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Prophylactic Drugs and Cytokine and Leptin Levels in Children With Migraine

Tugba Hirfanoglu, MD*, Ayse Serdaroglu, MD*, Ozlem Gulbahar, MD†, and Ali Cansu, MD*

The study objective was to evaluate levels of the cytokines tumor necrosis factor α, interleukin-1β, and interleukin-6 and of leptin, and then to determine the relationship between these levels and clinical responses in children with migraine after prophylactic therapy with one of four drugs. In all, 77 children who needed prophylactic drugs were treated with cyproheptadine, amitriptyline, propranolol, or flunarizine. Serum levels of the cytokines and leptin were measured before and 4 months after the treatment. Results were compared by drug for headache frequency, severity, and duration, the PedMIDAS score, and levels of each cytokine and of leptin. Each of the four drugs not only decreased the frequency and duration but also the severity of headache, and the PedMIDAS score. None of the drugs was found to be superior to others in terms of reduction in cytokine levels ($P > 0.05$). Both cyproheptadine and flunarizine (but not amitriptyline and propranolol) caused an increase in leptin levels ($P < 0.05$). These data suggest that cytokine levels are related to clinical responses, and might help in objective evaluation of clinical response in migraine. To our knowledge, the present study is the first trial to compare the effects of prophylactic drugs, cytokine levels, and leptin levels in children with migraine. **© 2009 by Elsevier Inc. All rights reserved.**

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Introduction

Migraine is a very common neurologic disorder in children, and is almost as common in adults [1-10]. Of children with migraine, 30% experience very frequent and disabling attacks requiring daily preventive medications. There are few reports from studies investigating the underlying mechanism of migraine and few controlled clinical investigations of treatment of childhood and adolescence migraine [3,4,8,11,12].

Although the mechanism leading to the typical headache in migraine is still unknown, the etiology of the disease is surely multifactorial, including factors such as alcohol consumption, smoking, nutrition, sleep disorders, stress, environmental change, exercise, and menstrual cycle [4,13-15]. The studies conducted on migraine mechanism pointed to genetic factors, immunoglobulin and complement levels, and differences in lymphocyte subsets and changes in cytokine profiles [14-17]. Cytokines, which have been demonstrated to induce headaches and migraine, are considered to be mediators of pain and inflammation [13]—although the findings remain controversial. A fluctuation of circulating cytokine levels in primary headaches is evident [14,15]. Recently, it has been reported that high leptin levels also cause an increase in pain sensitivity in mice, and that ibuprofen treatment leads to additional decreases in serum leptin concentrations [18].

Specifically, migraine may trigger inflammation due to personal tendency, and altered immunoglobulin, complement, and cytokine levels could cause headache in migraine and affect the response to medications [4,13,16,18].

Cyproheptadine (an antihistaminic and antiserotonergic), amitriptyline (a tricyclic antidepressant), propranolol (a serotonin receptor blocker), and flunarizine (a calcium channel blocker) have been the most widely used prophylactic agents in children with migraine [3,7,9,19]. Generally, patients are started on these agents and monitored on the basis of clinical criteria [3,7,11,19]. A limited number of reported studies have investigated the relationship between the effects of these drugs on cytokine levels and their clinical effects [20-24]. Thus, reliable criteria are lacking that could be used to evaluate relationships between clinical response and cytokine levels.

From the Departments of *Pediatric Neurology and †Biochemistry, Gazi University Faculty of Medicine, Ankara, Turkey.

Communications should be addressed to:
Dr. Hirfanoglu; Turgut Reis Cd; Serefli Sk; Doratli apt, 4/4; 06580 Mebusevleri; Ankara, Turkey.
E-mail: tugbahirfanoglu@yahoo.com, tluleci13@yahoo.com.
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The objective of the present study was to evaluate levels of tumor necrosis factor α, interleukin-1β, interleukin-6, and leptin and to determine the relationship between these levels and clinical responses in children with migraine after cyproheptadine, amitriptyline, propranolol, or flunarizine therapy.

Materials and Methods

This study included 77 children who came to the Department of Pediatric Neurology in Faculty of Medicine of Gazi University, between January 2005 and May 2007 because of migraine that was diagnosed according to ICD-II criteria [25] (68 patients without aura; 9 patients with aura). Mean age was 12.98 $\pm$ 3.11 years. The male-to-female ratio was 33:44.

