Interictal alterations of thalamic metabolic concentration ratios in migraine without aura detected by proton magnetic resonance spectroscopy

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Abstract  Aim of the work: To detect interictal changes of the metabolic concentration ratios in the thalami of patients with migraine without aura by using 1H-MRS.

Materials and methods: Twenty-two patients of migraine without aura were enrolled in this study in addition to 10 healthy controls. Patients were further divided into 2 subgroups: 12 with left-sided migraine and 10 with right-sided migraine. Patients were imaged interictally with multivoxel 1H-MRS for measuring the NAA/Cho, NAA/Cr, Cho/Cr, MI/NAA and Lac/NAA ratios in both thalami.

Results: Each of the mean NAA/Cho and NAA/Cr ratios was significantly decreased in patients compared to controls, and also on the contralateral side compared to the side of migraine in each subgroup of patients. In contrary, no significant difference was found in Cho/Cr, MI/NAA and Lac/NAA ratios between patients and controls, as well as on comparing both sides in each subgroup of patients. Furthermore, increased duration of illness and frequency of attacks were significantly associated with decreased NAA/Cho and NAA/Cr ratios as well as increased MI/NAA and Lac/NAA ratios.

Conclusion: Migraine without aura is associated with thalamic interictal changes in the mean metabolite concentration ratios, detected with multivoxel 1H-MRS.

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1. Introduction

Migraine is the most common neurological disease that affects women three times more than men (1). It is a chronic disabling primary headache disorder that affects about 15% of the population (2). It is characterized by recurrent episodes of pulsating or throbbing unilateral headache lasting from 4 to 72 h, and associated symptoms such as photophobia, phonophobia, nausea and/or vomiting. Migraine has an important genetic background but its exact aetiology is still unknown (3). Environmental factors are also clearly involved in precipitating migraine (4). It can be divided into two major subtypes: migraine without aura (common migraine) and migraine with aura (classic migraine). Migraine without aura is the most common, accounting for more than 80% of all migraines (5).

Advances in understanding how migraine alters brain function, structure or neurochemistry has the potential to renovate our understanding of the migraine brain and the progression to a chronic disease, thereby providing a base for new therapeutic approaches (6). The thalamus is a very important sensory station of pain conduction and processing of pain impulses through the brain. By using positron emission tomography, migraine without aura has been shown to produce activation in the thalamus during the headache phase of a migraine attack (7).

Magnetic resonance spectroscopy (MRS) is a well-established technique for the in vivo studies of biological systems. It can be used to explore the cerebral neurochemical changes in migraine patients that lie behind migraine susceptibility or that could drive the progress of acute migraine to a chronic illness following repeated attacks. Detection of the metabolic changes, may find a new diagnostic method for this disease, for we usually diagnose migraine from the history of illness and the symptoms. Also it can be used as a tool for effect analysis of the treatment (8).

We assumed interictal metabolic changes occurring in the thalamus of patients with migraine without aura are due to their increased sensitivity to pain. So, the aim of this work was to detect interictal changes of the metabolic concentration ratios in the thalami of patients with migraine without aura by using 1H-MRS.

2. Materials and methods

2.1. Study participants

Twenty-two migraineurs without aura (14 females and 8 males), in addition to 10 healthy age- and sex-matched controls (6 females and 4 males) with no history of neurological disease, mental disorder, or head trauma were the subjects of this study which was performed in the period from January 2012 to April 2013. The patients were recruited from the neurology department of our institution. The diagnosis of migraine without aura was carried out according to the definition of the Headache Classification Committee of the International Headache Society (5). Patients were further classified into two subgroups: 12 patients with left-sided migraine (migraine primarily affecting the left side of head) and 10 patients with right-sided migraine (migraine primarily affecting the right side of head). All patients were evaluated interictally (attack-free periods) at least one month from the last attack.

Patients with connective tissue disorders, coagulopathy or diabetes mellitus were excluded from this study. Also, none of the examined patients were taking prophylactic medications for their attacks at the study time. Additionally, brain magnetic resonance imaging (MRI) and electroencephalography (EEG) were unremarkable in all patients. An official permission to carry out the study was obtained from the responsible authorities and the administrative staff. Patients consent to participate in the study was obtained.

2.2. Imaging procedures

Both patients and controls were examined initially with MRI and then with multi-voxel 1H-MRS by using 1.5-Tesla MR unit (Signa Horizon SR 120; General Electric Medical Systems, Milwaukee, WI, USA) with a standard quadrature head coil. The MRI studies comprised the following sequences: multiplanar axial T1-weighted (T1WFSE) fast spin-echo with repetition time/echo time/number of excitations (TR/TE/NEX) of (500/14/2), multiplanar axial T2-weighted fast spin-echo (T2WFSE) with TR/TE/NEX of 4000/126/2, and axial fluid-attenuated inversion recovery (FLAIR) with TR/TE/NEX of 8000/142/1 and inversion time (TI) of 2200 milliseconds (ms).

