long-term lithium-treatment group that did not develop thyroid dysfunction, $P$ < 0.05, [a] Group 1 vs. Group 2, [b] Group 1 vs. Group 4; [c] Group 1 vs. Group 6

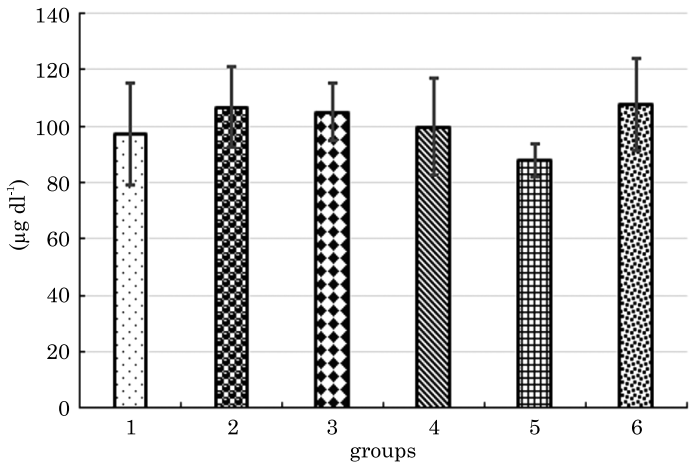


Fig. 3. The levels of iron in all study groups. Values are presented as the mean ± the standard deviation. Group 1 – non-treatment BD group, Group 2 – short-term lithium-treatment group without thyroid dysfunctions, Group 3 – short-term lithium-treatment group that developed hyperthyroidism, Group 4 – long-term lithium-treatment group that developed hypothyroidism, Group 5 – long-term lithium-treatment group that developed hyperthyroidism, and Group 6 – long-term lithium-treatment group that did not develop thyroid dysfunction

increased in Groups 2, 4, and 6 compared to Group 1 ($P < 0.05$ for all). Serum Fe levels were not significantly changed among all patient groups ($P > 0.05$).

## DISCUSSION

Bipolar disorder (BD), also known as manic-depressive illness, is a mental disorder with extreme mood swings from depression to mania. BD affects about 2.6 % of the U.S. population age 18 and older every year (Kessler et al. 2005), and it is the sixth leading cause of disability in the world (Colombo et al. 2012). Since it is a lifelong illness, its treatment will be long-term. Lithium is one of the most widely used medications for treatment of BD. It also has a broad spectrum of side effects, such as thyroid dysfunctions, and kidney damage (Dineen et al. 2017).

In our study, one to three of the patients who received short-term lithium therapy developed hyperthyroidism. Thyroid dysfunction, either hypothyroidism or hyperthyroidism, developed in about half the patients receiving long-term lithium treatment. The activity of the thyroid gland and the hypothalamic-pituitary-thyroid axis is important for the pathophysiology, clinical course and treatment of BD (Kraszewska et al. 2014). It may cause hypothyroidism or hyperthyroidism. There are several proposed mechanisms for the effects of lithium on the thyroid function. It is known that lithium is 3-4 times concentrated in the thyroid tissue compared to plasma. It may

interfere with various steps of the thyroid hormone production in the thyroid tissue, and with its effects on the hypothalamic-pituitary-thyroid axes (Lazarus 2009). For instance, lithium inhibits cyclic adenosine monophosphate (cAMP) activity induced by TSH, thereby decreasing T3 and T4 levels (Kusalic, Engelsmann 1999).

Oxidative stress is a process induced by endogenous and exogenous factors. Increasing evidence suggests that oxidative stress is linked to primary or secondary pathophysiological mechanisms of disease (Campos, Casado 2015, Valko et al. 2007). Thyroid hormones accelerate the basal metabolism in mitochondria, and cause changes in oxidative and antioxidant status due to their capacity to change the respiration rate. It has been proposed that changes in thyroid hormone levels may be one of the main physiological modulators of cellular oxidative stress (Campos, Casado 2015). Protein carbonyl derivatives are the most widely used markers of protein oxidation, and are used to evaluate diseases caused by oxidative stress (Dalle-Donne et al. 2003). Studies examining the relation of lithium treatment and oxidant status reported conflicting results (Dalle-Donne et al. 2003, Kiełczykowska 2006, Khairova et al. 2012). Although it has been reported that oxidative stress levels were decreased after lithium treatment in recent studies (Aliyazicioglu et al. 2007, Khairova et al. 2012), there are some reports in the literature showing increased MDA levels and decreased GSH content in an experimental study (Ahmad et al. 2011); decreased erythrocyte GSH levels in a BD patient treated with lithium (Engin et al. 2005). According to our results, erythrocyte GSH values decreased and plasma protein carbonyl levels increased in all patients receiving lithium treatment, compared to non-treated patients with BD, and appeared to be independent of thyroid function. We can argue that lithium has a prooxidant effect on tissues. Musik et al. (2017) also showed that lithium decreased GSH, GPx and selenium co-treatment showed a distinct protective effect, suggesting its effects by some selenoproteins which are abundant in the thyroid tissue. In addition, there were some conflicting results related with plasma/serum protein carbonyl levels in lithium treatment (Magalhaes et al. 2012, Akpinar et al. 2013, Andreazza et al. 2015). In our study, plasma and erythrocyte MDA levels were increased, and GSH levels were decreased in long-term lithium-treatment group that developed hypothyroidism compared to non-treated and short-term lithium-treatment two groups. Also, plasma and erythrocyte MDA levels were increased, and GSH levels were decreased in the long-term lithium-treatment group that did not develop thyroid dysfunction in comparsion with the two short-term lithium-treatment groups. However, there was no significant change in plasma and erythrocyte MDA levels in the long-term lithium-treatment group that developed hyperthyroidism compared to the two non-treated and short-term lithium-treatment groups. In addition, erythrocyte GSH levels were decreased in the long-term lithium-treatment group that developed hyperthyroidism compared to the two non-treated and short-term lithium-treatment groups. It is difficult

