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Electromyographical ischemic test, clinical symptoms related to neuromuscular hyperexcitability, and intra- and extracellular Mg^{++} levels in headache patients

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Abstract We observed the occurrence of neuromuscular hyperexcitability, assessed with electromyographic ischemic tests, in headache patients in relation to Mg^{++} levels in serum, red blood cells and mononuclear cells. Clinical symptoms most significantly associated with neuromuscolar hyperexcitability and magnesium derangements were also investigated. A total of 36 patients with migraine without aura (MwoA), 18 patients with episodic tension-type headache (ETTH) and 22 patients with chronic tension-type headache (CTTH) were examined during interictal periods. The electromyographic (EMG) ischemic test was positive in 91.7% of MwoA patients, in 27.7% of ETTH and in 13.6% of CTTH patients. In patients with MwoA, the Mg^{++} levels in red blood cells were significantly less than those in the other two groups of patients with tension-type headache (ANOVA, $p<0.00l$). Positive EMG ischemic tests were significantly associated with decreased erythrocyte Mg^{++} levels in MwoA patients (χ^2, $p<0.01$). The clinical symptoms in patients with MwoA most frequently suggest the presence of neuromuscular hyperexcitability and are more strictly associated with reduced red blood cell Mg^{++} levels. They include fasciculations (91.7%), asthenia (91.7%), blepharospasm (75.8%) and paresthesiae (47.2%). Anxiety and depression most often occurred in tension-type headache patients, but were not related to modifications in intra- and extracellular Mg^{++} levels.

Key words Magnesium deficiency • Electromyographical ischemic test • Neuromuscular hyperexcitability • Clinical assessment • Migraine • Tension-type headache

Introduction

Previous research has established the role of magnesium deficiency in migraine, in adults [1–7], children and adolescents [8, 9]. On the other hand, there is recurring evidence, since the early 1960s, that a relative or absolute lack of Mg^{++} is related to certain neurological conditions such as criptotetany, latent tetany, hyperventilation syndrome and spasmophilia [10–17]. The latter has recently been referred to as the central neuronal hyperexcitability syndrome (NHS) [18, 19]. Headache has often been described to be associated with the neuronal hyperexcitability syndrome (NHS), but no clear clinical characterization of this association is available [14–16].

In previous studies by our group, peripheral magnesium indices were determined and neurophysiological assessments were conducted to evaluate the occurrence of neuromuscular hyperexcitability in both adults and children suffering from migraine and tension-type headache. Positive electromyographic ischemic tests appeared to be associated with reduced Mg^{++} levels, particularly in red blood cells, in both adult and young migraine patients but not in tension-type headache patients [20, 21].

Several clinical symptoms, other than headache, have been associated with NHS. They concern the neuromuscular, respiratory and cardiovascular systems and also include neuropsychological disturbances [14, 22]. These symptoms have never been investigated in headache patients.

In the present research we extended our study to other groups of adult headache patients (migraineurs and patients with tension-type headache) in interictal periods. We measured neuromuscular hyperexcitability peripheral biochemical indices of Mg^{++} status and the occurrence of clinical symptomatology associated with NHS, in order to confirm the relationship between neuromuscular hyperexcitability and reduced Mg^{++} levels and to determine which clinical symptoms associated with the NHS recur most often.

Materials and methods

Patients

A total of 76 consecutive out-patients, attending the Headache Center of the Neuroscience Department of the University of Perugia, consented to participate in the study. In particular, 36 patients (30 women; mean age, 37.8 years; SD, 7.3 years) had migraine without aura (MwoA), 18 patients (14 women; mean age, 33.0 years; SD, 9.3 years) had episodic tension-type headache (ETTH) and 22 patients (19 women; mean age, 37.3 years; SD, 10.3 years) had chronic tension-type headache (CTTH). The clinical diagnosis was made according to the criteria laid down by the 1988 classification of the International Headache Society [23]. All 76 patients were assessed in the interictal period. None of the patients had taken any drugs for 5 days (ETTH or CTTH) or 10 days (MwoA) before blood sampling and neurophysiological evaluation. The patients did not suffer from other diseases (e.g. diabetes, kidney failure) nor had they been treated with drugs that interfere with Mg^{++} nutritional status (e.g. diuretics, antihypertensive agents).