2.3. 1H-MRS protocol

The 1H-MRS was performed via using two-dimensional multivoxel long-echo (TE of 144 ms) point-resolved spatially localized spectroscopy (PRESS) to assess the relative concentrations of metabolites with biological importance including: N-acetylaspartate (NAA), choline (Cho), creatine (Cr), lactate (Lac) and myo-inositol (MI) in both thalami of patients and control groups. In all patients, we used axial FLAIR images to locate the multivoxels for the 1H-MRS studies using a spin-echo (SE) mode sequence. The localized voxels of interests (VOIs) were located and distributed within different parts of both thalami as regions of interest (ROIs). Water resonance suppression was optimally achieved by using the chemical shift selective water suppression (CHESS) technique. In all patients, the used parameters were TR of 1000 ms, TE of 144 ms and 35 ms, FOV of 24 cm, 18 × 18 phase encoding matrices, 2.0 cm section thickness, 2500 Hz spectral width and 2048 data points. The MRS scan was initiated if the line width reported by the prescan process was less than 8 Hz. The MR spectra were obtained with a long TE of 144 ms as well as an additional short TE of 35 ms which was utilized to confirm the phase inversion associated with J-coupled metabolites of lactate, and amino acids but not of lipids, which may be helpful to discriminate lactate signals from lipid signals. The acquisition time for each sequence was 5 min and 54 s. The spectral data post-processing were carried out through the workstation spectroscopic analysis package using semi-automated software (Functool, Version 2.33, GE Medical Systems, Milwaukee, WI, USA). The spectra were displayed as grids of nominal voxel size 8.0 × 10 × 10 mm and overlaid on the FLAIR MR image used to plan the study.

The main metabolite resonances were limited to 2.02 ppm (ppm) for NAA, 3.02 ppm for Cr, 3.20 ppm for Cho, 1.33 ppm for Lac doublet, 3.56 for myo-inositol (MI) and 0.9–1.3 ppm for Lipids (lip). As a result of difficulties in calculation of the absolute metabolite concentrations, their relative
The results obtained were compared using the Chi-square test. Quantitative data were expressed in number (No) and percent (%). The means of these peak ratios were then calculated in the side of migraine and compared with those of the contralateral side, in each subgroup of patients. Also, they were calculated in the right thalamus and compared with the left thalamus, in the control group. Additionally, the mean peak ratios of both thalami in all patients were calculated and compared with those of the control group.

2.4. Statistical analysis

The calculated data were revised, coded, tabulated and statistically analysed using the SPSS for Windows version 18.0 software package (SPSS Inc, Chicago, IL). Quantitative data were expressed as mean ± standard deviation (SD), while qualitative data were expressed in number (No) and percent (%). The results obtained were compared using the Chi-square test (Chi) and the paired t-test (t). P-value lesser than 0.05 was considered statistically significant.

3. Results

Table 1 illustrates the demographic and clinical data of patients and control group. Regarding age and sex, there were no significant differences between the patients and control group (P > 0.05). The mean metabolite concentration ratios showed no significant differences between the two thalami in the control group (Table 2). However, the means of NAA/Cho and NAA/Cr ratios were significantly decreased in the thalami of the contralateral side of migraine when compared to the side of migraine in both left-sided migraine patients (P < 0.001 in each of NAA/Cho and NAA/Cr ratios) and right-sided migraine patients (P < 0.05 for each of NAA/Cho and NAA/Cr ratios). On the other hand, no significant difference was found in the means of Cho/Cr, MI/NAA and Lac/NAA ratios in the thalamus at the side of migraine compared with the contralateral side in both left-sided migraine patients (P = 0.53 for Cho/Cr ratio, P = 0.36 for MI/NAA ratio and P = 0.90 for Lac/NAA ratio) and right-sided migraine patients (P = 0.36 for Cho/Cr ratio, P = 0.90 for MI/NAA ratio and P = 0.79 for Lac/NAA ratio) (Table 3).

On studying both thalami collectively, each of the mean NAA/Cho and NAA/Cr ratios were significantly decreased in patients when compared to those of the controls (P = 0.003 and 0.027, respectively). On the other hand, the mean Cho/Cr, MI/NAA and Lac/NAA ratios showed no significant differences between patients and controls (P = 0.83, 0.10, and 0.09, respectively) (Table 4).

