Original Research Article

A Study of the Functional, Metabolic and Microstructural Brain Changes in Patients with Migraine Without Aura

Abstract

Background: migraine is a very common disease. Studying the pathological changes in the brain is important for understanding the mechanisms underlying migraine headache. Previous research work has given conflicting results. This study aimed to investigate the functional, metabolic and microstructural changes in the brain of migraine patients without aura.

Methods: this study included 42 migraine patients without aura in the interictal period and 11 age and sex matched controls. All participants were subjected to clinical assessment, assessment of the habituation to visual evoked potentials, assessment of the peak metabolic ratios by H-MRS and diffusion tensor imaging of the brain to test for regional microstructural changes.

Results: The amplitudes of VEPs showed significant reduction in control subjects ($P < 0.01$), but not in migraine patients after repeated stimulation and significant increase in migraine patients compared to controls ($P < 0.01$). H-MRS showed significant decrease of NAA/Cr ($P < 0.01$) and increase of Mi/NAA ($P < 0.001$) and Cho/Cr ($P < 0.05$) PMRs in the thalamus and occipital lobes in migraine patients compared to controls. DTI showed significant changes in the FA, AD, MD, RD values in the thalamus, occipital lobe and insula in migraine patients indicating microstructural changes in these areas. All changes showed significant correlation with the intensity, frequency and duration of migraine episodes, but not with the duration of migraine disease.

Conclusion: Migraine patients without aura showed increased excitability to visual stimulation and significant metabolite and microstructural brain changes that correlated with the severity, not the duration of the disease. These changes need to be confirmed in a large scale longitudinal studies.

Keywords: Migraine without aura; Visual evoked potentials; MRS, DTI, NAA/Cr, Fractional anisotropy

1. Introduction

Migraine is the most common neurological disorder. It is about three times as common in women as in men (WHO, 2011) with a life time prevalence of 15% in the general population in average [**1**]. The cost of lost work hours due to migraine attacks in USA was estimated to be very close to 20 billion US $ a year [**2**].

It is a disabling neurovascular disorder characterized clinically by moderate to severe unilateral or bilateral throbbing headache associated with nausea, increased sensitivity to light and sound. The condition may be associated also with disturbance of autonomic, emotional, cognitive or motor functions [**3**]. Several internal and external stimuli are known to precipitate migraine attacks including emotional stress, sleep changes, hormonal fluctuations, light flashes, certain odors and fasting [**4**].

It can be divided into two major subtypes: migraine without aura (common migraine) and migraine with aura (classic migraine). Migraine without aura is much more common, accounting for about 75% of all migraines [**5**].

Moreover, a significant decrease of N-acetylaspartate (NAA)/choline and NAA/creatine ratios was detected interictally in the thalamus of migraine patients without aura compared to controls. These changes were significantly related to the duration of illness and frequency of attacks. Interestingly enough, these ratios were significantly decreased in the headache side compared to the other [**6**].

Microstructural changes of the brain white matter in migraine patients were detected by means of Diffusion-Tensor MRI. These relatively new MRI modality has shown reduced fractional anisotropy, increased mean diffusivity and increased radial diffusivity in the right frontal white matter cluster of migraine patients.[**7**].

However, there is still a need to confirm the functional, metabolic and morphological changes in the brain in patients with migraine without aura and

Several clinical and experimental studies of the pathophysiology of migraine have indicated that activation and sensitization of the trigeminovascular system, specific brainstem nuclei and the diencephalon are involved in the generation of migraine attacks [**8**].

Although the exact neural and vascular mechanisms are not fully understood, it is believed that migraine attacks occur as a result of increased brain excitability that activates the trigeminovascular system. This leads to increase in the basal firing of first and second order neurons in trigeminocervical complex (TCC). Furthermore, it causes magnified response of TCC neurons to intracranial and extracranial stimulation of the trigeminal nociceptors in cranial blood vessels and pain sensitive dura. Genetic susceptibility is thought to play a role in this process [**9, 10**]. It has been hypothesized that alteration in the levels of excitatory or inhibitory neurotransmitters specially glutamate and GABA may be related to increased brain excitability in migraine (Ferrari et al 2015). Recent work has shown increased glutamate levels in the thalamus, anterior paracingulate cortex and occipital cortex in migraine patients without aura [**11, 12**].

evaluate the relation of these changes, if any, to the duration of migraine disease as well as the frequency, intensity and duration of migraine episodes.

These studies are very important because they form the basis for research work related to development and assessment of new therapeutic strategies aiming at abortion of or prophylaxis against migraine attacks, and also to the development of biological markers that can be used for evaluation of migraine severity and assessment of the response to treatment. So, this work was carried aiming to study these changes.

2. Subjects and methods

This cross sectional study was carried out in the Neuropsychiatry and Radiology departments in

Tanta University Hospitals in the period from 1st of December 2019 till the end of April 2022.

2.1. Participants

Fifty seven patients, of both sexes, diagnosed as migraine without aura and fourteen age and sex matched healthy volunteers were enrolled to this study. The migraine patients were recruited from the outpatient clinics and the healthy volunteers were selected from the relatives and companions of the patients not complaining of migraine to serve as control subjects.

Migraine without aura was diagnosed according to the criteria established by the current version of the International Classification of Headache Disorders; 3 beta version (ICHD-3 beta) published in 2013 (Headache Classification Committee of the International Headache Society, 2018). The patients were investigated in the interictal period at least 3 days after the last episode and 3 days before the next episode. Patients with any clinical or radiological evidence of intracranial disease, epilepsy, mental disorders or history of head trauma were excluded from the study, as well as those with signs of any systemic disease that can affect cerebral function or metabolism. In addition, patients receiving prophylactic anti-migraine medications in the preceding 3 months were not allowed to the study to avoid the probable confounding effects on neuroplasticity (Coppola et al 2016).

2.2. Methods

After taking the needed permissions from the research ethics committee and obtaining a written consent from participants, each participant was subjected to the following

2.2.1 Clinical assessment which included the following items

- Full history taking with special emphasis on the headache semiology, duration of the disease as well as the frequency, intensity and duration of episodes.
- Complete general and neurological examination
- Hamilton rating scale to exclude depression, as it has been demonstrated that depressive symptoms have an effect on the brain metabolism, microstructure and responsiveness [**13, 14**]. The scale consists of 17 items; each was given a score ranging from 0 to 4 according to the intensity of the symptom where 0 indicates absence of the symptom and 4 indicates the maximal intensity [**15**].
- Pain visual analogue

Assessment of the intensity of pain during migraine episodes using the pain visual analogue scale (VAS) described by Hawker et al [**16**]. It is formed of a continuous horizontal line of a length of 10 centimeters (100 mm). The left end of this line indicates a score of zero and has the label " No Pain" and the right end of the line indicates the score of 10 and has the label " Worst Imaginable Pain" [**17, 18**]. No numbers or descriptive labels were put at any point between these ends (Scott and Huskisson, 1976). The labels at the ends of the line were explained for the patient and the patient was asked to indicate a point on the line which represents her (his) pain intensity [**19**]. The score was determined by measuring the distance in mm from the zero point to the point marked by the patient [**18**]. The average of three scores was recorded for each patient. The following cut points were used for interpretation of the VAS scores; 0-4 indicates no pain, 5-44 indicates mild pain, 45-74 indicates moderate pain, 75-100 indicates severe pain [20]