Ischemic test for neuromuscular hyperexcitability

We made electromyographical recordings (Mystro MS6, Medelec, Surrey, UK) of the first interosseous muscle on the right arm, to test for neuromuscular hyperexcitability during the interictal period [22]. Subjects were asked to lie comfortably on a bed in a silent room, shielded from electrical interference. A sphygmomanometer placed on each subject's arm was inflated to a pressure of 270 mmHg. This pressure level was maintained for 10 minutes. From the fifth minute of ischemia and during the five minutes after deflation of the sphygmomanometer, the subject hyperventilated for 15 seconds each minute. Electrical activity in the muscle was recorded with a concentric needle electrode, during the period of ischemia and in the five minutes after the blood flow returned to normal. The EMG test was done blindly with respect to the clinical status of the subjects. The spontaneous electrical activity recorded was photographed on photosensitive paper. The test was considered positive (abnormal) when, after the strap of the sphygmomanometer had been loosened, spontaneous motor unit discharges in sequences of triplets and multiplets were observed for five minutes. The test was considered negative (normal) when a silent electromyographical trace was recorded after the strap of the sphygmomanometer was loosened with evidence of spontaneous electrical activity.

Blood sample collection and preparation

Before the electromyographical examination was carried out, blood samples were drawn to determine the Mg^{++} levels in serum and in red and mononuclear blood cells. In particular, for the determination of Mg^{++} content in serum, 5 ml blood was collected from the antecubital vein in glass tubes and centrifuged at 3000 rpm for 10 minutes to obtain serum specimens. With regard to the determination of Mg^{++} levels in red blood cells, plasma was eliminated from 10 ml heparinized blood samples by centrifugation, the buffy coat was removed, and the erythrocytes were washed in choline-Ringer's solution. Samples were then stored at -20° C and all processed together at the end of the protocol.

Intra- and extracellular Mg^{++} levels and routine laboratory analyses

The Mg^{++} levels in serum and red blood cells were determined by atomic absorption spectrophotometry. Standards were prepared from tritisol Mg^{++} standard solution (Carlo Erba, Milan, Italy, catalog no. 458891). An atomic spectrophotometer (Perkin Elmer 300) with a single-slot burner and a mixture of air and acetylene were used. The determination of Mg^{++} levels in mononuclear cells was performed following the method of Elin and Hosseini [24]. The mean values of Mg^{++} considered to be normal in our laboratory (determined from a group of 40 age-matched healthy subjects) were 1.00 (2 SD, 0.19) mmol/l for serum, 2.17 (2 SD, 0.10) mmol/l for red blood cells and 10.6 (2 SD, 3.4) mg/g DNA for mononuclear cells. Normal values were obtained from a group of 40 age-matched (age range: 18–45 years) apparently healthy subjects (20 males and 20 females) with no systemic or nervous system diseases, from personnel of our institute, medical and technical staff, and medical students. We used a value of 2 SD below the mean normal Mg^{++} concentration to define a cut-off point to distinguish abnormal Mg^{++} levels in headache patients. The cut-off points for Mg^{++} in serum, red blood cells and mononuclear cells were, therefore, 0.81 mmol/l, 2.07 mmol/l and 7.2 mg/g DNA, respectively.

Routine laboratory analyses included hematocrit, blood urea nitrogen, creatinine, glycemia, serum glutamic oxaloacetic transaminase, serum glutamic pyruvic transferase, total protein and serum levels of Na^{+} K^{+}, Ca^{++}. In all patients these laboratory tests were normal.