Statistical analysis of correlative data of our patients of migraine without aura demonstrated that increased duration of illness and frequency of attacks were significantly associated with decreased NAA/Cho ratio (r = −0.56, P = 0.004 and r = −0.65, P = 0.002, respectively), decreased NAA/Cr ratio (r = −0.67, P = 0.001 and r = −0.75, P = 0.001, respectively), increased MI/NAA ratio (r = 0.69, P < 0.001 and r = 0.58, P = 0.005, respectively) and increased Lac/NAA ratio (r = 0.76, P < 0.001 and r = 0.64, P = 0.003, respectively). Additionally, increased duration of illness and frequency of attacks were not significantly correlated with the Cho/Cr ratio (r = 0.13, P = 0.58 and r = 0.30, P = 0.17, respectively) (Table 5). Moreover, the family history was not significantly correlated with the changes in thalamic metabolite concentration ratios in patients with migraine without aura.

4. Cases

The figures (1 to 5) demonstrate a sample of selected cases of our study, each figure outlines one case.

A thirty year old healthy control male subject. Brain axial non-contrast T1WI (A) and axial T2WI (B) show apparently normal brain with a normal signal intensity of the brain parenchyma and good grey/white matter differentiation. The 1H-MRS multivoxel picture (C) and MRS spectrum of the right (D) and left (E) thalami at a long TE of 144 ms, in addition to 1H-MRS multivoxel picture (F) and MRS spectrum of the right (G) and left (H) thalami at a short TE of 35 ms demonstrate normal average NAA/Cho, NAA/Cr, Cho/Cr, Lac/NAA and MI/NAA peak metabolite concentration ratios of both thalami. In the right thalamus (D and G), the NAA/Cho is 1.36, NAA/Cr is 1.73, Cho/Cr is 1.27, Lac/NAA is 0.17 and MI/NAA is 0.57. In the left thalamus (E and H), the NAA/Cho is 1.33, NAA/Cr is 1.56, Cho/Cr is 1.17, Lac/NAA is 0.18 and MI/NAA is 0.38 (Fig. 1).

A thirty-four year old female patient with left-sided migraine without aura examined in the interictal period. Brain axial non-contrast T1WI (A) and axial T2WI (B) show apparently normal brain with a normal signal intensity of the brain parenchyma and good grey/white matter differentiation. The 1H-MRS multivoxel picture (C) and MRS spectrum of the right (D) and left (E) thalami at TE of 144 ms, in addition to 1H-MRS multivoxel picture (F) and MRS spectrum of the right (G) and left (H) thalami at TE of 35 ms reveal reduction of the NAA/Cr ratio of the right thalamus (E and H), they are: NAA/Cho = 0.91, NAA/Cr = 1.39, Cho/Cr = 1.53, Lac/NAA = 0.18 and MI/NAA = 0.51, (Fig. 2).

A thirty-seven year old female patient with right-sided migraine without aura examined in the interictal period. Brain axial non-contrast T1WI (A) and axial T2WI (B) show apparently normal brain with a normal signal intensity of the brain parenchyma and good grey/white matter differentiation. The 1H-MRS multivoxel picture (C), and MRS spectrum of the right (D) and left (E) thalami at TE of 144 ms, in addition to 1H-MRS multivoxel picture (F), and MRS spectrum of the right (G) and left (H) thalami at TE of 35 ms reveal reduction of the NAA/Cr and NAA/Cho ratios in the left thalamus.
(contralateral side of migraine) when compared to the right thalamus (side of migraine). However, the concentration ratios of Cho/Cr, Lac/NAA and MI/NAA show no significant difference when comparing the side of migraine with the contralateral side and when comparing those of the patients with normal controls. The peak concentration ratios of the contralateral left thalamus (E and H), are: NAA/Cho = 0.99, NAA/Cr = 1.27, Cho/Cr = 1.28, Lac/NAA = 0.25 and MI/NAA = 0.51, while; in the ipsilateral right thalamus (D and G), they are: NAA/Cho = 1.24, NAA/Cr = 1.76, Cho/Cr = 1.42, Lac/NAA = 0.24 and MI/NAA = 0.46, (Fig. 3).