The study also included 19 healthy control subjects (mean age, 12.26 $\pm$ 2.8 years) who were recruited from children who came to the Gazi University, Faculty of Medicine, Department of Healthy Children for routine care appropriate for their ages; they had no personal or family history of migraine, and no history of inflammatory, infectious, or immune disease or abnormal C-reactive protein plasma levels. The male-to-female ratio and sociodemographic features were also similar to those of the study group.

Inclusion criteria and indications for prophylaxis were recurrent migraine attacks (at least 3 per month) that significantly interfered with daily activities (based on the headache history and monthly headache diary, including headache findings for frequency, severity, duration, and PedMIDAS score) despite failure, overuse, or contraindication of acute therapy, comparative cost of acute and preventive therapies, patient and family preferences, and presence of uncommon migraine conditions [3,4,8,9]. Uncommon migraine was diagnosed based on ICD-II criteria. If patients needed to receive frequent acute therapy and visit the hospital, preventive therapy was considered.

Exclusion criteria were recurrent migraine at a frequency of fewer than 3 per month and that did not significantly interfere with daily activities; a medical history including the intake of prophylactic medication for migraine; and a history of inflammatory, infectious, or immune disease or abnormal C-reactive protein plasma levels. Patients having autoimmune disorders, patients with chronic migraine, and those with drug overuse were also excluded.

All patients received a comprehensive neurologic examination and were also evaluated for complete blood count, liver and renal function, electrolytes, as well as electrocardiography (and, if needed, electroencephalography and cranial magnetic resonance imaging).

According to these criteria and based on the information in the relevant literature, children under 12 years of age were started on cyproheptadine (n = 17) at 0.2 mg/kg per day. Children older than 12 years were started on amitriptyline (n = 21) at 0.5 mg/kg per day, or on propranolol (n = 20) at 10-40 mg/day), or on flunarizine (n = 19) at 5-10 mg/day [3,4,7,8]. All patients completed the 4-month treatment.

To evaluate the intensity of pain, the Facial Analog Scale was applied and the resulting point was completed to 10 and then recorded [26,27]. Treatment response was basically evaluated with the PedMIDAS instrument [28]. For each patient, a monthly headache diary including headache findings, frequency, severity, duration, and PedMIDAS was kept.

Serum tumor necrosis factor α, interleukin-1β, interleukin-6, and leptin levels of each patient were measured twice (i.e., before and after the 4-month treatment), to evaluate the response of the patient to the treatment. Blood samples were obtained during headache-free intervals and at least 4 days after the last attack. Peripheral blood samples (ethylene-diamine-tetraacetic acid anticoagulated) were drawn with the patient in supine position from an antecubital vein. They were immediately separated by centrifugation and the plasma was stored at -80°C. Tumor necrosis factor α, interleukin-1β, and interleukin-6 levels were studied using an enzyme-linked immunosorbent assay kit according to the manufacturer's instruction (RayBiotech, Norcross, GA); the sensitivity of the assays was 15 pg/mL, 0.3 pg/mL, 6 pg/mL, respectively. Leptin levels were studied using another enzyme-linked immunosorbent assay kit (AviBion; Orgenium Laboratories, Helsinki, Finland) with 1 ng/mL sensitivity.

Eight variables were compared for before and after the 4-month treatment: frequency, severity, and duration; PedMIDAS score; and levels of tumor necrosis factor α, interleukin-1β, interleukin-6, and leptin.

Statistical evaluations were made using the SPSS 11.5 software package (SPSS, Chicago, IL). Definitive analyses were conducted and mean ages of the patients were expressed in mean $\pm$ standard deviation. For other analyses, nonparametric tests were applied. To compare the values before and after treatment, a Wilcoxon signed test was used. Kruskal-Wallis one-way analysis of variance (P = 0.05) and Mann-Whitney U test (P = 0.01) were used to compare the before and after treatment values between the study group and the control group. The percentage change, calculated as %Change = [(BeforeTreatment $-$ AfterTreatment)/BeforeTreatment] $\times$ 100, was applied to the Kruskal-Wallis test to determine the best drug in terms of its effect on headache frequency, duration, and severity, the PedMIDAS score, and levels of tumor necrosis factor α, interleukin-1β, interleukin-6, and leptin after the treatment.

Spearman's rank correlation coefficients (ρ) were used when the continuous variables did not show a normal distribution in a test of normality. The ρ-value was applied to evaluate the relationships among headache frequency, duration, and severity, the PedMIDAS score, and levels of tumor necrosis factor α, interleukin-1β, interleukin-6, and leptin. A P-value of < 0.05 was considered significant.

