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Magnesium level in serum during acute ischemic stroke – the relationship with neurological status and Tau protein serum level

Magnesium ions (Mg^{2+}) play an important role in molecular pathogenesis of the ischemic stroke (IS). A significant reductions of Mg^{2+} levels in extracellular fluid (ECF) of the brain tissue was found in the animal models of stroke (1). Previous animal studies showed dose-dependent neuroprotection of Mg^{2+} administration in the focal cerebral ischemia (see 2 for review). The pathological stimulation of postsynaptic N-methyl d-aspartate (NMDA) and α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptors by neurotoxic glutamate during ischemia of the brain are thought to be key events in the evolution of neuronal cell death. Physiological extracellular Mg^{2+} level (up to 1 mmol/l) inhibits glutamate release (3) and specifically antagonizes NMDA receptors (4). Increasing the extracellular Mg^{2+} level above 1 mmol/l noncompetitively antagonizes NMDA conductance (5), whereas the reduction of extracellular Mg^{2+} enhances toxicity (6). Activation of NMDA receptor causes a 20-fold increase in the intracellular Mg^{2+} level (7). Magnesium ions present in intracellular fluid (ICF) buffer calcium ions (Ca^{2+}) required to initialing an apoptotic cascade and could help to uptake Ca^{2+} into endoplasmatic reticulum (*via* Mg^{2+}/Ca^{2+} ATPase) (8, 9). Magnesium infusions could improve cerebral flow through arteriolar vasodilatation either as a direct Ca^{2+} antagonist effect on vascular smooth muscles or as an antagonist on $PGF_{2\alpha}$, a strong vasoconstrictor (2). Unfortunately the IMAGES trial performed on a large group of patients with acute IS did not confirm that early treatment with a high dose of $MgSO_4$ can reduce the changes of death or disability. IMAGES trial concluded that Mg^{2+} administration may be beneficial in lacunar stroke (10, see 11 for review). However the supplementation of Mg^{2+} can be beneficial in other diseases with ischemic pathogenesis. The administration of $MgSO_4$ reduces the serum S100B protein level, well-known marker of astroglia damage, in patients after coronary artery bypass surgery (12). In our work we were wondering whether Mg^{2+}, together with other compounds, can be considered as a prognostic factor of patients outcome after IS. Additionally with Mg^{2+} we analyzed the serum Tau protein level as a marker of neurons' damage. Tau protein derives from neurons, predominantly axons and it is released after destruction of the cell structure (13, 14). According to our knowledge there are no publications focused on the correlation between the serum levels of Tau protein and Mg^{2+} in the stroke.

The purpose of our study was to evaluate whether the measurement of serum Mg^{2+} and Tau protein levels can be the prognostic factor for outcome after IS.

MATERIAL AND METHODS

Patients. Forty-seven patients admitted to the Stroke Unit at the Department of Neurology, Medical University of Lublin were prospectively enrolled into the study. Written informed consent was obtained from each patients (or from family members when necessary). The local Ethics Committee (Medical University of Lublin) accepted the protocol of the study.

Inclusion criteria were: (a) Diagnosis of IS based on history, physical examination and computed tomography (CT)-scan performed at admission to the hospital. (b) Admission to the hospital within the first 24 h from the onset of neurological focal symptoms.

Exclusion criteria were: (a) Regression of neurological symptoms within 24 h from the onset (Transient Ischemic Attack, TIA), (b) History or symptoms suggesting coexistence of cancer, rheumatoid, psychiatric, neurodegenerative or demyelinative diseases and nerve or muscle disorders, (c) Time of hospitalization shorter than 10 days, (d) Haemorrhagic transformation of ischemic focus confirmed by CT-scan on day 10 of stroke, (e) Prescription of thrombolytic therapy. History of stroke in the past was not an exclusion criterion.

Six primary enrolled patients met exclusion criteria; five of them were TIA patients, one was hospitalized shorter than 10 days. The final study group consisted of 41 patients, female 22, male 19, mean age 72.9 ± 11.08. Twenty patients developed arteriosclerotic stroke, nine cardioembolic, seven complex mechanism and five unknown pathogenesis. During the hospitalization the treatment was provided according to the standard protocol. It was unnecessary to administer magnesium ions as well as osmotic agents, such as mannitol, which could disrupt the blood brain barrier and affect the findings of the study (15).

Neurological examinations were performed on the admission (day 1) and day 10 of stroke based on the National Institute of Health Stroke Scale (NIHSS).