2.2.2 MRI studies

The studies have been performed at the Radiology Department, Tanta University Hospital, using a standard 1.5 Tesla MRI scanner (GE HealthCare, Sigma HDX., W) using a standard head coil. The

studies performed included the following examinations

- *Conventional brain MRI*

Axial T1, axial T2, axial FLAIR and coronal T2 sequences were obtained to exclude any brain pathology and to detect the tiny white matter hyperintensities that may be seen in migraine patients. The following parameters were used; T1W spine-sequence: TR 450, TE 15, matrix 80 x 81, FOV 230 X177, slice thickness 6 mm; T2W turbo spine-echo sequence: TR 3612, TE 100, matrix 208 x 127, FOV 230 X 177, slice thickness 6 mm; FLAIR (Fluid Attenuation Recovery) sequence: TR 6000, TE 120, matrix 240 x 111, FOV 230 X 184, slice thickness 6 mm. For standard and accurate axial slice positioning, the anterior and posterior commissural line (AC-PC line) was used as a reference for T2-weighted and FLAIR images

- *Non contrast high-resolution 3D T1-weighted sequence*

A 3D T1 spoiled gradient echo pulse sequence was acquired for accurate placement of the voxels for MRS and DTI studies. The following parameters were used; TR/TE/TI, 9.7/4.6/400 ms, flip angle (θ) = 35°, 124 slices 0.8 mm thick, 208 × 170 matrix, field of view (FOV) 23 cm 260 contiguous sections, acquisition time 5.25 min.

- *Magnetic Resonance Spectroscopy (MRS)*

Multi voxel MR spectroscopy (1-H MRS) was performed using a spin-echo mode sequence (SE) with long TE (144mm/sec) and short TE (35 mm/sec). Water suppression was achieved with chemical shift selection (CHESS) technique. The voxels were placed on the thalamus and occipital regions on both sides. The metabolites were identified including: N-acetylaspartate (NAA) at 2.0 ppm, creatine (Cr) at 3.0 ppm, choline (Cho) at 3.2 ppm, lipid at the range of 0.7-1.3 ppm, lactate at 1.33 ppm and myoinisitol at 3.56 ppm, and the NAA/Cr, Cho/Cr and MI/NAA peak metabolite ratios (PMRs) were estimated.

- *Diffusion tensor imaging of the brain*

Diffusion Tensor imaging consisted of a single shot, spin-echo echoplanar sequence in 40 encoding directions using the following parameters; diffusion weighting factor of 800s/mm2, TR 10951, TE 67, matrix 128 x 128, FOV 224 X 224 mm, number of excitations 2, slice thickness: 2.0/00 and flip angle 90 degrees. The following areas were studied; the thalamus, the occipital cortex and the, the insula.

Data processing and analysis :

All the diffusion-tensor MRI images were transferred to the workstation (Advantage workstation 4.7). Images were post-processed using the GE software devised for tractography. Fractional anisotropy (FA) maps and directionally-encoded color FA maps were obtained.

Neurophysiological studies which included the following examinations

2.2.3 Neurophysiological studies

- *Electroencephalogram:*

Thirty minute record was obtained for each case using international 10-20 system according to the standard parameters. It was done to exclude epileptiform discharges, as these discharges may affect the results of the study.

- *Visual evoked potential study :*

The pattern reversal method of stimulation was used in this study. It was performed using a visual stimulator which exhibits small and large checks on a screen placed at a distance of 1 meter from the patient. The small (of the size of 8') or large checks (of the size of 65') were arranged in checkerboard patterns of alternating white and dark checks. Repeated stimulation was done by reversing the white checks to dark and the dark checks to white. Both eyes were studied. At first adequate visual acuity was confirmed by Snellen's chart examination. The device used was manufactured by the Japanese company; Nihon Kohden Corporation with an evoked potential measuring system (model :

MEB-2300K, serial number : 00053) and an amplifier (model : JB-206B, serial number: 00329). The examination was done in a quiet room with dimmed light (5 lux) with the patient setting and completely relaxed. The visual field stimulated was 17 ¥ 13° and the contrast was 93%. The patients were instructed to focus on the fixation point in the middle of the checkerboard.

Responses were recorded from an area from the midoccipital lobe (located 5 cm above the inion) to the midfrontal lobe (the Fz, point defined by the International 10/20 system) and then averaged using a special software. The band pass filter was 2-250 Hz and the rejection level was set to 90 mV. For each one stimulation of a certain patient, 600 pattern reversals were presented continuously at a rate of 3 reversals per second (3 rps) and so 600 responses were recorded. The VEP operators were completely blinded to the diagnosis of the examined subjects.

The 600 responses of each stimulus were divided into six blocks of 100 responses. Calculations and averaging were done for the first and sixth blocks of responses. N70 (N1), P100 (P1), and N145 (N2) VEP peaks were visually identified by the operating neurophysiologist. Peak-to-peak amplitudes from N70 to P100 and from P100 to N145 were calculated. In addition the block ratio was calculated for each subject and for each group; it is the ratio of the amplitudes in block 6 to the amplitudes in block 1; this is the measure of habituation (**Omland et al, 2013**).

2.3 Statistical analysis

The following statistical tests have been used:

- *Unpaired T student test:*

This test was used to determine the statistical significance of the differences in the studied variables and parameters between the migraine patients and the control groups and between the subgroups of migraine patients.

- *Paired T student test:*

This test was used to determine the statistical significance of the differences in the studied parameters of evoked potentials between the successive blocks in each group.

- *Pearson Correlation test:*

This test was used to determine the degree and the statistical significance of correlation of the studied parameters in migraine patients with the frequency, intensity and duration of migraine episodes and the duration of the disease.

These tests are widely described elsewhere in the literature [**21, 22**]

3. Results

3.1 Demographic and clinical results

Fifteen of the fifty seven patients diagnosed as migraine without aura, and 3 of the 14 control subjects failed to complete the study. So, a net of 42 patients and 11 control subjects completed the study (Table 1). The age and gender distribution were comparable in patients and controls with no statistically significant difference (Table 2). The duration of the disease and the intensity, frequency and duration of migraine episodes, in addition to some important clinical signs are shown in table 3.

3.2 Visual Evoked Potentials (VEPs)

With small check stimulation, the block 6 N75 – P100 and P100 – N145 peak amplitudes ((measured in μV) and the B6/B1 ratio showed a statistically significant increase in the migraine group compared to the control group (Block 6: $P < 0.01$ & B6/B1 ratio: $P<0.01$; $P < 0.05$ respectively). Block1 amplitudes showed no significant difference. There was a statistically significant reduction in the block 6 compared to block 1 amplitudes in the control group ($P < 0.05$; $P <0.001$ respectively) but not in the migraine group. Large check simulation showed the similar results (Table 4).