Clinical assessment

All patients examined were asked to compile a questionnaire concerning the occurrence of the following symptoms in the previous two

months: asthenia, face flushing, extrasystoles, aerophagia, colitis, sexual disturbances, dysmenorrhea, hyperhydrosis, Raynaud's phenomenon in the hands, muscle cramps, sensation of lumps in the throat, blepharospasm, anxiety, depression, fasciculations and paresthesiae.

Statistical analysis

The statistical analysis consisted of ANOVA (with type I sum of squares to test for all effects and significance of interaction), Fisher's and chi-squared tests, performed using SAS procedures. Values of $p<0.05$ were considered to be statistically significant.

Results

The EMG test for neuromuscular hyperexcitability was performed on all patients and was well tolerated. During the period of ischemia the electromyographical recordings of all patients indicated no electrical activity; when the strap was loosened, neuromuscular hyperexcitability was observed in 33 (91.7%) migraineurs, in 5 (27.7%) ETTH patients and in 3 (13.6%) CTTH patients. The electrical activity recorded from a patient affected by MwoA is shown in Fig. 1.

The Mg^{++} levels in red blood cells were significantly less (ANOVA, $p<0.001$) in patients affected by migraine than in the other groups (Table 1). Significantly lower levels of Mg^{++}, although to a lesser extent, were also found in serum and mononuclear cells in migraine patients without aura ($p<0.05$ and $p<0.02$, respectively). By ANOVA, the variables of age (F=2.24, p=NS), Mg^{++} concentration in mononuclear cells (F=1.98, p=NS) and Mg^{++} concentration in serum (F=0.98, p=NS) were not associated with the headache diagnosis, whereas the concentration of Mg^{++} in red blood cells was related to the headache diagnosis, with decreased levels in the group of patients affected by migraine without aura (F=4.02, $p<0.02$).

Using the cut-off point for abnormal Mg^{++} concentrations, defined as a value of 2 SD below the mean normal value, we determined the occurrence of abnormally low Mg^{++} status in the 3 patient groups (Table 2). Abnormally low Mg^{++} status was found in the majority of patients in all groups. Regarding Mg^{++} in red blood cells, a significantly greater percentage of abnormal Mg^{++} status was found among migraineurs (91.7%) compared to patients with episodic or chronic tension-type headache (66.7% and 59.1%, respectively, $p<0.01$ by χ^2).

The frequency of neuromuscular hyperexcitability in migraine patients with abnormally low Mg^{++} levels is reported in Table 3.

All patients with low Mg^{++} status in serum had a positive ischemic test as did the majority of patients with low Mg^{++} in red blood cells and in mononuclear cells (93.9% and 96.4%, respectively).

Figure 2 shows, the frequency of symptoms in the three groups. Of interest is the high percentage of MwoA patients with fasciculations (91.7%), asthenia (91.7%), ble-