### Table 1  Demographic and clinical data of the examined patients and control group.

<table>
<thead>
<tr>
<th>Data</th>
<th>Patients (n = 22)</th>
<th>Control (n = 10)</th>
<th>Test statistics</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Females: males</td>
<td>1.75:1</td>
<td>1.5:1</td>
<td></td>
<td>0.843</td>
</tr>
<tr>
<td>Age in years (mean ± SD)</td>
<td>29.7 ± 3.9</td>
<td>26.8 ± 5.1</td>
<td></td>
<td>0.086</td>
</tr>
<tr>
<td>Positive family history of migraine [no (%)]</td>
<td>15 (68.2%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration of illness in years (Mean ± SD)</td>
<td>12.9 ± 7.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frequency of attacks/month (Mean ± SD)</td>
<td>3.5 ± 0.9</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Side of migraine**
- Right side [no. (%)]: 10 (45.5%)
- Left side [no. (%)]: 12 (54.5%)

### Table 2  Comparison of the metabolic ratios between the two thalami in the control group (n = 10).

<table>
<thead>
<tr>
<th>Metabolic ratio</th>
<th>Right thalamus</th>
<th>Left thalamus</th>
<th>Paired t-test</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAA/Cho</td>
<td>1.73 ± 0.24</td>
<td>1.65 ± 0.29</td>
<td>0.295</td>
<td>0.774</td>
</tr>
<tr>
<td>NAA/Cr</td>
<td>1.81 ± 0.37</td>
<td>1.79 ± 0.32</td>
<td>0.021</td>
<td>0.984</td>
</tr>
<tr>
<td>Cho/Cr</td>
<td>1.41 ± 0.36</td>
<td>1.40 ± 0.25</td>
<td>0.988</td>
<td>0.244</td>
</tr>
<tr>
<td>MI/NAA</td>
<td>0.40 ± 0.11</td>
<td>0.40 ± 0.15</td>
<td>0.595</td>
<td>0.566</td>
</tr>
<tr>
<td>Lac/NAA</td>
<td>0.29 ± 0.11</td>
<td>0.30 ± 0.12</td>
<td>0.714</td>
<td>0.493</td>
</tr>
</tbody>
</table>

### Table 3  Comparison of the metabolic ratios between the two thalami in the patients subgroup (n = 22).

<table>
<thead>
<tr>
<th>Metabolic ratio</th>
<th>Left thalamus (side of migraine)</th>
<th>Right thalamus (contralateral side)</th>
<th>Paired t-test</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAA/Cho</td>
<td>1.22 ± 0.25</td>
<td>0.91 ± 0.22</td>
<td>5.154</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>NAA/Cr</td>
<td>1.62 ± 0.32</td>
<td>1.31 ± 0.31</td>
<td>6.211</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Cho/Cr</td>
<td>1.36 ± 0.37</td>
<td>1.35 ± 0.33</td>
<td>0.645</td>
<td>0.532</td>
</tr>
<tr>
<td>MI/NAA</td>
<td>0.46 ± 0.13</td>
<td>0.50 ± 0.18</td>
<td>0.959</td>
<td>0.358</td>
</tr>
<tr>
<td>Lac/NAA</td>
<td>0.32 ± 0.22</td>
<td>0.31 ± 0.20</td>
<td>0.123</td>
<td>0.904</td>
</tr>
</tbody>
</table>

**Left-sided migraine patients (n = 12)**
- NAA/Cho: 1.22 ± 0.25
- NAA/Cr: 1.62 ± 0.32
- Cho/Cr: 1.36 ± 0.37
- MI/NAA: 0.46 ± 0.13
- Lac/NAA: 0.32 ± 0.22

**Right-sided migraine patients (n = 10)**
- NAA/Cho: 1.35 ± 0.31
- NAA/Cr: 1.71 ± 0.45
- Cho/Cr: 1.43 ± 0.38
- MI/NAA: 0.44 ± 0.18
- Lac/NAA: 0.27 ± 0.11

*: Significant

### Table 4  Comparison of the mean metabolic ratios between patients and controls.

<table>
<thead>
<tr>
<th>Metabolic ratio</th>
<th>Patients (n = 22)</th>
<th>Controls (n = 10)</th>
<th>Paired t-test</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAA/Cho</td>
<td>1.19 ± 0.32</td>
<td>1.69 ± 0.32</td>
<td>4.132</td>
<td>0.0003*</td>
</tr>
<tr>
<td>NAA/Cr</td>
<td>1.47 ± 0.42</td>
<td>1.85 ± 0.45</td>
<td>2.311</td>
<td>0.027*</td>
</tr>
<tr>
<td>Cho/Cr</td>
<td>1.37 ± 0.38</td>
<td>1.40 ± 0.32</td>
<td>0.218</td>
<td>0.828</td>
</tr>
<tr>
<td>MI/NAA</td>
<td>0.48 ± 0.19</td>
<td>0.37 ± 0.13</td>
<td>1.679</td>
<td>0.103</td>
</tr>
<tr>
<td>Lac/NAA</td>
<td>0.39 ± 0.11</td>
<td>0.32 ± 0.10</td>
<td>1.734</td>
<td>0.093</td>
</tr>
</tbody>
</table>