Table 1: Flow chart of the migraine patients and control subjects enrolled to the study

	Patients			Controls		
	Males	Females	Total	Males	Females	Total
Total Number enrolled	**15**	**42**	**57**	**4**	**10**	**14**
Total number excluded	**4**	**11**	**15**	**1**	**2**	**3**
Depression by Hamilton Scale	**2**	**3**	**5**	**-**	**1**	**1**
Claustrophobia	**-**	**1**	**1**	**-**	**-**	**-**
Impaired visual acuity	**1**	**1**	**2**	**-**	**-**	**-**
Refused SSEP	**-**	**2**	**2**	**-**	**-**	**-**
Covid 19 concerns	**-**	**3**	**3**	**1**	**1**	**2**
Transmission to far areas	**1**	**1**	**2**	**-**	**-**	**-**
Net number	**11**	**31**	**42**	**3**	**8**	**11**

The block 6 N75 – P100 and P100 – N145 peak amplitudes showed a statistically significant increase in the migraine patients with moderate to severe intensity episodes (n=29) compared to patients with mild intensity episodes (n=13) with small check ($P< 0.05$; $P< 0.01$ respectively) and large check ($P<0.01$) stimulation (Table 5). The block 6 N75 – P100 and P100 – N145 peak amplitudes of small and large check VEPs showed a statistically significant positive correlation with the frequency and duration of migraine episodes, but no correlation with the duration of the disease (Table 6).

3.3 MRS peak metabolic ratios

The right and left thalami in the migraine group showed a statistically significant decrease of NAA/Cr ratio ($P < 0.01$), and a statistically significant increase of MI/NAA ($P < 0.001$) and Cho/Cr ratios ($P < 0.05$) compared to the control group, and so showed the occipital lobes ($P < 0.01$, $P < 0.001$, $P < 0.05$ respectively); shown in Table 7 and Figures 1 & 2.

Both thalami, as well as the occipital lobes, in the moderate/severe intensity subgroup (n=29) showed a statistically significant decrease of NAA/Cr ratio ($P < 0.05$; $P < 0.001$), and a statistically significant increase of MI/NAA ($P < 0.001$) and of the Cho/Cr ratio ($P < 0.001$) compared to the mild intensity subgroup (n=13) (Table 8).

The NAA/Cr, MI/NAA and Cho/Cr peak metabolite ratios in the right and left thalami of migraine

patients showed statistically significant correlation with the frequency and duration of migraine episodes, but no correlation with the duration of the disease; NAA/Cr ratio: negative correlation ($P < 0.01$, $P < 0.05$ respectively), MI/NAA and Cho/Cr ratios: positive correlation ($P < 0.001$). The right and left occipital lobes also showed significant negative correlation of NAA/Cr, and significant positive correlation of MI/NAA and Cho/Cr peak metabolite ratios with the frequency and duration of migraine episodes, but no correlation with the duration of the disease (Table 9).

Table (2): The age and gender distribution of patients and controls

		Patients (n= 42)	Controls (n=11)	Statistical Test
Age	**Mean ± SD**	**28.6 ± 6.5**	**27.8 ± 6.3**	**T = 0.372**
	Range	**16 -41**	**18 – 40**	**P > 0.05**
Gender	**Males**	**11 (26.2%)**	**3 (27.3%)**	***X*2 =**
	Females	**31 (73.8%)**	**8 (72.7%)**	**P > 0.05**

3.4 Diffusion Tensor Imaging (DTI)

Fractional anisotropy (AF) and axial diffusivity (AD) showed a statistically significant increase in the thalamus ($P < 0.001$) and decrease in the occipital lobes ($P < 0.001$) and the insula ($P < 0.001$, $P < 0.01$ respectively) in migraine patients compared to controls, (Table 10, 11 & Figure 3). Mean diffusivity (MD) and radial diffusivity (RD) showed a statistically significant decrease in the thalamus ($P < 0.001$) and increase in the occipital lobes ($P < 0.001$) and insula ($P < 0.01$) in migraine patients compared to controls (Table 12, Table 13). The changes in AF and MD were significantly greater in the moderate/severe ($P < 0.05$ and $P < 0.01$) compared to the mild intensity subgroups of migraine patients (Table 14).

All DTI measures showed statistically significant correlation with the frequency and duration of migraine episodes, but no significant correlation with the duration of the disease. FA correlated positively in the thalamus and negatively in the occipital lobes and insula, and RD correlated in the opposite direction (Tables 15, 16).

Table (3) : Clinical signs of migraine patients

		N = 42
Duration of the disease	Mean ± SD	7 ± 3.73
	Range	6 months – 16 years
Frequency of episodes	Mean ± SD	5.88 ± 2.07
	Range	2 - 9
Intensity of episodes	Mean ± SD	57.6 ± 21.99
	Mild episode (5-44)	13 (31%)
	Moderate episode (45-74)	17 (40%)
	Severe episode (75-100)	12 (29%)
Duration of episodes	Mean ± SD	21.7 ± 9.6
	Range	4 – 34 hours
Clinical signs	Unilateral headache	11 (26.2 %)
	Throbbing headache	30 (76.2 %)
	Photophobia	30 (71.4 %)
	Nausea and/or Vomiting	25 (59.5 %)
	Triggering factors	29 (69 %)

Table (4) : The N75-P100 and P100-N145 peak to peak amplitudes of VEPs with PRS

Waves of VEPs		Patients (N = 42)	Controls (N = 11)	T Value
N75 – P100 Small checks (8)	Block 6	13.8 ± 1.75	10.85 ± 2.93	3.2**
	B6/B1 Ratio	1.04 ± 0.27	0.76 ± 0.23	3.46**
	T Value	1.32	2.82**	
P100 – N145 Small checks (8)	Block 6	14.88 ± 2.28	12.27 ± 2.57	3.07**
	B6/B1 Ratio	0.94 ± 0.14	0.79 ± 0.19	2.45*
	T Value	0.57	5.77***	
N75 – P100 Large checks (8)	Block 6	14.11 ± 2.73	11.3 ± 2.37	3.39**
	B6/B1 Ratio	0.965 ± 0.15	0.74 ± 0.22	3.2**
	T Value	1.6	3.28**	
P100 – N145 Large checks (8)	Block 6	15.11 ± 2.91	11.5 ± 3.59	3.08**
	B6/B1 Ratio	0.968 ± 0.23	0.78 ± 0.24	2.33*
	T Value	1.55	2.92**	

Peaks: in microvolt, PRS: pattern reversal stimulation, *: P < 0.05, **: P < 0.01, ***: P < 0.001

Table (5) : Amplitudes of VEPs in the moderate/severe versus mild intensity subgroups of migraine patients

VEP Waves in Block 6		Moderate/Severe (VAS >= 45) (N = 29)	Mild (VAS: <45) (N = 13)	T Value
N75 – P100	Small Checks	14.2 ± 1.59	12.88 ± 1.79	2.304*
	Large Checks	14.9 ± 2.37	12.33 ± 2.73	2.93**
P100 – N145	Small Checks	15.62 ± 1.7	13.24 ± 2.62	3.01**
	Large Checks	16.01 ± 2.4	12.92 ± 2.85	2.48**

Table (6) : Correlation of the amplitudes of VEPs with the duration of disease, frequency and duration of migraine episodes

VEP Waves in Block 6		Disease Duration (In years) 7.33 ± 3.83	Frequency (MEs/month) 5.88 ± 2.07	Duration of MEs (In hours) 21.7 ± 9.6
N75 – P100	Checks (8) 13.8 ± 1.75	R: 0.273 (T: 1.79)	R: 0.458** (T: 3.26)	R: 0.563*** (T: 4.31)
	Checks (65) 14.11 ± 2.73	R: - 0.02 (T: 0.098)	R: 0.64*** (T: 5.26)	R: 0.546*** (T: 4.12)
P100 – N145	Checks (8) 14.88 ± 2.28	R: - 0.07 (T: 0.42)	R: 0.585*** (T: 4.56)	R: 0.572*** (T: 4.42)
	Checks (65) 15.11 ± 2.91	R: - 0.14 (T: 0.9)	R : 0.459** (T: 3.27)	R: 0.46** (T: 3.28)

R: Correlation Coefficient, T: Calculated T, MEs: Migraine Episodes, **: P < 0.01, ***: P < 0.001.