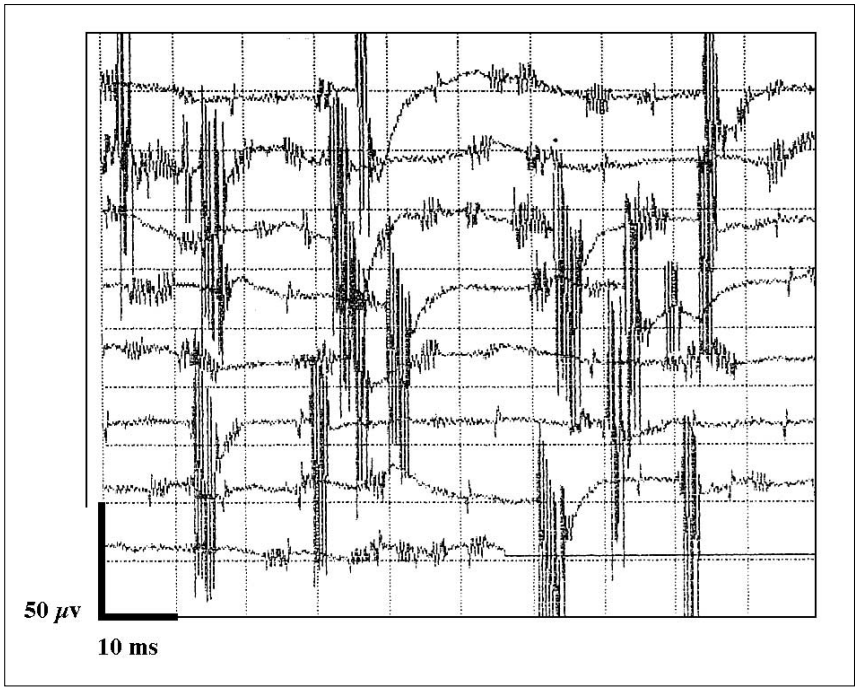

Fig. 1 Typical abnormal recording of a patient with migraine without aura and with neuromuscular hyperexcitability. Recording began 5 min after stopping ischemia. Shown are 5 traces of 100 ms each. Sequences of multiplets are apparent

Table 1 Mg^{++} concentrations in serum, red blood cells and mononuclear cells of patients with MwoA, ETTH, or CTTH. Values are mean (SE)

	MwoA	ETTH	CTTH	Normal values[a]
Serum, mM	0.85 (0.13)	0.91 (0.14)*	0.96 (0.23)*	1.03 (0.19)[4]*
Red blood cells, mM	2.02 (0.32)	2.14 (0.23)[2]*	2.13 (0.37)[3]*	2.17 (0.10)[2]*
Mononuclear cells, mg/g DNA	7.14 (1.53)	9.33 (1.34)[4]*	9.29 (1.59)[5]*	10.63 (1.89)[2]*

[a] as defined by our laboratory
* $p<0.05$; [2]* $p<0.001$; [3]* $p<0.002$; [4]* $p<0.04$; [5]* $p<0.03$ vs. MwoA

Table 2 Frequency and percentage of patients with abnormally low Mg^{++} status, defined as a Mg^{++} more than 2 SD below the normal value

	Serum Mg^{++}	Red blood cells Mg^{++}	Mononuclear cells Mg^{++}
MWA, n=36	24 (66.7)	33 (91.7)	28 (77.8)
ETTH, n=18	12 (66.7)	12 (66.7)	13 (72.2)
CTTH, n=22	12 (54.5)	13 (59.1)	17 (77.3)
Total, n=76	48 (63.2)	58 (76.3)	58 (76.3)

Table 3 Percentage of low magnesium levels in migraine patients with neuromuscular excitability (n=33)

	Abnormal low Mg^{++} levels	Percentage with respect to migraine patients with positive ischemic tests (n=33)
Serum Mg^{++}	n=24	72.2%
Red blood mononuclear cell Mg^{++}	n=31	93.9%
Mononuclear cell Mg^{++}	n=27	81.1%