*: Significant
A forty-two year old female patient with left-sided migraine without aura examined in the interictal period. Axial non-contrast T1WI (A) and axial T2WI (B) show apparently a normal brain with a normal signal intensity of the brain parenchyma and good grey/white matter differentiation. The 1H-MRS multivoxel picture (C), and MRS spectrum of the right (D) and left (E) thalami at TE of 144 ms, in addition to 1H-MRS multivoxel picture (F), and MRS spectrum of the right (G) and left (H) thalami at TE of 35 ms reveal reduction of the NAA/Cr and NAA/Cho ratios in the right thalamus (contralateral side of migraine) when compared to the left thalamus (side of migraine). However, the concentration ratios of Cho/Cr, Lac/NAA and MI/NAA show no significant difference in comparing the side of migraine with contralateral side as well as in comparing those of the patients with normal controls. The peak concentration ratios in the contralateral right thalamus (D and G) are: NAA/Cho = 0.93, NAA/Cr = 1.47, Cho/Cr = 1.42, Lac/NAA = 0.18 and MI/NAA = 0.57, while; in the ipsilateral left thalamus (E and H), they are: NAA/Cho = 0.87, NAA/Cr = 1.65, Cho/Cr = 1.33, Lac/NAA = 0.17 and MI/NAA = 0.47, (Fig. 4).

A twenty-five year old male patient with left-sided migraine without aura examined in the interictal period. The 1H-MRS multivoxel picture (A), and MRS spectrum of the right (B) and left (C) thalami at TE of 144 ms, in addition to 1H-MRS multivoxel picture (D), and MRS spectrum of the right (E) and left (F) thalami at TE of 35 ms reveal reduction of the NAA/Cr and NAA/Cho ratios in the right thalamus (contralateral side of migraine) when compared to the left thalamus (side of migraine). However, the concentration ratios of Cho/Cr, Lac/NAA and MI/NAA show no significant difference in comparing the side of migraine with contralateral side as well as in comparing those of the patients with normal controls. The peak concentration ratios in the contralateral right thalamus (D and G) are: NAA/Cho = 0.87, NAA/Cr = 1.22, Cho/Cr = 1.40, Lac/NAA = 0.24 and MI/NAA = 0.80, while; in the ipsilateral left thalamus (E and F), they are: NAA/Cho = 0.90, NAA/Cr = 1.47, Cho/Cr = 1.63, Lac/NAA = 0.21 and MI/NAA = 0.55, (Fig. 5).

5. Discussion

Migraine is a neurobiological headache disorder which causes significant individual and societal burden due to pain and environmental sensitivities, resulting in disability and lost productivity (9). Its attack manifests itself from childhood (usually 8–12 years) to old age, with a decline among women during the postmenopausal years (3,9,10). One potential mechanism for changes in neuronal excitability in the migraine brain includes an abnormality in the release of excitatory amino acid neurotransmitters (10) that may predispose patients to attacks or could be a consequence of a chronic migraine state (11).

Neuroimaging has led to advances in the description of migraine mechanisms and in the identification of secondary structural and functional effects of migraine (3). Altered cerebral blood flow and neurotransmitter systems have been identified during and between headache episodes in migraine with and without aura (9). Functional imaging techniques have revolutionized the field of neuroscience research. The 1H-MRS is a non-invasive technique that can detect several essential metabolites that have remarkable biological importance, since their alterations may be indicative of some dysfunctions or illnesses (12,13) and would provide a target for potential interictal therapies that may decrease the severity and/or frequency of migraine and provide a basis for evaluating changes that may take place in the transition to chronic migraine (10).

Table 5

<table>
<thead>
<tr>
<th>Metabolic ratio</th>
<th>Positive family history of migraine</th>
<th>Duration of illness in years</th>
<th>Frequency of attacks/month</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAA/Cho</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>r</td>
<td>0.151</td>
<td>−0.557</td>
<td>−0.647</td>
</tr>
<tr>
<td>P-value</td>
<td>0.125</td>
<td>0.004*</td>
<td>0.0018*</td>
</tr>
<tr>
<td>NAA/Cr</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>r</td>
<td>0.311</td>
<td>−0.667</td>
<td>−0.7547</td>
</tr>
<tr>
<td>P-value</td>
<td>0.159</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Cho/Cr</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>r</td>
<td>0.048</td>
<td>0.125</td>
<td>0.303</td>
</tr>
<tr>
<td>P-value</td>
<td>0.831</td>
<td>0.581</td>
<td>0.171</td>
</tr>
<tr>
<td>MI/NAA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>r</td>
<td>0.374</td>
<td>0.687</td>
<td>0.577</td>
</tr>
<tr>
<td>P-value</td>
<td>0.086</td>
<td>&lt;0.001*</td>
<td>0.0054</td>
</tr>
<tr>
<td>Lac/NAA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>r</td>
<td>0.317</td>
<td>0.758</td>
<td>0.644</td>
</tr>
<tr>
<td>P-value</td>
<td>0.151</td>
<td>&lt;0.001*</td>
<td>0.0025</td>
</tr>
</tbody>
</table>