Table (7) : MRS peak metabolite ratios in the migraine and control groups

Areas and Metabolites		Patients (N = 42)	Controls (N = 11)	T Value
Right Thalamus	NAA/Cr	1.82 ± 0.153	1.94 ± 0.088	3.38**
	MI/NAA	0.491 ± 0.0789	0.372 ± 0.054	5.85***
	Cho/Cr	1.2 ± 0.19	1.08 ± 0.13	2.45*
Left Thalamus	NAA/Cr	1.86 ± 0.162	1.99 ± 0.124	2.89**
	MI/NAA	0.482 ± 0.084	0.368 ± 0.067	4.75***
	Cho/Cr	1.31 ± 0.16	1.18 ± 0.17	2.29*
Right Occipital Lobe	NAA/Cr	1.41 ± 0.167	1.57 ± 0.128	3.45**
	MI/NAA	0.469 ± 0.0797	0.364 ± 0.053	5.21***
	Cho/Cr	1.108 ± 0.176	1.01 ± 0.114	2.24*
Left Occipital Lobe	NAA/Cr	1.46 ± 0.154	1.62 ± 0.132	3.45**
	MI/NAA	0.455 ± 0.078	0.358 ± 0.075	3.79***
	Cho/Cr	1.12 ± 0.161	1.03 ± 0.115	2.11*

NAA: N acetyl aspartate, Cr: creatine, Cho: choline, MI: myoinositole, *: P < 0.05, **: P < 0.01, ***: P < 0.001.

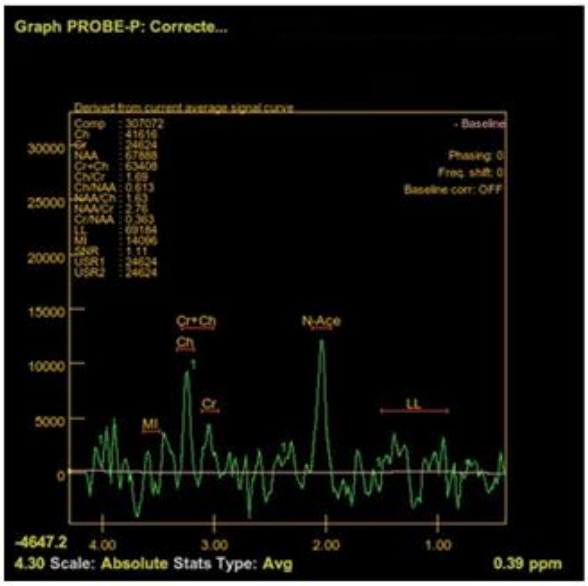

Figure 1 (A): MRS of the right thalamus in a female patient with migraine; 26 years old, with 5 year history of migraine, frequency of MEs: 6 per month, VAS score: 68. MRS shows decrease of the NAA peak with reduction of NAA/Cr ratio and increase of the Mi/NAA ratio

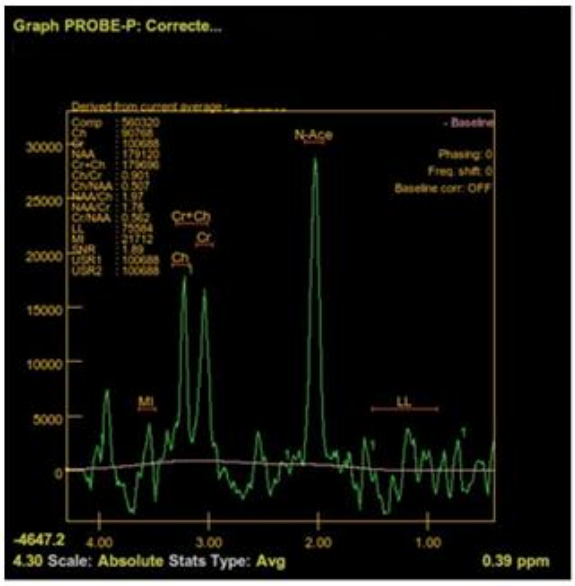

Figure 1 (B): MRS of the right thalamus in a female control; 28 years old. MRS show average NAA peak with relative increase of NAA/Cr ratio and decrease of Mi/NAA ratio.

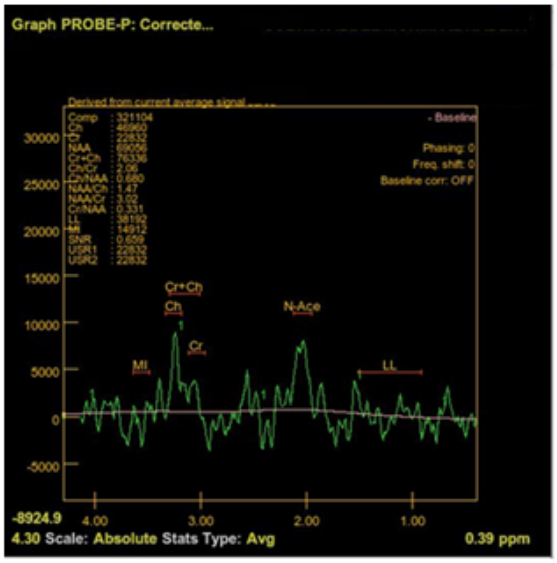

Figure 2 (A): MRS of the left occipital lobe in a female patient; 30 years old, with 7 year history of migraine, frequency of MEs: 7 per month, VAS score: 64. MRS shows decrease of the NAA peak with reduction of NAA/Cr ratio and increase of the Mi/NAA ratio

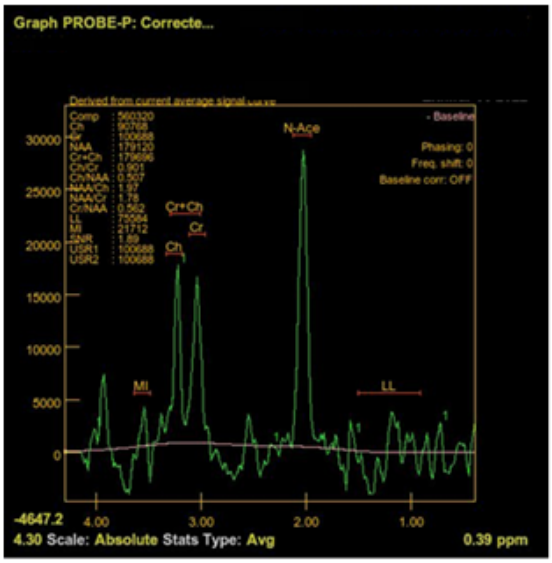

Figure 2 (B): MRS of the left occipital lobe in a female control; 33 years old. MRS show high NAA peak with relative increase of NAA/Cr ratio and decrease of Mi/NAA ratio. .

