Serum concentrations of neuron-specific enolase in pediatric migraine

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Recent studies suggest that migraine might be a progressive disease that causes neuronal damage, rather than being a benign headache disorder. The objective of the present study was to investigate the concentrations of neuron-specific enolase (NSE) in pediatric migraineurs in order to identify possible neuronal damage. Forty-one children and adolescents with migraine (mean age: 14.58±2.35 years, range: 7-17 years, 12 with aura) and 30 control subjects were included. Serum NSE levels were measured during the attack and repeated at least 7 days thereafter in the patients, and measurements were obtained once in the control group. There were no significant differences in NSE concentrations with respect to values during the attack versus pain-free period or between the patient and control groups. NSE levels did not differ according to the clinical variables, including the presence of aura, severity and duration of headaches, nor with the length of migraine. In conclusion, our study showed that NSE levels did not change during migraine attack in pediatric patients. Further studies with different markers are warranted to assess possible neuronal injury in pediatric migraine.

Key words: childhood migraine, neuronal injury, headache, pathophysiology.

Migraine is a chronic paroxysmal disorder characterized by recurrent episodes of headache with associated symptoms. Migraine is the most frequent type of headache among primary headaches in children[1]. Although it has been a known disease for a long time, the pathophysiology of migraine is not yet completely delineated. Many theories, such as vascular, neuronal or hypoxic, have been put forward in the formation of attacks. The occurrence of the attacks has been explained by cortically spreading depression that develops secondarily to cortical hypersensitivity, by vascular changes caused by cortical spreading depression and by stimulation of the trigeminovascular system[2]. United theory, central inhibition disorders, vulnerability to oxidative stress, and migrainous endotheliopathy are theories in discussion[2-6].

Recent publications suggest that migraine is a progressive disease rather than being a benign headache disorder[7]. Particularly, some publications suggest that there is an association between migraine and stroke[8]. There are some lesions similar to silent infarct in the brain in those with a migraine diagnosis, and some specific disorders such as CADASIL (cerebral autosomal dominant arteriopathy, subcortical infarct, leukoencephalopathy) and MELAS (mitochondrial encephalomyopathy, encephalopathy, lactic acidosis, and stroke-like episodes) support this hypothesis[9,10]. The risk of stroke during a typical migraine attack has been found to be 0.5–1.5% among all ischemic strokes in wide series studies[11-13]. Migrainous infarction has been suspected in the etiology in 1.4%-13% of individuals presenting with stroke[12,13]. These studies suggest that permanent damage might appear during migraine attacks.

The blood-brain barrier (BBB) deteriorates in many circumstances, including neoplastic diseases, hypertensive encephalopathy, status epilepticus, and stroke[14,15]. There are several studies in the literature that show a disruption in BBB during migraine attack[16-18]. The

elevation of some neuronal markers, such as serum matrix metalloproteinase-9 and S100B proteins in serum, during migraine attack has also supported this hypothesis[19,20].

Neuron-specific enolase (NSE) is an enzyme localized in neurons in the brain and it plays a crucial role in glycolysis[21-23]. Its biological half-life is approximately 24 hours[24]. Raised serum concentrations of NSE reflect neuronal cell injury as described in cerebrovascular incidences and trauma, and this increase has been positively correlated with the degree of the damage[25-27]. For this reason, NSE is assumed to be useful to estimate neuronal injury and clinical outcome in various neurological conditions associated with neuronal damage[28-30]. Although the best method is to determine NSE concentration in cerebrospinal fluid (CSF), it has been demonstrated that serum concentrations could be informative in disorders involving BBB disruption[31,32]. It was reported that maximal levels of NSE were reached in the serum between 7 and 18 hours after stroke onset[33].

Neuron-specific enolase (NSE) serum concentration has been determined in a few studies performed in adult migraineurs[19,34]. No elevation of NSE has been found in these studies, although an elevation in CSF NSE levels was found in a study carried out in a few migraine patients[35]. NSE concentrations have not been previously investigated in pediatric migraine patients.

In this study, the serum concentrations of NSE were determined in pediatric migraineurs during and after the migraine attack in order to identify possible neuronal damage and to gain further insight into migraine pathophysiology.

Material and Methods

Patients

The migraine group consisted of 41 consecutive children and adolescents attending the child neurology outpatient clinic at Başkent University Ankara Hospital and fulfilling the criteria for migraine without aura and with aura, according to the International Classification of Headache Disorders, 2nd Edition[36]. The control group was composed of healthy children with no history of headache or pain and no history of migraine ascertained with detailed questioning in the first-degree relatives. All patients and control subjects received a complete physical and neurologic examination. The migraine group was administered a structured interview concerning the characteristics of the headache, associated symptoms and medications. Based on a 10-point verbal pain severity scale, the headache at the time of enrollment was defined as mild (score 1-3), moderate (4-6), severe (score 7-8), or very severe (score 9-10).