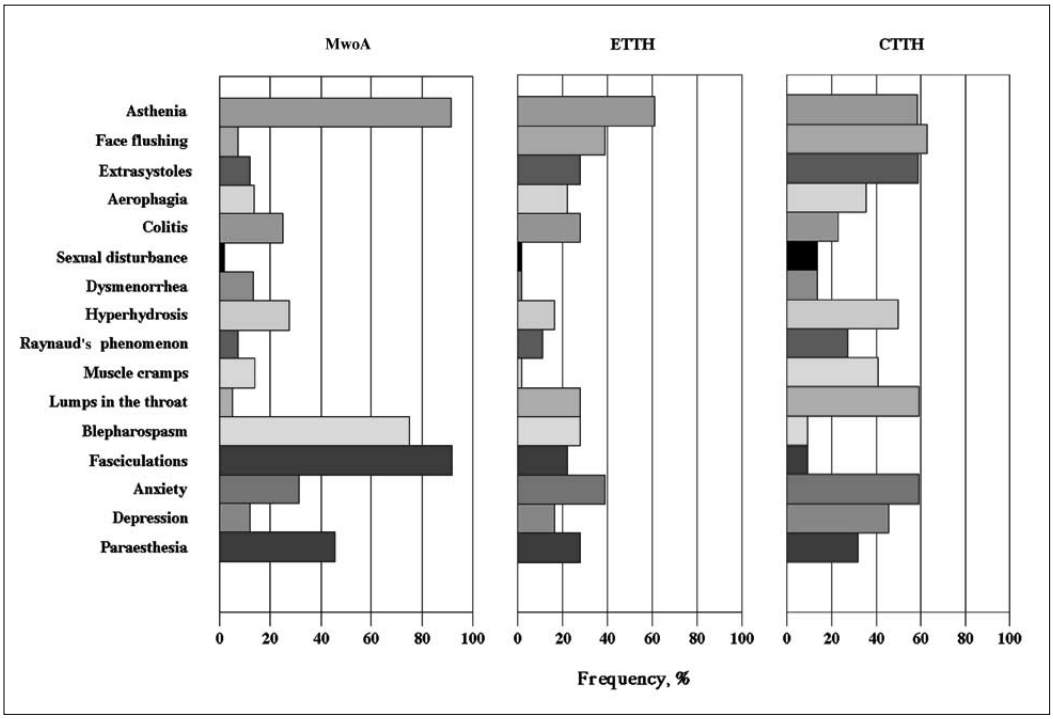

Fig. 2 Frequency of clinical symptoms reported by 36 patients with MwoA, 18 patients with ETTH, and 22 patients with CTTH

pharospasm (75.8%) and paraesthesiae (47.2%). In this group anxiety and depression occurred in 30.6% and 11.1% of the cases, respectively. The symptom most frequently reported by episodic tension-type headache patients was asthenia (61.1%). Anxiety was present in 38.9% of patients with episodic tension-type headache, depression in 16.7%. Chronic tension-type headache patients more often reported face flushing (63.6%) extrasystoles (59.1%), asthenia (59.1%), anxiety (59.1%), sensation of lump in the throat (59.1%), hyperhydrosis (50.0%) and depression (45.5%).

Among the clinical symptoms reported, reduced Mg^{++} concentration in mononuclear cells was not related to any symptoms, whereas low levels of Mg^{++} in serum were associated with the presence of blepharospasm ($F=4.45$, $p<0.03$) and fasciculations ($F=4.66$, $p<0.01$) in patients with MwoA. Reduced Mg^{++} concentration in red blood cells of patients with MwoA was related to the occurrence of asthenia ($F=24.5$; $p<0.00l$) and extrasystoles ($F=18.9$, $p<0.001$). In the same patient group, low Mg^{++} levels were also associated with the presence of paraesthesiae ($F=32.6$, $p<0.00l$); blepharospasm ($F=45.4$, $p<0.00l$) and fasciculations ($F=36.2$, $p<0.001$).

No other symptoms, including anxiety and depression, were associated with variations in the levels of magnesium in migraineurs. There was no association between symptomatology and Mg^{++} levels in patients with episodic or chronic tension-type headache.

Discussion

In the present study, electromyographical ischemic tests for neuromuscular hyperexcitability were positive in 91.7% migraineurs, but only in 27.7% of episodic tension-type headache patients and in 13.6% of chronic-tension headache patients, all assessed in interictal periods. This indicates the greater occurrence of a state of neuromuscular hyperexcitability in migraineurs. Migraineurs also had lower Mg^{++} levels in serum and, to a greater extent, in red blood and mononuclear cells, compared to other headache patients. In particular, a high percentage of migraineurs (91.7%) showed Mg^{++} levels below the norm in red blood cells; this finding concurs with the results of previous research by us and other groups [5–9, 20, 21]. This marker seems to be a more sensitive index of short-term variations of magnesium than mononuclear cell Mg^{++} which, although considered a better indicator of the entity of the body's Mg^{++} storage, does not appear to be related to the modifications in the functional state of some systems, such as the nervous system, and muscle [25].