Table (8) : The peak metabolite ratios in the moderate/severe versus mild intensity subgroups

Areas and Metabolites		Moderate/Severe VAS >= 45 (N = 29)	Mild VAS: <45 (N = 13)	T Value
Right Thalamus	NAA/Cr	1.772 ± 0.088	1.921 ± 0.214	2.42*
	MI/NAA	0.526 ± 0.0596	0.412 ± 0.057	5.89***
	Cho/Cr	1.286 ± 0.146	1.008 ± 0.139	5.9***
Left Thalamus	NAA/Cr	1.81 ± 0.174	1.972 ± 0.195	2.57*
	MI/NAA	0.498 ± 0.0485	0.446 ± 0.0412	3.57***
	Cho/Cr	1.362 ± 0.138	1.194 ± 0.142	3.85***
Right Occipital Lobe	NAA/Cr	1.324 ± 0.088	1.592 ± 0.154	5.86***
	MI/NAA	0.502 ±0.0655	0.395 ±0.0549	5.47***
	Cho/Cr	1.19 ± 0.124	0.92 ± 0.121	6.68***
Left Occipital Lobe	NAA/Cr	1.391 ± 0.121	1.614 ± 0.158	4.53***
	MI/NAA	0.486 ± 0.058	0.386 ± 0.0564	5.26***
	Cho/Cr	1.198 ± 0.135	0.946 ± 0.148	5.24***

AA: N acetyl aspartate, Cr: creatine, Cho: choline, MI: myoinositole. *: P < 0.05, **: P < 0.01, ***: P < 0.001

Table (9) : Correlation of the peak metabolite ratios with the duration of migraine, frequency and duration of migraine episodes

Areas and Metabolites		Disease Duration (in years) 7.33 ± 3.83	Frequency (MEs/month) 5.88 ± 2.07	Duration of MEs (In hours) 21.71 ± 9.55
Right Thalamus	NAA/Cr 1.82 ± 0.153	R: 0.07 (T: 0.43)	R: - 0.48** (T: 3.4)	R: - 0.41* (T: 2.84)
	MI/NAA 0.491 ± 0.0789	R: 0.05 (T: 0.31)	R: 0.593*** (T: 4.65)	R: 0.592*** (T: 4.64)
	Cho/Cr 1.2 ± 0.19	R: 0.05 (T: 0.31)	R: 0.594*** (T: 4.67)	R: 0.588*** (T: 4.6)
Left Thalamus	NAA/Cr 1.86 ± 0.162	R: 0.08 (T: 0.51)	R: - 0.46** (T: 3.28)	R: - 0.427** (T: 2.99)
	MI/NAA 0.482 ± 0.084	R: 0.11 (T: 0.7)	R: 0.64*** (T: 5.27)	R: 0.576*** (T: 4.46)
	Cho/Cr 1.31 ± 0.16	R: 0.07 (T:0.44)	R: 0.61*** (T: 4.87)	R: 0.632*** (T: 5.16)
Right Occipital	NAA/Cr 1.82 ± 0.153	R: - 0.18 (T: 1.19)	R: - 0.54*** (T: 4.03)	R: - 0.498*** (T: 3.64)
	MI/NAA 0.491 ± 0.0789	R: 0.1 (T: 0.63)	R: 0.58*** (T: 4.54)	R: 0.545*** (T: 4.11)
	Cho/Cr 1.2 ± 0.19	R: 0.15 (T: 0.95)	R: 0.613*** (T: 4.9)	R: 0.553*** (T: 4.2)
Left Occipital	NAA/Cr 1.86 ± 0.162	R: - 0.12 (T: 0.76)	R: - 0.62*** (T: 4.997)	R: - 0.463** (T: 3.3)
	MI/NAA 0.482 ± 0.084	R: 0.11 (T: 0.7)	R: 0.67*** (T: 5.7)	R: 0.528*** (T: 3.93)
	Cho/Cr 1.31 ± 0.16	R: 0.17 (T: 1.1)	R: 0.69*** (T: 6.03)	R: 0.537*** (T: 4.03)

NAA: N acetyl aspartate, Cr: creatine, Cho: choline, MI: myoinositole, *: P < 0.05, **: P < 0.01, ***: P < 0.001.

Table (10) : DTI measured Fractional Anisotropy (FA) in the brain of migraine patients and controls

Brain Areas		Patients (N = 42)	Controls (N = 11)	T Value
Thalamus	Right	0.35 ± 0.046	0.26 ± 0.032	6.1***
	Left	0.37 ± 0.043	0.28 ± 0.035	6.4***
Occipital lobes	Right	0.36 ± 0.045	0.47 ± 0.043	7.5***
	Left	0.33 ± 0.042	0.46 ± 0.047	8.3***
Insula	Right	0.18 ± 0.027	0.25 ± 0.033	6.5***
	Left	0.17 ± 0.024	0.24 ± 0.032	6.8***

DTI: diffusion tensor imaging, ***: P < 0.001.

Table (11) : DTI measured axial diffusivity (AD) in the brain of migraine patients and controls

Brain Areas		Patients (N = 42)	Controls (N = 11)	T Value
Thalamus	Right	0.88 ± 0.077	0.76 ± 0.074	4.6***
	Left	0.92 ± 0.086	0.79 ± 0.083	4.5***
Occipital lobes	Right	0.74 ± 0.083	0.85 ± 0.073	4.3***
	Left	0.76 ± 0.072	0.88 ± 0.069	5.1***
Insula	Right	0.81 ± 0.076	0.89 ± 0.67	3.4**
	Left	0.82 ± 0.074	0.91 ± 0.085	3.2**

DTI: diffusion tensor imaging, **: P < 0.01, ***: P < 0.001.

Table (12) : DTI measured mean diffusivity (MD) in the brain of migraine patients and controls

Brain Areas		Patients (N = 42)	Controls (N = 11)	T Value
Thalamus	Right	0.75 ± 0.071	0.88 ± 0.072	5.4***
	Left	0.74 ± 0.078	0.87 ± 0.064	5.1***
Occipital lobes	Right	0.84 ± 0.073	0.73 ± 0.075	4.4***
	Left	0.85 ± 0.079	0.71 ± 0.086	4.9***
Insula	Right	0.88 ± 0.074	0.81 ± 0.061	3.2**
	Left	0.86 ± 0.065	0.80 ± 0.063	3.8**

DTI: diffusion tensor imaging, **: P < 0.01, ***: P < 0.001.

Table (13) : DTI measured radial diffusivity (RD) in the brain of migraine patients and controls

Brain Areas		Patients (N = 42)	Controls (N = 11)	T Value
Thalamus	Right	0.76 ± 0.082	0.91 ± 0.073	5.5***
	Left	0.74 ± 0.079	0.88 ± 0.071	5.3***
Occipital lobes	Right	0.88 ± 0.075	0.76 ± 0.081	4.4***
	Left	0.87 ± 0.071	0.73 ± 0.078	5.4***
Insula	Right	0.89 ± 0.065	0.81 ± 0.069	3.5**
	Left	0.9 ± 0.064	0.82 ± 0.069	3.5**

DTI: diffusion tensor imaging, **: P < 0.01, ***: P < 0.001.