Patients with chronic systemic disease, neuroendocrine tumor, traumatic brain injuries, stroke, multisystemic trauma, chronic neurologic and psychiatric problems, hypertension, anemia, and other chronic forms of headaches were excluded. None of the study subjects had taken analgesics or other medications for at least one week prior to the study.

The study was approved by the Başkent University Faculty of Medicine Ethics Committee, and a written informed consent was obtained from all parents.

Analysis of Neuron-Specific Enolase

Blood samples (3 ml) were collected from a peripheral arm vein into a standard biochemistry Vacutainer. The migraine patients had been informed about the study during their clinic visits and they were instructed to inform the investigators immediately at the onset of migraine attack. To exclude the potential influence of a previous attack, a minimum 72-hour pain-free interval before the current attack was required for the enrollment of the patient. It was reported that maximal levels of NSE were reached in serum between 7 and 18 hours after stroke onset. First blood samples of the migraine patients were obtained within 6 to 24 hours of the headache attack having at least moderate severity. Second samples were taken seven days after the attack to serve as the baseline values for each patient. Patients having another attack in this interval or whose second blood samples could not be obtained were excluded from the study. Serum NSE concentration was obtained once in the control group.

The samples were transferred to the biochemistry laboratory within one hour for biochemical analysis and centrifuged immediately. None of the samples were hemolyzed. A commercially

Table I. Demographic Characteristics of the Study Groups

		Migraine	Control	P	Migraine without aura	Migraine with aura	p
Age (yr±SD)		14.5±2.4	14.1±2.1	p>0.05	14.4±2.5	15±1.9	p>0.05
Sex	Female	25	18	p>0.05	17	8	p>0.05
	Male	16	12		12	4	
Total		41	30		29	12	

available electrochemiluminescence immunosorbent assay, Elecsys® (Roche Diagnostics GmbH, Mannheim) was used for the determination of NSE concentration. This laboratory method is available as a daily routine in our hospital, and the results are obtained on the same day. The 95% confidence interval was 15.7-17.0 ng/ml. The error ratio of the test was <0.5 ng/mg.

Statistical Analysis

All statistics were calculated using the SPSS software (Statistical Product and Services Solutions, version 16.0, SPSS Inc, Chicago, IL, USA). Results for all measurements are expressed as mean ± standard deviation (SD), and median values were also given when a nonparametric test was used for the statistical analysis. Descriptive statistics were used for age, sex and migraine type. In the analysis of categorical data, Fisher's exact test and likelihood ratio test were used. The adjustment of permanent variables was controlled with Shapiro-Wilks test. Wilcoxon signed rank test was used for comparison for the two time points of measurement ("migraine attack" and "pain-free period") within the migraine group. For comparisons between the patients and controls, Mann-Whitney U tests were conducted. In comparison of more than two groups, Kruskal-Wallis one-way variant analysis was done. Pearson correlation analysis was used to evaluate the correlation of NSE level with age and sex.

Values of statistical significance are indicated as p<0.05.

Results

The mean age of the 41 migraine patients was 14.5±2.4 years (range: 7-17 years), and 25 were female; the mean age of the control group was 14.1±2.1 years, and 18 were female. Age and sex distributions of the patient and control groups were similar (p>0.05) (Table I). In the migraine group, 12 patients had migraine with aura; 8 were female. Age and sex distributions of the patients between the migraine subgroups according to the presence of aura were similar (p>0.05 for both, Mann-Whitney U and Fisher's exact tests, respectively) (Table I). The disease duration was similar between the migraine subgroups, and there was no significant difference in the attack severity and duration (likelihood ratio test, p>0.05 for all).

NSE levels in the migraine group during the attack and the pain-free period were 15.73±6.06 ng/ml and 14.66±5.6 ng/ml, respectively. There was no significant difference in values between the migraine attack and pain-free periods (p=0.347, Wilcoxon signed ranks

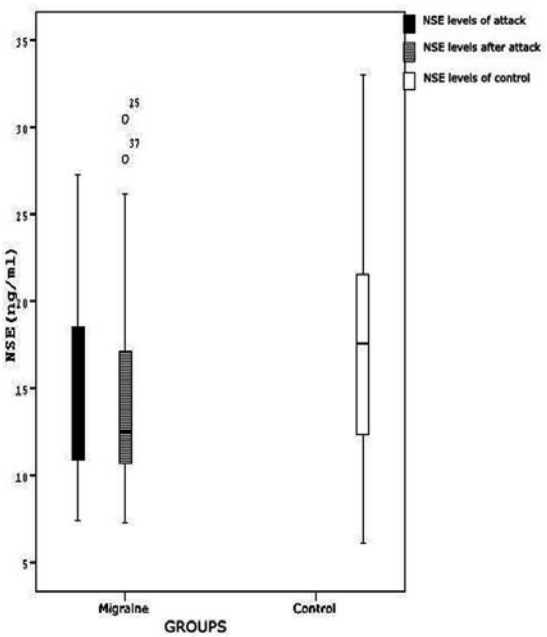

Fig. 1. Neuron-specific enolase (NSE) concentrations in the migraine patients and control group. Box plots show median, interquartile values and 10th and 90th percentiles (horizontal bar indicates median). Circles indicate outliers and numbers denote case number of outlier.

test). NSE levels in the control group were 17.37±6.64 ng/ml. There were no significant differences in NSE values between the migraine attack period and the control group (p=0.233, Mann-Whitney U test) or between the pain-free period and the control group (p=0.057, Mann-Whitney U test) (Fig. 1).

