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Ionized magnesium in plasma and erythrocytes for the assessment of low magnesium status in alcohol dependent patients

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ABSTRACT

Background: Studies on the homeostasis of magnesium in alcohol-dependent patients have often been characterized by low hypomagnesemia detection rates. This may be due to the fact that the content of magnesium in blood serum constitutes only 1% of the average magnesium level within the human body. However, the concentration of ionized magnesium is more physiologically important and makes up 67% of the total magnesium within a human organism. There are no data concerning the determination of the ionized fraction of magnesium in patients addicted to alcohol and its influence on mental health status.

Methods: This study included 100 alcohol-dependent patients and 50 healthy subjects. The free magnesium fraction was determined using the potentiometric method by means of using ion-selective electrodes. The total magnesium level was determined by using a biochemical Indiko Plus analyzer. In this study, different psychometric scales were applied.

Results: Our results confirm the usefulness of ionized magnesium concentrations in erythrocytes and plasma as a diagnostic parameter of low magnesium status in alcohol-dependent patients.

Conclusions: The lower the concentration of ionized magnesium, the worse the quality of life an alcohol-dependent person might experience. In the case of total magnesium, no such correlation was determined.

1. Introduction

In recent years, magnesium disturbance among alcohol-dependent patients has been observed as an increasing issue. Many psychopathological symptoms may be linked to a deficit of this bio-element. Low magnesium status contributes significantly to the prevalence of states of depression, anxiety disorders, and the aggressive behaviors during social interactions (Rio et al., 2013; Serefko et al., 2013; Rayssiguier et al., 2010; Poikolainen and Alho, 2008).

This growing interest is focused around the possibility of identifying intracellular elements, such as ionized magnesium, which constitutes 67% of the total pool of magnesium in the body. In this form, it actively affects several cell functions, including the activity of more than 300 enzymes. The intracellular content of this biologically active form provides a more complete picture of the body's condition. Total magnesium in blood serum constitutes only 1% of the total pool of magnesium in the human body. The determination of extracellular or total intracellular magnesium concentration does not fully reflect the status of this bioelement in the human organism. It confirms the validity of the determination of ionized magnesium, both in erythrocytes and in blood plasma as a diagnostic parameter. Knowledge of this relationship between ionized and total magnesium concentrations in erythrocytes, along with knowledge of extracellular environment, gives a much fuller picture of the biochemical changes that take place in a human organism (Szewczyk et al., 2008; Malon et al., 2004; Martin et al., 2004; Fawcett et al., 1999; Rude, 1989).

Reduced magnesium status in alcohol-dependent patients has, to a large extent, a negative effect on heart rates, the nervous system, and a person's psyche (Serefko et al., 2013; Rayssiguier et al., 2010).

Basic mechanisms of magnesium deficiency and its consequences in

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alcohol-dependent people include the following: (1) diets low in magnesium, (2) poor absorption and losses (vomiting, diarrhea), (3) excessive urinary excretion and losses with sweat, (4) stressful situations that result in increased production of aldosterone and catecholamine hormones, an increase in excretion of antidiuretic hormone and significant loss of taurine, (5) shortages of ions and vitamins, and (6) iatrogenic loss due to the use of diuretics, some antibiotics, the adrenal cortex products, etc. (Kulkarni et al., 2012; Sobral-Oliviera et al., 2011; Iezhitsa and Spasov, 2008; Lieber, 2000; Elisaf et al., 1995).

McCollister et al. (1963) and Siegenthaler (1987) illustrated the impact of chronic alcoholism on magnesium levels. A number of scientists and researchers confirmed the occurrence of a reduced level of magnesium in alcohol-dependent people. Wu and Kenny determined total and ionized magnesium concentrations in the serum of patients admitted to hospitals. No differences were observed in the total magnesium concentration of both groups. However, the concentration of the ionized magnesium fraction was lower in the group of patients who were under the influence of alcohol (Wu and Kenny, 1996). Other authors have obtained similar results (Rylander et al., 2001; Altura and Altura, 1999; Hristova et al., 1997; Altura et al., 1995).

Other studies have shown a significant reduction of magnesium within the blood serum of alcohol-dependent people who have been diagnosed with an alcoholic fatty liver when compared to a control group (Turecky et al., 2006). Hypomagnesemia has also been observed in cases when a patient is diagnosed with acute pancreatitis caused by alcohol consumption (Liamis et al., 2001). Patients with liver cirrhosis caused by alcohol abuse were also found to have a lower level of magnesium in the serum (Aagaard et al., 2002; Tokmak et al., 2016). In several studies, the measurements of the total magnesium in blood serum were characterized by hypomagnesemia detection (Poikolainen and Alho, 2008; Stasiukyniene, 2002; Kisters et al., 1998).

There are no data on the ionized magnesium fraction in blood cells of alcohol-dependent people within the scientific literature. Therefore, the determination of the ionized fraction of magnesium in the blood plasma and the erythrocytes of alcohol-dependent patients was the goal of this study. In addition, we investigated whether there were significant differences between alcohol-dependent patients with decreased and normal concentrations of particular magnesium fractions.