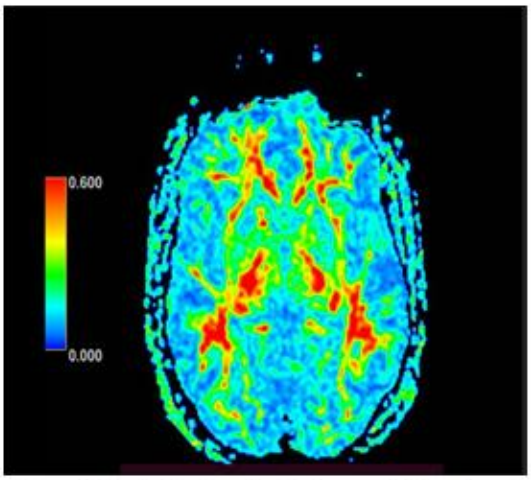

Figure 3 (A): A female patient 25 years old, duration of migraine: 4 years, frequency of MEs: 5 per month, VAS score : 60. DTI shows increase of the FA in the thalamus

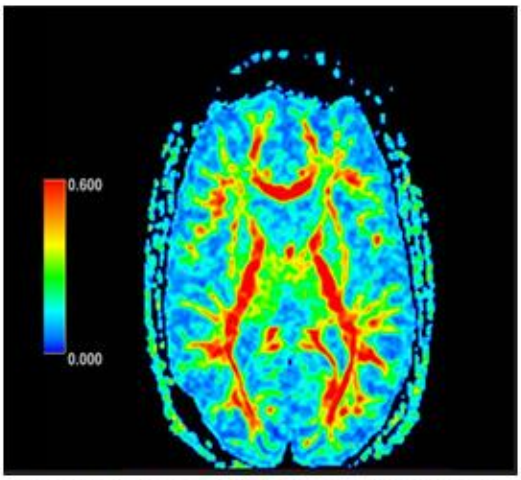

Figure 3 (B): A male patient 34 years old, duration of migraine: 6 years, frequency of MEs: 4 per month, VAS score : 60. DTI shows decrease of FA in the occipital lobes

Table (14) : FA and MD in the moderate/severe versus mild intensity subgroups of migraine patients

Brain Areas		Moderate/Severe VAS >= 45 (N = 29)	Mild VAS: <45 (N = 13)	T Value
Thalamus	FA	0.285 ± 0.032	0.249 ± 0.037	3.2**
	MD	0.823 ± 0.065	0.891± 0.062	3.18**
Occipital lobes	Right	0.349 ± 0.032	0.391 ± 0.047	3.14**
	Left	0.868 ± 0.058	0.81 ± 0.063	2.8**
Insula	Right	0.173 ± 0.034	0.196 ± 0.028	2.3*
	Left	0.885 ± 0.067	0.804 ± 0.076	3.3**

*: P < 0.05, **: P < 0.01.

Table (15) : Correlation of FA in the brain areas with the duration of migraine and frequency and duration of migraine episodes

Brain Areas	Duration of migraine (In years) 7.33 ± 3.83	Frequency of Episodes (Per month) 5.88 ± 2.07	Duration of episodes (In hours) 21.7 ± 9.6
Thalamus 0.28 ± 0.035	R: 0.294 T: 1.95	R: 0.445** T: 3.14	R: 0.548*** T: 4.14
Occipital lobe 0.33 ± 0.042	R: - 0.275 T: 1.81	R: - 0.424** T: 2.96	R: - 0.532*** T: 3.97
Insula 0.17 ± 0.024	R: - 0.257 T: 1.68	R: 0.415** T: 2.88	R: - 0.535*** T: 4

**: P < 0.01, ***: P < 0.001.

Table (16) : Correlation of RD in the brain areas with the duration of migraine and frequency and duration of migraine episodes

Brain Areas	Duration of migraine (In years) 7.33 ± 3.83	Frequency of Episodes (Per month) 5.88 ± 2.07	Duration of episodes (In hours) 21.7 ± 9.6
Thalamus 0.28 ± 0.035	R: - 0.284 T: 1.87	R: - 0.473** T: 3.4	R: - 0.45** T: 3.2
Occipital lobe 0.33 ± 0.042	R: 0.295 T: 1.95	R: 0.458** T: 3.3	R: 0.462** T: 3.2
Insula 0.17 ± 0.024	R: 0.227 T: 1.47	R: 0.422 T: 2.9	R: 0.404** T: 2.8

**: P < 0.01, ***: P < 0.001.

4. Discussion

In spite of extensive research work and a great number of studies, the mechanisms underlying the development of the attacks of migraine headache, are still not clearly understood [23] and a great amount of controversy still exists regarding the physiological, metabolic and microstructural changes in the brain of migraine patients [**24, 25, 26, 27**] .

So, this study was carried out to investigate the these brain changes in the interictal period in the same patients and detect if there is any correlation of these changes with the duration of the disease and/or the intensity, frequency and duration of

migraine episodes. The majority of previous studies have included migraine patients with aura or a mixture of patients with and without aura and some studies even included patients with chronic migraine. Specific studies of migraine patients without aura were remarkably few [**25, 27, 28, 29**]. So, our study included only migraine patients without aura to investigate these brain changes in this particular clinically entity. We aimed to get specific results that are more amenable for later comparisons with further studies.

This is a blind controlled cross sectional study. The patients and controls are well matched for age and sex. Females constituted about three fourths of the patient sample which is consistent with general female to male ratio in migraine patients in many localities in the world [**30, 31**]. The age ranged from 16 to 41 years in migraine patients and from 18 to 40 years in controls which is perfectly matched.

Patients and controls with significant depressive symptoms, according to Hamilton scale, or EEG epileptiform activity were ruled out from the study to avoid the confounding effect of depression [**14**] and epilepsy [**32**] on the microstructural changes in the brain.

Visual Evoked Potentials

Visual evoked potentials constitute a measure of central brain excitability [**33, 34**]. Normally, repeated peripheral stimulation leads to decrease in the amplitude of the evoked potentials; a physiological phenomenon called habituation [**34**]**,** most probably resulting from fatigue of synaptic transmission due to exhaustion of the neurotransmitters [35]

Increased central excitability in migraine patients, expressed as maintenance of the amplitude of evoked potentials after repeated stimulation, called loss of habituation, or more markedly by increase of the amplitude of evoked potentials, called potentiation, has been revealed in previous clinical and experimental studies [36, 37].

This study showed occurrence of habituation, with significant decrease of peak to peak amplitudes, in control subjects, and loss of habituation in migraine patients. These findings are consistent with the results of study of **Schoenen et al [38],** the first to show loss of habituation and even potentiation of VEPs in migraine patients. Results consistent with our study, have been demonstrated by several following studies (**36, 39-44**). The habituation deficit in migraine has been attributed to increased cortical excitability with exaggerated response to visual stimulation [**45**]. It has also been suggested that cortical dysfunction results from a disorder in thalamic function; abnormal thalamic activation of the cortex may lead to reduction of the activity of the lateral inhibitory circuits [**46**]. Moreover, the visual and trigeminal nerve pathways converge with each other on the thalamus and on the visual cortex [**47**]. So, over reactivity of trigemniovascular system may explain the increased response to visual stimulation, and this may form the base for clinical photophobia and triggering of migraine episodes by light impulses [**48, 49**].