r = Correlation coefficient.
* Significant.
to which it is made worse by environmental stimuli, such as light. These perceptions give the beginning to understand that the thalamus may be a target for current and future migraine treatments (14).
Fig. 2 (A–H) represents a thirty-four year old female patient with left-sided migraine without aura examined in the interictal period. Brain axial non-contrast T1WI (A) and axial T2WI (B) show apparently a normal brain with a normal signal intensity of the brain parenchyma with good grey/white matter differentiation. The 1H-MRS multivoxel picture (C) and MRS spectrum of the right (D) and left (E) thalami at TE of 144 ms, in addition to 1H-MRS multivoxel picture (F) and MRS spectrum of the right (G) and left (H) thalami at TE of 35 ms reveal reduction of the NAA/Cr and NAA/Cho ratios in the right thalamus (contralateral side of migraine) when compared to the left thalamus (side of migraine). However, the Cho/Cr, Lac/NAA and MI/NAA ratios show no significant difference in comparing the side of migraine with contralateral side as well as in comparing those of the patients with normal controls. The peak concentration ratios in the contralateral thalamus (D and G), are: NAA/Cho = 0.72, NAA/Cr = 1.15, Cho/Cr = 1.58, Lac/NAA = 0.30 and MI/NAA = 0.63, while in the ipsilateral thalamus (E and H), they are: NAA/Cho = 0.91, NAA/Cr = 1.39, Cho/Cr = 1.53, Lac/NAA = 0.18 and MI/NAA = 0.51.
Fig. 3  (A–H) represents a thirty-seven year old female patient with right-sided migraine without aura examined in the interictal period. Axial non-contrast T1WI (A) and axial T2WI (B) show apparently a normal brain with a normal signal intensity of brain parenchyma and good grey/white matter differentiation. The 1H-MRS multivoxel picture (C), and MRS spectrum of the right (D) and left (E) thalami at TE of 144 ms, in addition to 1H-MRS multivoxel picture (F), and MRS spectrum of the right (G) and left (H) thalami at TE of 35 ms reveal reduction of the NAA/Cr and NAA/Cho ratios in the left thalamus (contralateral side of migraine) when compared to the right thalamus (side of migraine). However, the concentration ratios of Cho/Cr, Lac/NAA and MI/NAA show no significant difference when comparing the side of migraine with the contralateral side and when comparing those of the patients with normal controls. The peak concentration ratios of the contralateral left thalamus (E and H), are: NAA/Cho = 0.99, NAA/Cr = 1.27, Cho/Cr = 1.28, Lac/NAA = 0.25 and MI/NAA = 0.51, while; in the ipsilateral right thalamus (D and G), they are: NAA/Cho = 1.24, NAA/Cr = 1.76, Cho/Cr = 1.42, Lac/NAA = 0.24 and MI/NAA = 0.46.
Fig. 4  (A–H) represents a forty-two year old female patient with left-sided migraine without aura examined in the interictal period. Axial non-contrast T1WI (A) and axial T2WI (B) show apparently a normal brain with a normal signal intensity of the brain parenchyma and good grey/white matter differentiation. The 1H-MRS multivoxel picture (C), and MRS spectrum of the right (D) and left (E) thalami at TE of 144 ms, in addition to 1H-MRS multivoxel picture (F), and MRS spectrum of the right (G) and left (H) thalami at TE of 35 ms reveal reduction of the NAA/Cr and NAA/Cho ratios in the right thalamus (contralateral side of migraine) when compared to the left thalamus (side of migraine). However, the concentration ratios of Cho/Cr, Lac/NAA and MI/NAA show no significant difference in comparing the side of migraine with contralateral side as well as in comparing those of the patients with normal controls. The peak concentration ratios in the contralateral right thalamus (D and G) are: NAA/Cho = 1.03, NAA/Cr = 1.46, Cho/Cr = 1.42, Lac/NAA = 0.18 and MI/NAA = 0.57, while; in the ipsilateral left thalamus (E and H), they are: NAA/Cho = 1.24, NAA/Cr = 1.65, Cho/Cr = 1.33, Lac/NAA = 0.17 and MI/NAA = 0.47.
The NAA is considered as a marker of neuronal, and in particular, axonal integrity, and because it is synthesized and located prevalently in neural mitochondria where it seems to be involved in mitochondrial/cytosolic carbon transport, it has been taken as a marker of mitochondrial functioning. Furthermore, some studies(7,15,16) have evaluated the role of NAA in the mitochondrion, suggesting that NAA may act as an acetyl donor for myelin synthesis(15). The synthesis rates of NAA were showed to be highly linear with rates of oxygen consumption and ATP synthesis. When migraine attacks occur, oxygen consumption is changed in neurons of the thalamus, which will be reflected as the concentrations of NAA and Cr (16). Also, Cr is an important indicator of energy metabolism and Cho is a marker of membrane turnover (17).