The majority of migraine patients (93.9%) with neuromuscular excitability had red blood cell levels of magnesium below the norm. The percentages of migraine patients with positive ischemic tests and mononuclear cells and serum Mg^{++} levels below the cut-off point defining the normalcy were lower (81.1% and 72.2%, respectively). This finding further supports the superiority of the determination of Mg^{++} levels in erythrocytes not only as a sensitive indicator of magnesium nutritional status, but also as an indirect marker of muscular excitability, compared to the magnesium concentration in other body compartments such as mononuclear cells and particularly serum [18, 21, 22, 26, 27].

In the wide symptomatological spectrum related to NHS, several clinical symptoms were associated with reduced indices of Mg^{++} status in migraineurs. In particular, low Mg^{++} levels in serum were significantly related to the presence of blepharospasm and fasciculations in MwoA patients. Reduced Mg^{++} concentration in red blood cells were significantly associated with the occurrence of asthenia, paraesthesiae, blepharospasm and fasciculations in the same patients. Thus, these symptoms regarding the neuromuscular apparatus should be checked in MwoA patients as further evidence of NHS.

As far as mood disturbances are concerned, it should be pointed out that neuromuscular hyperexcitability, demonstrated by the positive results of EMG ischemic tests, was associated in previous research with high levels of anxiety and/or stress [5, 28]. Therefore we investigated in our patient groups the occurrence of anxiety and depression. Anxiety and depression occurred more often in patients with tension-type headache, particularly those with the chronic form. However, reduced Mg^{++} levels did not contribute to the presence of these neuropsychological disturbances, because the difference in the levels of Mg^{++} status between patients with and without anxiety or depression in migraineurs and tension-type headache patients was minimal.

In conclusion, this study confirms the validity of EMG ischemic testing in discriminating a condition of neuromuscular hyperexcitability which is associated with reduced levels of magnesium, particularly in red blood cells. This reduction not only intervenes in determining and precipitating migraine attacks, but also contributes to the clinical symptoms referred to by migraine patients, particularly those related to the neuromuscular system. EMG ischemic tests, together with the determination of intracellular magnesium levels, especially in red blood cells, should be particularly recommended for migraine patients with clear evidence of neuromuscular hyperexcitability suggested by the occurrence of symptoms regarding the neuromuscular apparatus. Such a diagnostic approach would lead to better patient management and to pursuing magnesium therapy selectively, i.e. only in patients with documented deficiency who could benefit from it with a significant improvement in terms of reduced frequency and intensity of attacks.

In light of recent genetic theories, mutations in postsynaptic or particular aplotypes of Ca^{++} channels of P/Q type could provide the basis for neural instability and excitability in migraine [29–31]. As an alternative hypothesis, altered functional status may favor the onset of cortical spreading depression, which is believed to initiate migraine attacks. Considering the recurrent nature of migraine, we hypothesize that the functional effects on central neuronal activity may not be due per se to a presumed but yet unidentified mutation, but that some unknown factor may intervene in precipitating migraine attacks by switching the abnormal (perhaps genetically determined) channel off and on. Reduced magnesium levels both at the central and peripheral levels could contribute to reducing the threshold of this channel to be switched on.

P/Q Ca^{++} channels have also been found at neuromuscular junctions where they control stimulus-induced acetylcholine release. Impairment of neuromuscular transmission, which may be due to a dysfunctioning P/Q Ca^{++} channel in motor nerve endings, has been suggested by Ambrosini et al. [32]. The authors performed single-fiber electromyography in migraineurs and demonstrated abnormal jitter or impulse blocking related to the severity of the clinical spectrum of migraine. Minor variations in electromyographic recordings were in fact observed in patients with mild migraine without aura whereas the most relevant changes emerged in the most severe forms of migraine with prolonged aura [33]. The genetic basis of this disturbance is at the moment under investigation and the results are awaited [34].

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