Biochemical procedures. Venous blood samples were obtained during the first 24 h of stroke and on day 10 after symptoms onset. After centrifugation the serum was stored in -60°C for maximum 8 months.

Commercially available enzyme-linked immunosorbent assay (ELISA) kit was applied to evaluate serum Tau protein (Innotest, Innogenetics NV, Belgium) according to the producer instructions. The detection limit of Tau protein assay was 60 pg/ml and it has corresponded to the lowest standard applied in the calibration curve. All values below the detection limit were rendered zero and they were not applied for calculations. After Tau protein evaluation the study group was divided into two separate groups depending on the presence of Tau protein in the serum. The patient group in which Tau protein was detected was marked "Tau+", the remaining was assigned as "Tau-". All values below the detection limit were rendered zero and they were not applied for calculations. The optical density was determined by using a microplate reader set to 450 nm.

Magnesium ions react in an alkaline medium with the metallochrome dye calmagite to form a chromophore which absorbs waves at 520 nm. To evaluate the magnesium level in patients serum the commercial Magnesium Calmagite Colorimetric Method (Randox, Cat. MG 573) was used according to the producer manual. Briefly, samples were prepared by mixing 10 µl of serum with 1 ml of the Working Reagent. After incubation for 60 seconds the absorbance of sample was measured at 520 nm, zeroing against the reagent blank. Calculation of the magnesium level was set with absorbance of magnesium standard (1 mmol/l). The magnesium ions level was measured two times, on day 1 and 10 of stroke.

Statistical methods. The values of magnesium or Tau protein serum levels on day 1 and 10 were compared. Wilcoxon matched-pairs signed-ranks test was used. For Gaussian distribution paired t-test was applied. On order to compare unpaired populations with nonparametric distribution Mann-Whitney test was applied. The Spearman Rank Correlation was used as nonparametric correlation. Statistically significant values were considered when $p < 0.05$. Statistical analysis was performed with the use of the computer-assisted statistical program GraphPad InStat v. 3.06. (San Diego, USA).

RESULTS

The median value of the magnesium serum level was increased on day 10 in comparison to the onset of stroke (see Tab. 1). Among all patients only at 10 of them (group A) the magnesium level was decreased on day 10 [1.27 mmol/l (1.08–1.37) vs. 1.04 mmol/l (0.96–1.19) on days 1 and 10 respectively, median values (1^{st} – 3^{rd} quartiles), $p = 0.002$, Wilcoxon test]. At 31 patients (group B) the magnesium level increased on day 10 of stroke in comparison to day 1 [0.92 mmol/l (0.14) vs.1.19 mmol/l (0.16), mean (SD) on days 1 and 10 respectively, $p < 0.0001$, Paired t test]. There was no correlation between the mean Mg^{2+} level and mean NIHSS score (Fig. 1). However, mean NIHSS score was not significantly higher in patients with the decrease of magnesium level (group A) in comparison to those with the increase of the magnesium level on day 10 of stoke (group B) (11.13 vs. 8.75, median values for NIHSS score of group A and B respectively, $p = 0.08$, Mann-Whitney test).

Table 1. Serum level of magnesium and Tau protein on day 1 and 10.
Median values and 1^{st} – 3^{rd} quartiles

	Day 1	Day 10	Wilcoxon test
Magnesium (mmol/l)	0.95 (0.87–1.06)	1.14 (1.06–1.27)	p = 0.0014
Tau (pg/ml)	78.11 (60.79–89.96)	98.48 (81.22–200.87)	p = 0.002

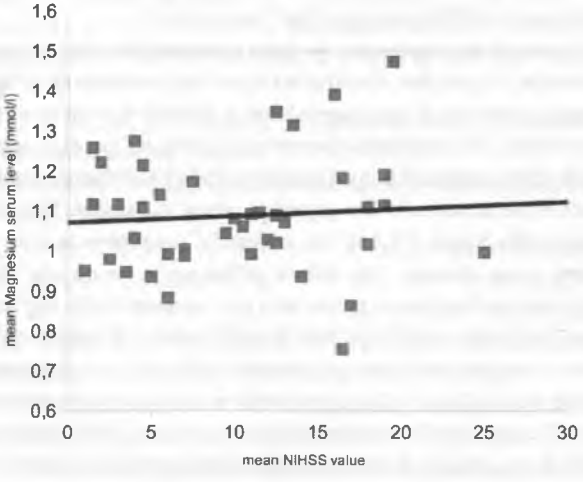

Fig. 1. The absence of relationship between the mean Mg^{2+} serum level and NIHSS value; $r = 0.04$, $p > 0.1$, Spearman Rank Correlation