As far as we are aware, imaging studies specifically designed to explore the issue of laterality of migraine are very seldom (3). Gu et al. (17), revealed no significant difference in the NAA/Cho, NAA/Cr and Cho/Cr ratios between both sides in the control group; while in the patients group, there was a significant decrease of NAA/Cho in the left thalamus irrespective of the side of migraine. They concluded that the left thalamus has higher values of norepinephrine which is correlated.

Fig. 5  (A–F) represents a twenty-five year old male patient with left-sided migraine without aura examined in the interictal period. The 1H-MRS multivoxel picture (A), and MRS spectrum of the right (B) and left (C) thalami at TE of 144 ms, in addition to 1H-MRS multivoxel picture (D), and MRS spectrum of the right (E) and left (F) thalami at TE of 35 ms reveal reduction of the NAA/Cr and NAA/Cho ratios in the right thalamus (contralateral side of migraine) when compared to the left thalamus (side of migraine). However, the concentration ratios of Cho/Cr, Lac/NAA and MI/NAA show no significant difference in comparing the side of migraine with contralateral side as well as in comparing those of the patients with normal controls. The peak concentration ratios of the contralateral right thalamus (B and E) are: NAA/Cho = 0.87, NAA/Cr = 1.22, Cho/Cr = 1.40, Lac/NAA = 0.24 and MI/NAA = 0.80, while; in the ipsilateral left thalamus (C and F), they are: NAA/Cho = 0.90, NAA/Cr = 1.47, Cho/Cr = 1.63, Lac/NAA = 0.21 and MI/ NAA = 0.55.
with oxygen metabolism and when a migraine attack starts, the neurons in the left thalamus could be more vulnerable for oxygen consumption (18). However, they recommended further studies to support these explanations and to confirm their results. In contrary, we found a significant decrease in the NAA/Cho and NAA/Cr ratios at the contralateral side of migraine when compared to the ipsilateral side of migraine in both left-sided ($P < 0.001$ for each of NAA/Cho and NAA/Cr ratios) and right-sided migraine patients. On the other side, there were non-significant differences of thalamic Cho/Cr, MI/NAA and Lac/NAA ratios of contralateral side when compared to the side of migraine in both left-sided and right-sided migraine patients.

According to the existing trigeminovascular theory of migraine, activation of the trigeminal nucleus caudalis in the brainstem results in vasodilatation and release of vasoactive neuropeptides with transmission of central pain signals to the thalamus. According to the study of Afridi et al. (19) who studied the laterality of brainstem activation in migraine by using positron emission tomography (PET), the pontine and hypothalamic activations were ipsilateral, while the activation was contralateral in the thalamus in the right-sided and left-sided migraine groups which persisted after pain was controlled by medications. Moreover, on the basis of previous studies (19,20), the efferent pathways arising in the spinal trigeminal nucleus may be subdivided into some groups one of which is bilateral, but predominantly crossed, ascending fibre tracts to the midbrain and thalamus (21). The trigeminal nucleus caudalis, in addition to acting as a relay site to the thalamus, also exerts a predominantly facilitatory influence on the relay to the thalamus (20).

In the current study, there was a significant decrease in the mean NAA/Cho and NAA/Cr ratios as well as a non significant decrease in the Cho/Cr ratio in patients when compared to those of the control group. In the study of Gu et al. (17), they found that only NAA/Cr ratio was significantly reduced in migraine patients when compared with normal control. Furthermore, four other small studies (12,22–24) which used 1H-MRS in migraine patients interictally have described controversial results. The studies of Sarchielli et al. (12) and Dichgans et al. (22), who studied metabolite changes in migraine patients interictally with the MRS voxels placed in the grey matter of the cerebellum, reported a reduction in NAA level in migraine patients interictally. Whereas, the studies of Watanabe et al. (23) and Marci et al. (24), who examined the alteration of metabolite ratios in migraine patients interictally with the MRS voxels were placed in the grey matter of the occipital lobe, found no changes in the NAA peak.