The consent of the Ethical Committee of Faculty of Medicine in Gazi University was obtained, and family informed consent forms were signed by the families of each patient.

Results

The mean age was 10.2 $\pm$ 2.7 years at the beginning of participation in this study and 12.98 $\pm$ 3.11 after completion of the monthly headache diary. Before treatment, the mean headache frequency was 6.5 $\pm$ 2.4 (range, 3-15) per month; the mean severity was 8.16 $\pm$ 1 on a 10-point scale, low to high; the mean duration was 3.4 $\pm$ 1.35 hours; and the mean PedMIDAS score was 62.18 $\pm$ 11.9, with no distribution applied according to drug group.

The findings for headache frequency, duration, and severity, the PedMIDAS score, and levels of tumor necrosis factor α, interleukin-1β, interleukin-6, and leptin are presented, by drug, in Table 1.

Cyproheptadine

With cyproheptadine, there was a significant decrease in frequency, severity, and duration of headache and in PedMIDAS scores after treatment ($P < 0.05$). The PedMIDAS score was correlated with headache frequency (ρ = 0.743, P = 0.001), but not with severity or duration ($P > 0.05$).

Cytokine levels (tumor necrosis factor α, interleukin-1β, interleukin-6) significantly decreased and leptin levels increased after treatment ($P < 0.05$), compared with levels before treatment with cyproheptadine. For comparison with the control groups before treatment only tumor necrosis factor α and interleukin-6 levels were significantly higher ($P < 0.01$). After treatment, no differences were found among tumor necrosis factor α, interleukin-1β, and interleukin-6 levels between the control group and the study group ($P > 0.01$).

There was a significant correlation between interleukin-6 levels and headache severity (ρ = 0.558, $P < 0.05$). No correlations were observed for the other cytokines (tumor

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Table 1. Clinical response and serum cytokine (pg/mL) and leptin (ng/mL) levels in children before and after treatment with one of four drugs for migraine*

Variable	Before Treatment		After Treatment		*P* value†
	Mean ± S.D.	Median (range)	Mean ± S.D.	Median (range)	
Headache frequency, no./month					
Cyproheptadine	6.5 ± 1.94	6 (4-12)	1.29 ± 0.46	1 (1-2)	0.000
Amitriptyline	8.14 ± 2.1	8 (5-14)	1.47 ± 0.51	1 (1-2)	0.000
Propranolol	6.3 ± 2.87	6 (3-15)	1.6 ± 0.48	2 (1-2)	0.000
Flunarizine	5.1 ± 1.52	5 (3-7)	1.36 ± 0.49	1 (1-2)	0.000
Headache duration, hours					
Cyproheptadine	3.9 ± 1.12	3.5 (2.5-6)	1.47 ± 0.44	1.5 (1-2)	0.000
Amitriptyline	3.6 ± 1.62	3 (1.5-7)	2.11 ± 0.72	2 (1-4)	0.001
Propranolol	2.9 ± 1	2.7 (1.5-6)	2.3 ± 0.49	2 (1.5-3)	0.001
Flunarizine	3.14 ± 1.38	3 (2-7)	2.68 ± 0.86	2.5 (2-5)	0.017
Headache severity, 10-point scale, low to high					
Cyproheptadine	8.58 ± 0.93	9 (7-10)	3.2 ± 1.6	2 (2-6)	0.000
Amitriptyline	7.6 ± 1	8 (6-10)	4.7 ± 1.14	5 (3-6)	0.000
Propranolol	8.2 ± 1.01	8 (7-10)	3.75 ± 1.48	4 (2-6)	0.000
Flunarizine	8.2 ± 0.99	8 (7-10)	4.36 ± 0.76	5 (3-5)	0.000
PedMIDAS score, low to high disability‡					
Cyproheptadine	59 ± 11	57 (48-90)	10.35 ± 3.6	10 (4-17)	0.000
Amitriptyline	63 ± 12.73	59 (47-95)	6.6 ± 2.87	5 (4-12)	0.000
Propranolol	62 ± 11.1	59 (49-88)	5.6 ± 1.42	6 (4-7)	0.000
Flunarizine	64 ± 12.92	65 (45-99)	6.2 ± 2.42	6 (3-10)	0.000
Tumor necrosis factor α, pg/mL					
Cyproheptadine	18.9 ± 4.3	17 (15-28)	15.9 ± 1.14	15 (15-18)	0.001
Aamitriptyline	19.7 ± 4.47	19 (15-32)	16.5 ± 2	15.7 (15-22.8)	0.001
Propranolol	17.88 ± 3.5	17.2 (15-28.9)	16 ± 1.42	15.5 (15-20.67)	0.002
Flunarizine	18.7 ± 4.8	17 (15-32)	16.7 ± 2	16 (15-22.8)	0.003
Interleukin 1β, pg/mL					
Cyproheptadine	8.76 ± 15.1	0.3 (0.3-52.24)	5.7 ± 8.9	0.3 (0.3-30)	0.018
Amitriptyline	6.3 ± 11.8	0.3 (0.01-36.8)	5.8 ± 10.9	0.3 (0.01-33.4)	0.028
Propranolol	5.4 ± 7.9	1.4 8 (0.3-28.6)	4.5 ± 7.2	0.8 (0.3-25.6)	0.028
Flunarizine	10.3 ± 13.4	6.3 (0.3-55.5)	6.4 ± 6.5	6.3 (0.3-21.6)	0.027
Interleukin 6, pg/mL					
Cyproheptadine	16.1 ± 13.4	9.2 (6-49.5)	9.3 ± 6.2	96 (5.7-23.6)	0.080
Amitriptyline	9.4 ± 5.3	6 (6-20.9)	7.6 ± 3.7	6 (4-20.9)	0.036
Propranolol	17.3 ± 14.6	11.4 (6-54.6)	8.3 ± 6.4	6 (2.4-32.7)	0.003
Flunarizine	12.3 ± 8	9.1 86-34.6)	9 ± 4.8	6 (6-22.6)	0.012
Leptin, ng/mL					
Cyproheptadine	3.1 ± 0.6	2.9 (2.4-4.3)	6.6 ± 3.3	5.3 (2.8-13.4)	0.001
Amitriptyline	3.3 ± 1	3 (2.4-6.7)	4.2 ± 2.6	2.8 (2.2-12.3)	0.170
Propranolol	4.4 ± 2.6	3.4 (2.5-11.3)	3.9 ± 2.2	3.2 (2.3-11.3)	0.241
Flunarizine	3 ± 0.7	2.9 (2-4.2)	5.8 ± 3.7	3.7 (2.8-12.8)	0.001