According to the mentioned above value of detection limit, we noticed Tau protein in samples obtained from 20 (48.8%) of 41 patients (Tau+ group). In all Tau+ patients Tau protein was present at

both evaluated time-points. The median of the Tau protein level was increased on day 10 in comparison with the stroke onset. There was no difference between Mg^{2+} serum levels evaluated in Tau+ and Tau- groups (1.081 and 1.085 mmol/l, median values for Tau+ and Tau- groups respectively, $p > 0.1$, Mann-Whitney test). In addition, there was no correlation between the mean magnesium serum level and mean Tau protein level during acute phase of IS ($r = 0.009$, $p > 0.1$, linear regression). Mean NIHSS score was significantly higher in patients from Tau+ group in comparison with Tau- [14.75 (17.75–7.88) vs. 6.38 (10.5–3.38), median values ($1^{st} - 3^{rd}$ quartiles) for Tau+ and Tau- groups respectively, $p = 0.0048$, Mann-Whitney test].

DISCUSSION

The fluctuations of elements homeostasis can be the reason of numerous diseases (16). Magnesium ions as the essential constituents of ATP-Mg^{2+} complex play the key role in the metabolism of brain tissue. Magnesium has neuroprotective properties, which antagonizes Ca^{2+} in several biological processes especially during the apoptosis (see 17 for review). Dose-dependent neuroprotective effect was observed in the animal models of focal cerebral ischemia (2). Inhibition of glutamate had brought the hope that magnesium could have been used in the stroke treatment. Unfortunately promising neuroprotective features of Mg^{2+} were not confirmed in clinical trial with over 2,500 individuals performed in acute IS (10,18). Despite the lack of clear evidences that administration of magnesium has a neuroprotective effect in the IS, the influence on glutamate/NMDA pathway could be interesting. The absence of therapeutic potential with simultaneous positive results of experimental data suggests the complex mechanism of magnesium ions action. The last small study concerning the Mg^{2+} measurement during the first 2 days of stroke revealed higher NIHSS value in patients with low magnesium ions level in the serum (19). Our study partially confirmed above observations, however we noticed that not the Mg^{2+} serum level but its decline up to day 10 of stroke seems to be a bad prognostic factor. Unfortunately, the difference did not reach the statistical significance and this hypothesis has to be confirmed on a larger group of patients. We did not find the correlation between the mean NIHSS score and Mg^{2+} serum level.

During the next part of the experiment we have compared the Mg^{2+} serum level with some marker of neuron's damage. To gain that objective we chose Tau protein as an indicator of brain tissue damage and biochemical predictor of neurological status. NIHSS score was significantly higher in Tau+ patients. Unfortunately, the hypothetical correlation between the Mg^{2+} and Tau protein level was not found, in spite of the magnesium involvement in brain tissue homeostasis.

Separate problem is the presence of Tau protein in serum of less than 50% of all stroke patients during the acute phase of the disease (13,14). The reason for Tau protein scarcity in serum of above 50% of stroke patients is not obvious. The lack of difference in serum Mg^{2+} levels of Tau+ and Tau- group suggested that the Tau protein presence is not connected with Mg^{2+} metabolism. Bitsch et al. noticed that the Tau protein could have been found in serum of patients with IS whose lesion underwent spontaneous reperfusion in areas of ischemic focus due to endogenous thrombolysis. In those events reperfusion would allow the Tau protein to be washed out from the destroyed parenchyma (13). Theoretically Mg^{2+} can be involved in the reperfusion process, which facilitates to wash up the Tau protein from brain tissue, due to its vasodilatation effect mostly as a direct Ca^{2+} antagonist (2). However, the role of Mg^{2+} in the spontaneous reperfusion during IS is not clear.

CONCLUSIONS

1. The presence of the Tau protein (but not its level) in the serum predicts the neurological status during acute IS.

2. The decrease of the Mg^{2+} serum level during the acute phase of stroke seems to correspond with worse neurological status during acute IS. The opposite effect could cause the increase of the Mg^{2+} serum level, therefore the next studies performed on a larger group of individuals have to focus on fluctuations of the Mg^{2+} serum level during IS.

