Evaluation of intracellular magnesium and calcium concentration in patients with migraine

Durdi Qujeq, *PhD,* ***Mehdi Zandemami,*** *MSc,*
Alijan A. Ahanger, *MD,* ***Mohammed E. Shahabadin,*** *MSc.*

It is now clear that migraine is associated with magnesium (Mg) deficiency.[1] In the literature, low serum and tissue contents of total Mg have been reported in patients with migraine.[2] Investigators have reported a strong correlation between serum Mg levels and the calcium (Ca)/Mg ratio in migraine.[3] Serum Mg concentration does not reflect the intracellular content. In contrast, the cellular content has been assessed as an index of Mg status.[4] The objective of the present study was to examine the possible use of the Mg and Ca content of lymphocytes as an index for Mg and Ca status.

The control group consisted of 24 healthy volunteers who came for a routine check up, did not have any disorder, and did not suffer from migraine. The study group consisted of 21 patients who were followed up in the hospital with the diagnosis of migraine. Patients were diagnosed with migraine according to the International Headache Society (HIS) criteria,[5] and international classification of headache disorders. Each patient underwent an extensive neurological and physical examination. The patients with migraine headache with aura were distinguished from those without aura. All examinations on migraine patients were performed while they were not having a headache attack. Patients and controls were asked to lie at rest during examinations. Inclusion criteria were a history of migraine, moderate to severe pain on admission, and a normal systemic and neurological examination. Exclusion criteria included use of anti migraine medication during the attack. Informed written consent was obtained from all the subjects who donated their blood, patients and control subjects, and the study was carried out with the approval of Babol University Ethics Committee. Neither the controls nor the patients were taking any medication known to interfere with any Ca and Mg status. All subjects were matched for lifestyle and dietary habits. Patients were not eligible for inclusion in the study if they had undergone previous treatment for migraine. Also, we excluded those who were on medications. This study was conducted at the School of Medicine, University Hospital of Babol, Babol, Iran, between 2008 and 2010.

Venous blood was taken in the morning after an overnight fast for at least 12 hours. Blood was drawn into tubes containing heparin. Lymphocytes from 2.5 ml of heparinized blood were separated on Ficoll-Paque (Pars Azma Teb Co., Tehran, Iran) 1.077-1.080 gr/cm^3 by centrifugation at 1800g for 30 minutes. The lymphocyte containing interface was removed, and 200-300 µl of samples were subsequently transferred into 10 ml tubes. Samples were mixed with 50 µl of 10% Triton X-100 (Merck, Darmstadt, Germany) then stored for 24 hours at -20°C. Then, 20 µl of nitric acid (HNO_3) was added. These were centrifuged for 15 minutes at 2000 RPM, and then 200 µl of the top layer was removed. The Mg and Ca in lymphocytes were determined by atomic absorption spectrophotometry at 285 nm (Shimadzu AA-670, Kyoto, Japan). The results were expressed as mean ± SD to compare between means of 2 groups. Using the Statistical Program for Social Sciences (SPSS Inc., Chicago, IL, USA) version 16, the statistical significance was determined using the Mann-Whitney U test for data, a significant difference was assumed at $p<0.05$.

Migrainers had higher lymphocyte Ca levels in comparison with normal subjects. However, no significant differences were found comparing patients affected by migraine and controls. **Table 1** shows the comparison of lymphocyte Mg and Ca in normal individuals and the patients.

Earlier studies have suggested that Mg plays a role in the development of headaches.[1,2] As reported by many other investigators, Mg deficiency is proposed to play a role in the pathophysiology of migraine.[2,3] In recent years, cellular and free Mg concentrations have been assessed in patients with migraine.[2,3] Defective energy metabolism has been documented in migraine headaches associated with low intracellular Mg.[3,4] In previous studies, impaired Mg metabolism has been reported in migraine. Low serum levels of Mg have been reported in patients with migraine.[3,4] Some of the findings in these reports have been contradictory, as both normal, and low levels of Mg were found in the same sample of patients with migraine.[4] As reported

Table 1 - Comparison of lymphocyte magnesium and calcium in normal individuals and patients with migraine.

Group	Magnesium level	Calcium level
Normal group	8.944 ± 0.865	10.369 ± 0.912
Patient group	8.917 ± 0.743	11.261 ± 0.931

Data are presented as mean±SD of serum calcium and magnesium (µg/ml).