2. Material and methods

The experimental group consisted of 100 patients dependent on alcohol, while the control group consisted of 50 healthy subjects.

The last alcohol binge was 55.11 ± 15.76 days, while the number of days that alcohol-dependent patients consumed alcohol within the last four weeks was 13.75 ± 1.06 days. The eligibility of patients was determined by psychiatrists, as well as on the basis of the scales measuring alcohol dependency.

A standardized clinical interview was also carried out. People participating in the study were properly consented. The study was approved by the Bioethics Committee of Medical University of Warsaw KB/88/2014 of April 15, 2014.

The blood samples were collected in vacuum tubes containing lithium heparin, centrifuged for 20 min at 4000 rpm, and then the plasma was separated. The top layer of lymphocytes was removed (buffy lymphocytes coat). The blood processing was carried out on the basis of which a sample reflecting the intracellular environment was obtained. The distilled and deionized water and TRIS/TES buffer of pH 7.2 (1 + 1 + 1, sample + water + buffer) were added to the isolated erythrocytes. Then, the sample was lysed in an ultrasonic bath for 20 min (ultrasonic cleaner "Polsonic" sonic–0.5, Poland). The determination of ionized magnesium fractions, both in plasma and in red blood cells, was performed using the potentiometric method by applying ion-selective electrodes (ISE) and a clinical Analyzer Microlyte 6 (KONE, Espoo, Finland) (Malon and Maj-Zurawska, 2005). In the first stage, an electrode examination was performed. The analyzer automatically calibrated using three standard solutions. The moment the calibration was completed, the control serum (Nortrol, Thermo, Espoo, Finland) was used three times in order to validate the working electrodes. Under the above-mentioned conditions, the analyzer automatically converted the measured potential of the ion-selective electrode into the concentration of ionized magnesium. In the case of erythrocytes, new standard solutions were used in which the concentration of individual ions were similar to concentrations in the tested samples (Malon et al., 2002). On the basis of a series of measurements of the new standard solutions, the calibration curve was performed.

Taking the ionic strength of the sample into consideration, the concentration of ionized magnesium in erythrocyte lysates was calculated. For each pattern and sample, three measurements were collected. Every four hours a new calibration was carried out, as well as the exchange of standard solutions and completion of new calibration solution measurements (Malon et al., 2004).

Total magnesium in plasma and erythrocytes (lysate) was determined by using automatic, biochemical Indiko Plus analyzer (Thermo).

In the study, different questions evaluating the degree of alcohol and cigarette dependence were used and the following psychometric scales were applied: (1) Barratt Impulsiveness Scale (BIS–11) was used to measure impulsivity (Jakubczyk and Wojnar, 2009), (2) Brief symptom inventory (BSI) questionnaire was used to assess psychological quality of life (Mohammadkhani et al., 2010), (3) Quality of life questionnaire, the short form health survey (SF–36), was used to evaluate physical and psychological quality of life (Burholt and Nash, 2011), (4) Sleep Disorders Questionnaire (SDQ–7) was used to assess sleep disorders (Lomelí et al., 2008), and (5) NEO Five Factor Inventory (NEO-FFI) used to evaluate parameters such as neuroticism, diligence, extraversion, agreeableness, openness on experience (Renner, 2002)

Statistical analysis was conducted using the IBM SPSS Statistics 22 package. The nonparametric test for independent groups (Mann–Whitney *U* test) was used in order to check if there were statistically significant differences between the groups in terms of specific fractions of magnesium. The Spearman correlation was used to check if there were any significant correlations between concentrations of specific magnesium fractions. The difference in occurrence of "low" values between two studied groups was assessed by a Chi-square test.

3. Results

Regarding the results of ionized magnesium obtained in the group of 50 healthy people and in the group of 100 alcohol-dependent people, there were statistically significant differences in the concentration of free magnesium in the plasma of both groups: $U = 976.5$; $p < 0.001$ and in erythrocytes $U = 185.5$; $p < 0.001$ (Table 1). The average concentration of ionized magnesium in the plasma of the alcohol-dependent patients was 0.51 ± 0.01 mmol/L, while in the control group

Table 1
Concentration of ionized magnesium and total magnesium in plasma and erythrocytes in the group of alcohol-dependent group and in the control group.

	Alcohol-dependent group (n = 100)	Control group (n = 50)
Ionized magnesium in plasma (mmol/L)	0.51 ± 0.01** Me = 0.53	0.66 ± 0.02 Me = 0.7
Ionized magnesium in red blood cells hemolysates (mmol/L)	0.36 ± 0.01** Me = 0.36	0.71 ± 0.02 Me = 0.7
Total magnesium in plasma (mmol/L)	0.81 ± 0.01* Me = 0.82	0.84 ± 0.01 Me = 0.85
Total magnesium in red blood cells (mmol/L)	1.64 ± 0.08 Me = 1.5	1.60 ± 0.1 Me = 1.44

Note: Mann-Whitney *U* test; M ± SE.
* $p < 0.01$.
** $p < 0.001$, Me = median.

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