3. The construction of biomarker panel containing serum Tau and Mg^{2+} levels is difficult to apply due to the lack of clear correlations between serum levels of above mentioned compounds.

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SUMMARY

Previous studies indicated magnesium ions (Mg^{2+}) as an important factor in the molecular pathogenesis of stroke. In this work we analyzed whether serum Mg^{2+} level, together with Tau protein level (the marker of neuron's damage), can be considered as prognostic factors of patients outcome after ischemic stroke. The final study group consisted of 41 ischemic stroke patients. Neurological examination (NIHSS) and blood samples were obtained at the onset and the end of acute phase of stroke (days 1 and 10). ELISA kit was applied to evaluate serum Tau protein. To evaluate Mg^{2+} level the commercial Magnesium Calmagite Colorimetric Method was used. The median value of magnesium serum level was increased on day 10 in comparison to the onset of stroke. There was no correlation between the mean Mg^{2+} level and mean NIHSS score, however average NIHSS score was higher in patients with the decline of the Mg^{2+} level, but the result was not statistically significant ($p = 0.08$). Tau protein was noticed in samples obtained from 20 (48.8%) of 41 patients (Tau+ group). Tau protein level significantly increased on day 10 of stroke in comparison with day 1. There was no difference between mean Mg^{2+} levels evaluated in Tau+ and Tau- groups. In addition, the correlation between the mean Mg^{2+} serum level and mean Tau protein level during the acute phase of ischemic stroke was not observed. Mean NIHSS score was significantly higher in patients from Tau+ group in comparison with Tau- group. Our observation indicated that the presence of Tau protein (but not its level) in serum can predict the neurological status in the acute phase of stroke. The decrease of the Mg^{2+} serum level seems to correlate with the neurological status of patients with ischemic stroke. The last result has to be confirmed on the larger group of patients.

Poziom magnezu w surowicy krwi podczas ostrej fazy niedokrwiennego udaru mózgu – związek z neurologicznym stanem pacjentów i stężeniem białka Tau

Istotnym czynnikiem biorącym udział w patogenezie udaru mózgu są jony magnezu (Mg^{2+}). W pracy sprawdzono, czy stężenie Mg^{2+} w surowicy krwi, razem z markerem uszkodzenia neuronów – białkiem Tau, może być czynnikiem prognostycznym u pacjentów z udarem niedokrwienym mózgu. Grupa badana składała się z 41 pacjentów. Krew żylna pobierana była na początku i końcu ostrej fazy udaru mózgu (1 oraz 10 dzień udaru). W tych samych dniach oceniano stan kliniczny pacjentów za pomocą skali NIHSS. Stężenie białka Tau oznaczano za pomocą metody ELISA, natomiast stężenie Mg^{2+} za pomocą metody kolorymetrycznej. Analizując całą badaną grupę stwierdzono, że średnie stężenie jonów magnezu w surowicy krwi wzrosło w dziesiątej dobie udaru w porównaniu

z pierwszą dobą. Jednak u 10 pacjentów z grupy badanej odnotowano istotny statystycznie spadek stężenia Mg^{2+} w surowicy. Nie stwierdzono korelacji pomiędzy średnim stężeniem Mg^{2+} oraz średnim wynikiem NIHSS (wyższy wynik wskazuje na poważniejszy stan kliniczny). Średni NIHSS był większy u pacjentów, u których doszło do spadku stężenia Mg^{2+} w ostrej fazie udaru, jednakże wynik był nieistotny statystycznie ($p = 0.08$). Białko Tau zostało znalezione w surowicy krwi u 20 (48,8%) pacjentów. Stężenie Tau wzrosło w 10 dniu w porównaniu do dnia 1. Nie znaleziono różnic statystycznych pomiędzy stężeniem Mg^{2+} u pacjentów z obecnym i nieobecnym białkiem Tau w surowicy krwi. Nie znaleziono również zależności pomiędzy średnim stężeniem Mg^{2+} a stężeniem białka Tau w ostrej fazie udaru. Pacjenci z obecnym białkiem Tau w surowicy krwi w ostrej fazie udaru mózgu uzyskali wyższy wynik w skali NIHSS, wskazujący na poważniejszy stan kliniczny w porównaniu z grupą pacjentów bez obecnego białka Tau. Przyszłe badania powinny jednoznacznie wyjaśnić, czy spadek stężenia Mg^{2+} w surowicy krwi obserwowany w ostrej fazie przyczynia się do gorszego rokowania u pacjentów z udarem niedokrwiennym mózgu.