Disclosure. This work was supported by a grant (#2001210025) from the Babol University of Medical Sciences Research Department. The authors declare no conflicting interests, and this study was not supported or funded by any drug company.

by many other investigators, total and ionized Mg has been found to be reduced in the serum of patients with migraine. It is clear that evaluation of the Mg status is complicated by the fact that most of the Mg is stored in tissues, so that serum values are not truly representative. As is well known, total blood Mg tests measure total Mg. To detect these deficiencies, a non-invasive and sensitive test assessing Mg status is needed. We investigated the possible utility of lymphocyte Mg and Ca level as an index of these ion statuses. Our results indicate that intracellular Mg is more relevant to the migraine problem. These findings demonstrated that intracellular levels of Mg and Ca may accurately reflect ion status. Measurement of intracellular Mg and Ca concentrations are sensitive methods for demonstrating ion deficiency. The lymphocyte Mg seems a better indicator of Mg status than serum values; these results confirm findings of other investigators. Our current findings are consistent with extensive neurological and physical examination results. Also, our results were in good agreement with those reported previously.[3,4]

Magnesium is known to play an important role in the regulation of Ca entry in neural cells. Magnesium is a cofactor for enzyme reactions involved in the pathways of energy metabolism. Calcium ions regulate many important physiological processes. Calcium plays an important role in signal transduction. Intracellular Ca is the prime inorganic messenger for regulation of cell function. Intracellular Ca serves a vital role in controlling the function of all cells. Calcium is the most abundant mineral in humans, although Ca is predominantly extra cellular, it plays an important regulatory role within cells. Changes in the intracellular concentration of Ca triggers cellular process. Calcium is involved in activating selected enzymes.[3,4]

Low Mg levels on Ca channels might play a pivotal role in the biochemical events leading to the neural and vascular modification associated with a migraine attack. Magnesium plays an important role in numerous biochemical processes. Magnesium has been proposed to play a role in many theories of migraine pathogenesis. Latter studies have shown that migraine suffers have low Mg levels in the lymphocytes.[3,4] In our present study, we find some interesting similarities and differences between our findings and those reported earlier. We have suggested that these inconsistencies may be due to small sample size, and different methodology.

Our observation that lymphocyte Mg levels in the migraine group were decreased compared with the control group is in agreement with the results obtained by other authors. The molecular mechanisms leading to Mg deficiency are still unknown. This study confirms previous investigations, which showed the relationship between migraine and Mg metabolism. Also, our results may support the hypothesis that Mg deficiency plays a role in the migraine attacks. Our current findings also are consistent with recent results obtained from patients with migraine. The molecular mechanisms responsible for the magnesium deficit in migraine remain unclear. However, our present findings, taken with previous results in patients with migraine showing low free lymphocyte magnesium support the notion that lymphocyte Mg and Ca concentrations are indicators of Mg status. Measurement of intracellular and ionized Mg and Ca concentrations are sensitive methods for demonstrating Mg deficiency. Serum or extra cellular Mg and Ca concentrations do not reflect the intracellular content. In contrast, the lymphocyte Mg level seems a better indicator of Mg status than serum values.

Our study has some limitations. Beyond all these positive results and suggestions, some limitations, and methodological flaws of our study should be mentioned. Our small sample size might have led to loss of the power of statistical analysis, and our patient profile represents some differences from the general migraine population. Further studies on intracellular ions are needed to clarify the mechanisms responsible for the Mg deficit in migraine.

In conclusion, assessment of intracellular and ionized Mg and Ca concentrations are sensitive methods for demonstrating Mg deficiency in migraine.

Received 26th April 2011. Accepted 12th September 2011.

From the Cellular and Molecular Biology Research Center (Qujeq), the Department of Biochemistry and Biophysics (Qujeq, Zandemami, Shahbadin), Faculty of Medicine, the Department of Neurology (Ahanger), Yahyanejad Hospital, the Department of Neurology (Ahanger), Aytoleh Rohani Hospital, Babol University of Medical Sciences, Babol, and the Department of Biochemistry (Shahbadin), Faculty of Medicine, Kashan University of Medical Sciences, Kashan, Iran. Address correspondence and reprint requests to: Dr. Durdi Qujeq, Associate Professor, Cellular and Molecular Biology Research Center (CMBRC), Babol University of Medical Sciences, Babol, Iran. Tel. +98 (111) 2190569. Fax. +98 (111) 2226109. E-mail: d.qujeq@mubabol.ac.ir / dqujeq@hotmail.com

References

1. Thomas J, Thomas E, Tomb E. Preliminary communication: serum and erythrocyte magnesium concentrations and migraine. ***Magnesium Res*** 1992; 5: 127-130.
2. Facchinetti F, Sances G, Botella P, Genazzani AR, Nappi G. Magnesium prophylaxis of menstrual migraine: effects on intracellular magnesium. ***Headache*** 1991; 31: 298-301.
3. Altura BM, Altura BT, Gupta RK. Alcohol intoxication results in rapid loss in free magnesium in brain and disturbances in brain bioenergetics: relation to cerebrovasospasm, alcoholi-induced strokes, and barbiturate anesthesia-induced deaths. ***Magnes Trace Elem*** 1991-1992; 10: 122-135.
4. Goto K, Yasue H, Okumura K, Matsuyama K, Kugiyama K, Miyagi H, et al. Magnesium deficiency detected by intravenous loading tests in variant angina pectoris. ***Am J Cardiol*** 1990; 65: 709-712.
5. International Headache Society. Headache Classification Committee of the International Headache Society: classification and diagnostic criteria for headache disorders, cranial neuralgias and facial pain. ***Cephalgia*** 1997; 18 (Suppl): 43-110.