In the migraine patients with aura, NSE levels during the attack were 12.75±5.11 ng/ml and during the pain-free period were 15.84±4.36 ng/ml, and there was no significant difference between them (p=0.239, Wilcoxon signed ranks test). These values were also found to be statistically similar to the values of the control group (Mann-Whitney U test, $p<0.05$ for both).

In the migraine without aura group, NSE levels during the attack were 16.24±5.95 ng/ml and during the pain-free period were 14.07±6.10 ng/ml (p=0.056, Wilcoxon signed ranks test). No significant differences in NSE concentrations were detected between the values of the patients without aura and the controls (Mann-Whitney U test, $p<0.05$ for both).

Neuron-specific enolase (NSE) levels did not differ according to the clinical variables including severity and duration of the headache attack or the length of migraine (Table II). Finally, NSE levels were not correlated with age or sex.

Table II. NSE Concentrations During the Attack and Post-Attack Periods According to Severity and Duration of the Migraine Attack and Disease Duration

		NSE level during attack (ng/ml)					NSE level in post-attack period (ng/ml)					P
		n	Mean	Median	Min	Max	n	Mean	Median	Min	Max	
Severity	Moderate	6	16.7±8.2	15.0	8.1	27.3	6	15.7±5	13.9	10.3	24.1	0.917
	Severe	20	16±4.5	15.8	8.5	26.5	20	15±5.5	12.9	7.9	28.2	0.390
	Very severe	15	14.9±7.2	12.5	7.5	34.2	15	13.7±6.3	11.9	7.3	30.5	0.426
	P	0.417					0.295					
Duration of migraine	<1 year	14	16.5±8.2	15.4	7.4	34.2	14	15.9±5.2	13.6	9.3	25.3	0.875
	1-3 years	10	13.3±5	12.2	8.5	25.6	10	12.1±2.7	10.9	9.9	18.1	0.799
	>3 years	17	16.4±4.1	17	9.9	24.6	17	15±6.9	12.5	7.3	30.5	0.156
	P	0.191					0.196					
Duration of headache attack	1-4 hr	22	15.9±6.2	15.5	7.5	34.2	22	13.8±4.1	12.7	7.9	24.1	0.149
	4-12 hr	14	16.4±6.3	13.9	9.2	27.3	14	16.4±7	14.8	7.3	30.5	0.925
	12-24 hr	5	12.6±4.3	12.4	8.4	19.8	5	13.2±7.2	9.9	9.5	26.2	0.893
	P	0.404					0.812					

NSE: Neuron-specific enolase.

Discussion

Neuron-specific enolase (NSE) is a noninvasive marker for neuronal injury and was described to be elevated in CSF and serum in different neurological diseases, including acute ischemic stroke, subarachnoid bleeding, Creutzfeldt-Jacob disease, hypoxic brain damage, head injuries, and status epilepticus[15,37-40]. The elevation in this enzyme level has been associated with the degree of the damage in cerebrovascular disorders[27,31]. Lafon-Cazal et al.[41] stated that even transient neuronal damage can cause NSE elevation in serum. Increased serum concentrations of NSE also suggest a disruption of the BBB[32,37,38].

Neuron-specific enolase (NSE) serum concentration has been determined in a few studies performed in adult migraineurs, and no elevation in NSE was found in these

studies[19,34]. There were also two studies investigating the NSE concentration in the CSF of patients with migraine. These studies showed conflicting results, but it is difficult to make a conclusion since they included a very small number of patients with migraine[34,35].

Neuron-specific enolase (NSE) concentrations have not been investigated previously in pediatric migraine patients. In this study, we aimed to establish whether or not there was an elevation in serum NSE levels during migraine attacks. Although the best method would be to determine NSE concentration in the CSF, it is deferred due to ethical reasons. NSE concentrations were determined in migraine patients during the attack and the interictal period, and no elevation was found during the attack. These values were also similar with those of the control group. These results are in concordance with the results of the above-mentioned studies performed in adults[19,34]. We also investigated NSE concentrations according to the clinical variables, including severity and duration of the headache attack and length of the migraine, and did not find any elevation even in the more severe form of the disease, which further emphasizes the consistency of our results.

There are some publications suggesting an association between migraine and aura and stroke[7,8]. Thus, one might assume that neuronal damage is more likely to occur in patients with migraine with aura than in patients with migraine without aura. However, our finding of similar results between the migraine subgroups does not support this hypothesis.

In conclusion, the present study showed that NSE levels did not change during migraine attacks in pediatric patients. Further studies with different markers are warranted to assess possible neuronal injury in pediatric migraine.

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