The thalamus is the main switching station in the brain for sensory information which may be reflected by the change of metabolite concentrations. Moreover, migraine is characterized interictally by dysfunctional thalamo-cortical connections. The NAA/Cr ratio is generally considered to be a marker of neuronal health which is reduced in conditions where there is neuronal loss or dysfunction.

As a result of pain felt by migraineurs, the neurons in the thalamus are dysfunctional due to possible deafferentation with subsequent diminution in NAA which is related to the reduction in neuronal/axonal viability with the possibility of the presence of either immature or dysfunctional neurons (25). This explanation can provide the reason why there was a decrease in NAA/Cr and NAA/Cho ratios which was shown interictally in our patients with migraine without aura. The Cho/Cr ratio can be used as an indirect marker of myelination and cell membrane metabolism (26). Our results go with the findings of previous studies (3,26) which revealed a reduction in Cho level in the interictal period of migraine. Furthermore, the current results seem to support the findings obtained by previous studies (3,27,28), as we found a non-significant increase of both Lac/NAA and MI/NAA ratios in the patient group when compared to the control group. The studies of Reyngoudt et al. (3) and Dyjak et al. (27) reported a non-significant increase in the Lac/NAA ratio in patient group versus controls. The increased MI could be related to disrupted calcium homeostasis which strengthens the hypothesis of the presence of dysfunctional calcium channels in subgroups of migraineurs (28).

The relations between each of the duration of illness and frequency of headache attacks in migraine patients with the cerebral metabolic alterations are rarely investigated (26,29). The present study revealed a significant inverse correlation between the duration of illness and frequency of headache attacks with the thalamic NAA/Cho and NAA/Cr ratios. On the other hand, we found that increased duration of illness and frequency of headache attacks were associated with increased MI/NAA and Lac/NAA ratios. On the other hand, we observed that, the family history was not significantly correlated with the changes in thalamic metabolite concentration ratios in patients with migraine without aura. In a previous study done by Lai et al. (26), they reported a significant inverse correlation between dorsal pons NAA/Cr ratios and headache frequency among all migraine patients but they did not find correlations between NAA/Cr ratio in dorsal pons and the other variables such as family history, age, gender, disease age onset and duration of illness. However, Schmitz et al. (29) demonstrated that the disease duration and frequency of headache attacks in migraineurs have an influence on the brain structure and integrity. Also, they stated that migraineurs with a high frequency of migraine attacks or long duration of illness were associated with both brain structure and diffusion abnormalities.

Although, we feel that our study offers an important insight into the pathophysiology of the interictal period of migraine without aura which focused on the thalamus to be a goal for migraine treatments especially interictally, but unfortunately, we had some limitations in our study. First, while patients with migraine could not tolerate the MR scanning during the attack; we performed the 1-HMRS scanning in the attack-free (interictal) periods and we examined only migraine without aura patients while other types of migraine were not involved. Second, because of the limitation on scanning time which was imposed by the need to collect high-quality multivoxel 1H-MRS measurements, the areas of the brain we evaluated differed from those in some of the preceding studies, which to a large majority had interest in the occipital cortex. It is possible that variation in the assessed brain areas may be responsible for any difference between our results and previous studies. However, in accordance with a previous study (30) the alterations in energy metabolism in migraineurs affect the whole body as well as muscle tissues, therefore any metabolic abnormalities should be present in the entire cortex, and it is unlikely that voxel location should have influenced our results. Furthermore, the small number of patients in this study was another limitation. So we recommend further extended study on a lar-
ger number of patients investigated interictally in both supratentorial as well as infratentorial brain areas that might be involved in the pathogenesis of migraine in addition to including other types of migraine for future management of this disease more properly.

In summary, migraine without aura (common migraine) is associated with changes in the mean metabolite concentration ratios in the thalami of migraineurs in their interictal (attack-free) period. These changes can be detected through imaging with 1H-MRS which is a non invasive technique. They include a significant reduction in the NAA/Cho and NAA/Cr ratios with non significant decrease in the concentration ratio of Cho/Cr. On the other hand, the concentration ratios of Lac/NAA and MI/NAA are non-significantly increased in migraineurs. These changes of the metabolite concentration ratios are more in the thalamus of the contralateral side rather than the ipsilateral side of the headache. Decreased NAA/Cho and NAA/Cr ratios as well as increased Lac/NAA and MI/NAA ratios are significantly correlated with increased frequency of migraine attacks and increased duration of illness.

Conflict of interest

The authors declare no conflict of interest.

References