On the other hand, several other studies showed the absence of the habituation deficit in VEPs in migraine patients [**50-53**]. However, these studies applied low frequency of pattern reversal [54] relative to our study and other consistent studies; this may explain the controversy in the results. Further controversy was excited by **Omland et al, [54],** who demonstrated the occurrence of habituation in migraine patients even with high frequency stimulation, a finding which was reproduced by the same team 3 years later [**25**]**,** thus arguing against the presumed explanation of variation in stimulus frequency.

More recently, a large multicentered study [**55**] demonstrated a significant decrease in habituation in migraine patients compared to the controls which is going with the results of our study. So, the controversy extended over numerous studies for more than 25 years. This may be due to technical

factors, differences in the duration or the severity of the disease or difference in the timing of the test relative to the episodes or to the intake of abortive drugs as triptans. Furthermore, VEPs can be affected by mood [**56**], attention [**57**], fatigue [**58**], and the degree of focusing on visual stimuli [**59, 60**]. In our study, these factors were seriously considered and for example, patients with depressive symptoms were excluded while other studies didn't report the mood state of their patients, and so some of their patients might have depressive symptoms which could have affected VEPs.

It was assumed that the habituation deficit for VEPs in migraine may be correlated with the duration of the disease or the intensity, the frequency or the severity of the migraine episodes. In addition, the controversy observed in the reported results of the different studies makes it necessary to study the effect of these clinical parameters on the response to visual stimulation.

This study showed a statistically significant positive correlation of the habituation deficit with intensity, the duration and the frequency of migraine episodes, but no correlation was detected with the duration of the disease.

Although, only few studies reported the relation of the clinical parameters to the results of VEPs, the results of some studies were consistent with our study; visual excitability correlated with the severity of headache [**53**] and the frequency of migraine episodes [**61**]**. However,** contrary to our study, positive correlation was detected with the duration of the disease [**54**]**,** but the evidence indicates that the longer duration in that study reflected also the greater severity of the disease.

Magnetic resonance spectroscopic examination (MRS)

Few studies have examined metabolite changes in migraine patients without aura and **the** results were mostly inconsistent and difficult to compare due to variations in the methods of examination, examination of different areas of the brain and inclusion of clinically heterogeneous patient groups [**26**]. In the current study, 1 H-MRS was used for estimation of the peak metabolite ratios (PMR), and it showed significant changes in the NAA/Cr, MI/NAA and Cho/Cr PMRs in the thalamus, the visual areas on the occipital lobe and the insula, and these changes showed significant correlation with the intensity of headache attacks, the frequency and duration of migraine episodes, but no correlation with the duration of the disease.

Some previous studies showed results consistent with our study; NAA was reduced in the occipital lobe in 44 patients interictally, although this was more evident in migraine with aura [**62**], NAA was reduced and Cho was elevated in the left thalamus in 20 migraine patients [**28**], NAA/Cr and NAA/Cho PMRs were significantly decreased in the thalamus on both sides in 20 migraine patients without aura, and there was a trend for increase in the MI/NAA PMR in the right thalamus [**6**]. The results of some other studies were not going with our results; no significant change of NAA, MI or Cho was detected in the occipital lobes in 2 studies of migraine patients [**11, 63**]. However, this differences may be explained by the less severe migraine in the cases included in these studies. In a more recent study [**64**], NAA/Cr PMR was significantly decreased, but Cho/Cr PMR showed no significant change in both thalami of migraine patients.

Few studies have investigated the correlation of the metabolic changes with the clinical parameters of migraine. Significant correlation of the metabolite changes in the thalamus with the frequency of episodes and the duration of the disease was revealed in three consecutive studies [**6, 65, 66**]**.** Moreover, in the study of **Gu et al (64),** **i**mprovement of the clinical parameters of migraine was associated with increase of the NAA/Cr PMR which is consistent with the current study.

A more recent 1H-MRS stud **[67}** showed significant decrease of NAA/Cr and significant increase of

MI/NAA and Cho/Cr metabolic ratios in the occipital lobe in migraine patients without aura interictally compared to controls and these changes showed significant correlation with the frequency of the disease which is consistent with the results of the current study. However, in contrary to our study, the metabolic changes correlated with the duration of the disease.

It was strongly proposed that NAA level can be considered a marker also for mitochondrial function and decreased concentrations of NAA in migraine patients may indicate mitochondrial dysfunction [**68, 69**]**.** Choline (Cho) is a very important for cell membrane metabolism [**70**], but its relation to the pathophysiology of migraine or to its clinical phenotype is still to be investigated. Myoinositole (MI) has a role in regulation of calcium channel activity and increased MI may indicate a disorder in calcium equilibrium [**65**].

A defect in mitochondrial energy metabolism has been suggested to play a role in the pathogenesis of migraine headache [**24**], and an accumulating evidence indicates that the brain in migraine patient is working at a rate higher than normal due to subnormal energy reserve [**71**]. The visual cortex is particularly more sensitive to the energy defect in migraine patients as it has a relatively less neuronal/glial cells ratio. [**28].** Reduced energy reserve may explain the susceptibility to headache episodes in migraine patients and may explain the low NAA concentration level in the thalamus and occipital lobes found in our study and the previous studies.

It was found that the degree of the metabolic changes and hence the severity of energy defect and the mitochondrial dysfunction correlate significantly with the severity of migraine disease [**24**]. This may explain the controversies in the metabolic findings of the different studies which may be due to the small or heterogeneous patients groups Moreover, this is consistent with results of our study; the metabolic changes correlated significantly with the severity of the disease. Furthermore, the energy defect in migraine patients may lower the threshold for development of migraine attacks and this renders the patient susceptible to the different triggers [**71**]. So, it logically follows that more energy defect, indicated in our study by more reduction in NAA, will be associated with higher frequency of episodes.

So, 1H-MRS estimation of NAA in the thalamus and occipital visual cortex may be used as an indirect measure of mitochondrial function in migraine patients and thus, a marker for the disease severity, progress and response to prophylactic therapy. Although, indirect, it has several advantages from the clinical aspect; it is more easy and practical and widely available in health centers.

The Cho/Cr PMR was increased in our study. Previous studies have shown inconsistent results; no change in occipital lobe [11], decrease [**72**], increase in the occipital lobe [**67**]. It is difficult to compare these studies due to the small number of cases and/or the different clinical phenotypes. It may be proposed that choline level is dynamic; changes from time to time or changes along the migraine cycle. This needs further investigation. Choline levels have been linked to number and activity of glial [**73**]**.** However, for understanding the role of choline level changes in migraine, it seems that longitudinal studies should be carried out to estimate the Cho/Cr PMR in different stages of the migraine cycle and along several months of the course of the disease.

Myoinositole/Cr PMR was increased in our study. Previous studies has also demonstrated increase of MI/Cr ratio in in the thalamus of migraine patients without aura [**6**]**.** It is important for regulation of calcium channels [**74**], and a relation of Ca^{++} regulation to the pathophysiology of migraine was recently suggested an experimental study [**26**].

Diffusion tensor imaging

Diffusion weighted spin-echo, single-shot echo planner imaging (EPI) has been used in this study. It is the most common pulse sequence for DTI; it is

available in most MRI scanners, in addition to being easy and fast [**75**].