to say if there is a direct relationship between oxidative stress and thyroid dysfunction after Li treatment. But it is quite possible that it is associated with Li treatment. However, these finding might have been affected by some other factors. such as the severity of BD disease, selected study population and sample size.

Trace elements are known to have vital roles in different metabolic processes in the body. Some of these elements are cofactors of antioxidant enzymes and they are related with oxidant/antioxidant balance (Negi et al. 2012, Shazia et al. 2012, Kiełczykowska et al. 2018). In our study, serum Zn and Cu was high in patients who received short-term or long-term treatment but did not develop thyroid dysfunction. Zn was also high in patients with hyperthyroidism who received short-term treatment. In addition, serum Cu levels were high in patients with hypothyroidism who received long-term treatment. There are few reports showing relationships between trace elements and thyroid dysfunction caused by lithium treatment of BD in the literature. It has been shown that Zn supplementation attenuated the adverse effects caused by lithium on thyroid functions (Li et al. 2017). This finding supports our results showing high Zn values in groups of patients who do not have thyroid dysfunction after both short-term or long-term lithium treatment. It was also reported that erythrocyte Cu level increased with lithium treatment, but there was no significant change in Zn and Fe levels (Kiełczykowska et al. 2018). In our study, Zn and Cu levels changed due to lithium treatment of BD, and they showed association with thyroid dysfunctions. Noevertheless, it is difficult to detect their temporal relations. More studies are needed to elucidate this problem.

Increased risk of cardiovascular disease is one of the leading causes of shorter life expectancy in patients with BD compared to the general population (Kaleliogl u et al. 2018). Increased plasma and whole blood viscosity levels are associated with mortality due to cardiovascular diseases (Peters et al. 2017). It has been reported that thyroid hormone levels change the lipid profile of the blood (Rizos et al. 2011), and so, it shows some effects on blood rheology (Carallo et al. 1996). Viscosity, a fundamental parameter in blood rheology, is a major determinant of shear stress caused by blood flow, thus playing an important role in maintaining vascular homeostasis. Blood viscosity is mainly determined by hematocrit levels, plasma viscosity, deformability and aggregation of erythrocytes (Chen et al. 2012). Plasma viscosity is affected by concentrations of plasma proteins and lipoproteins, such as fibrinogen and immunoglobulins (Cowan et al. 2012). Studies have reported the relationship between the risk of cardiovascular disease in BD, but the effect of the lithium-induced thyroid dysfunction on hemorheological markers and related mechanisms have not been fully evaluated. In our study, plasma and blood viscosity were higher in all long-term lithium treated group compared to untreated and short-term lithium treated groups. We found that the lowest hemoglobin level was in the long-term lithium treated group who developed hypothyroidism. In addition, fibrinogen

levels were found to be higher in the long-term lithium treated group with thyroid dysfunction (either hypothyroidism or hyperthyroidism) than in the untreated group. In our literature research, we found few studies evaluating blood rheology in psychiatric disorders (Wong et al. 2008, Le Melledo et al. 2011, Kalelioglu et al. 2018). It is reported that patients with panic disorder have decreased plasma volume and increased hemoglobin and hematocrit levels (Le Melledo et al. 2011). Wong et al. 2008 informed higher whole blood viscosity in patients with unipolar depression than in controls. Kalelioglu et al. (2018) declared that whole blood viscosity values were higher in BD group than in control. To the best of our knowledge, this is the first study of hemorheological markers in thyroid dysfunction due to lithium treatment in BD. Our results suggest that hemorheological parameters may have a role in the risk of cardiovascular disease as a result of the development of thyroid dysfunctions in BD patients receiving lithium treatment.

In conclusion, the findings of our study showed that lithium treatment in patients with BD caused thyroid dysfunctions presenting as either hypothyroidism or hyperthyroidism; the consequences also depend on the duration of lithium treatment. More likely, lithium itself is prooxidant and increased oxidative stress may interact with other factors, like trace elements, proteins and lipids in different combinations depending on duration of lithium treatment, its doses, and severity of the disease. Also, whole blood viscosity, plasma viscosity, fibrinogen, Zn, and Cu levels were affected by lithium treatment. It should be mentioned that the main limitation of our study was the cross-sectional design and a relatively small sample size. Thus, it is necessary to confirm these findings by further studies.

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**Conflict of interest**

The authors declare that they have no conflict of interest.

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