In the current study, DTI examination revealed significantly increased fractional anisotropy (FA) and axial diffusivity (AD) and decreased mean diffusivity (MD) and radial diffusivity (RD) in the thalamus, and the opposite changes in the occipital lobes and insula. In addition, these changes showed a significant correlation with frequency, intensity and duration of migraine episodes, but not with the duration of the disease. Previous studies reported inconsistent and conflicting results; decrease of FA in the occipital cortex and thalamus [**76**]**,** decreased FA, increased MD and increased RD in the thalamus and insula with no correlation with the severity or duration of the disease [**77**], decreased FA and increase of MD in the thalamus, occipital lob and insula [**78**], increase of FA in the thalamus bilaterally [**79**]**.** Therefore, the results of several studies have been consistent our study. However, contradictory findings have been also detected by other studies. There was significant decrease of the FA in the thalamus in two studies [76, 80]. The AD and MD were decreased in the thalamus with no change in RD in one study [**81**]. In another study**,** in agreement with our study, it was found that MD and RD were decreased in the thalamus**,** but, on contrary, the AD was decreased [**82**]**.**

Clinical and experimental data indicate that the thalamus is a key structure in migraine pathophysiology [**79**]. The thalamic nuclei have extensive connections with many important cortical and brainstem areas and constitute an essential part in many of the neural networks [**83**]. So, it is involved in many of the clinical and neurophysiologic features of migraine as allodynia [**84**], photophobia [**85**] and exacerbation of headache by light [**86**]. Reduction of the thalmocortical is believed to play a role in the habituation deficit for most sensory modalities, and all sensory modalities show abnormal responses in migraine patients [**87**].

The DTI parameters in the visual area of the occipital lobe also show controversies. A study of pediatric migraine patients demonstrated decrease of MD, AD and RD of WM tracts in the occipital areas of the cerebral cortex with no correlation with duration or frequency of the disease [**82**].

The insula also showed decrease of FA and AD and increase of MD and RD in migraine patients of our study, similar to that obtained by **Gomez-**Beldarrain **et al [88].** The insula is involved in several cerebral functions; sensory, cognitive, autonomic, emotional and behavioral [**89**]. The posterior insular cortex receives nociceptive signals from the thalamic neurons including the trigeminovascular neurons, involved in migraine episodes [**49, 90**], and it was suggested that the insula has a role in the processing and integration of pain sensation [**91, 92**].

Furthermore, morphological changes have been detected in the insula in patients with high frequency of migraine episodes and it was suggested that highly frequent repeated migraine episodes cause abnormal functioning of the insula [**93**]. This goes in line with our results; microstructural changes have been detected in the insula with correlation with the episode frequency and severity. Disrupted structure and function of the insula was linked to vestibular symptoms in migraine [94], olfactory hypersensitivity in between the migraine attacks [**95**] and autonomic symptoms [**96**]. Therefore, the insula is involved in many aspects of migraine and this may explain the microstructural changes detected in the insula in our study.

The controversy in the results of DTI studies is difficult to explain. This has been attributed to different techniques of examination (TBSS versus voxel based), inclusion of mixed groups of migraine patients, difference in the position of the patients relative to the migraine cycle at the time of examination, difference in associated clinical features and other factors

As regards to the correlation of DTI parameters with the frequency and duration of the disease, the

results have been also conflicting. In our study, there was a significant correlation of DTI parameters with severity of the disease; the frequency, intensity and duration of migraine episodes, but no correlation was detected with the duration of the disease. Several studies have shown correlation with the frequency of migraine attacks [**13, 81, 88, 97**], some with the duration of the disease, in addition, [**66, 81**]**,** and others showed correlation only with the duration of the disease [**98**]**,** whereas several other studies didn't show correlation with either the frequency of the episodes or the duration of the disease [**78, 82, 99, 100, 101**]**.**

In our study, the abnormalities of evoked potentials and the metabolic changes also showed significant correlation with the frequency of the disease. We believe it is logic that high frequency disease, i.e. the more severe disease, is associated with more severe physiological, metabolic and microstructural changes and supporting evidence has been shown in discussion of the physiological and metabolic changes. However, this is still to be confirmed in further studies.

As regard to the duration of the disease, some studies showed no correlation, just as we have shown, but others detected significant correlation. This must be discussed in view of the following data. First, migraine is generally a benign self-remitting disease with decrease of the frequency of attacks in a large proportion of cases as the age advances [**26, 102**]. Significant correlation with the duration of the disease implies that migraine is a progressive disease with accumulation of the pathology overtime. This would make the prevalence of chronic migraine much greater, which is not the case. Second, The predictors of transformation to chronic migraine include, among several factors, the frequency of migraine episodes, but not the duration of the disease [**103, 104**]. Therefore, it is the severity, not the duration, of the disease which is associated with more significant physiological, metabolic and microstructural changes. Third, whether the metabolic and microstructural changes are a cause or a consequence of recurrent migraine episodes and whether these changes are reversible or permanent is still a matter of great debate [102]. Fourth, reduced FA and other DTI changes may be the result of white matter damage induced by migraine attacks [**81, 98**].

In view of these findings we propose that migraine without aura is not a single clinical entity, but it is heterogeneous; it comprises at least two distinct forms; a benign remitting form which will resolve with time with decrease of the frequency of episodes with age and a progressive form which accumulate pathological changes over time with increase of the frequency of migraine episodes and may be conversion to chronic migraine. We suggest that inclusion of different proportions of the 2 forms in different studies is the cause of the conflicting results.

Fractional anisotropy is sensitive to several types of pathological changes, but not specific. AD is sensitive to axonal pathology; decrease of AD may indicate axon loss or impairment of axonal integrity. RD is more specific to myelin structure and high RD may indicate demyelination or axon loss. High MD may indicate edema **[75, 105].** However, the interpretation of the collective set of FA, AD, MD and RD values is complex, and it is still difficult to translate this set of abnormalities into specific pathological disorders in migraine [**97**]**.** Moreover, DTI values are affected by each other [**75]** and change along the migraine cycle [**106**]. In addition, FA is known to decrease gradually with age [**99**].

In spite of the conflicting results of other studies and the difficult interpretation of DTI measures, we think that our results are significant and are reproducible and are indicative of pathological changes in migraine and can be employed, after confirmation in larger studies with longitudinal design, in the diagnosis and follow up of the progress of migraine and in research work for the treatment of the disease

5. Conclusion

The results of this study indicate occurrence of functional, metabolic and regional microstructural changes in the brain of migraine patients. VEPs indicated reduced habituation to repeated visual stimulation, and H-MRS examination indicated decrease of NAA/Cr and increase of MI/NAA and Cho/Cr PMRs in the thalamus and occipital lobe. Although the microstructural abnormalities reflected by changes in DTI values are still not well defined, these changes are highly suggestive of regional microstructural change. The brain changes showed correlation with severity of migraine disease but not with the duration of the disease. These changes may, at least in part, reflect mitochondrial dysfunction in migraine. The NAA/Cr PMR in the thalamus may serve as a biological marker that can be used in the assessment and follow up of mitochondrial dysfunction in migraine patients without aura and the response to prophylactic treatment. Further longitudinal studies, comprising examination of the patients in different stages of migraine cycle, are still needed to confirm the results of this study and facilitate employing of these results in clinical practice.

ETHICAL APPROVAL

As per international standard or university standard, a written ethical approval has been collected and preserved by the authors.

Consent

As per international standard or university standard, patient(s) written consent has been collected and preserved by the author(s).

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