* Sample size $N = 77$ (cyproheptadine, $n = 17$; amitriptyline, $n = 21$; propranolol, $n = 20$; flunarizine, $n = 19$). Cyproheptadine was prescribed only for children under 12 years of age; the other three drugs, only for children older than 12.

† Wilcoxon signed test.

‡ The PedMIDAS grading scale for disability due to migraine: 0-10, little to none; 11-30, mild; 31-50, moderate; >50, severe disability.

Abbreviation:
S.D. = Standard deviation

necrosis factor α, interleukin-1β) with headache frequency, duration, severity, or PedMIDAS score, before or after treatment ($P > 0.05$).

Flunarizine

Before treatment, the mean value for headache frequency was 5.1 ± 1.52 per month; for duration, 3.14 ± 1.38 hours; for severity, 8.26 ± 0.99; and for PedMIDAS score, 64 ± 12.92. After treatment, these values decreased to 1.3 ± 0.49 per month, 2.68 ± 0.86 hours, 4.3 ± 5, and 6.26 ± 2.42, respectively ($P < 0.05$). The PedMIDAS score was not correlated with frequency, duration, or severity of the headaches ($P > 0.05$).

Within the treatment group, levels of all the cytokines (tumor necrosis factor α, interleukin-1β, and interleukin-

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6) significantly decreased, and leptin levels increased, compared with levels before treatment ($P < 0.05$). After treatment, interleukin-1β levels were statistically higher ($P < 0.01$) than those of the control group. Compared with the control group, no difference was observed for levels of tumor necrosis factor α, interleukin-6, and leptin in the treatment group before or after treatment ($P > 0.01$).

No correlations were observed between cytokine levels and the frequency, duration, and severity of headaches and the PedMIDAS score ($P > 0.05$).

Amitriptyline and Propranolol

The same statistical results were determined for both amitriptyline and propranolol in terms of clinical response (Table 1).

Headache frequency, duration, severity, and PedMIDAS score were significantly lower at 4 months after the patients started to take amitriptyline or propranolol ($P < 0.05$). No correlations were found between the frequency, duration, and severity of headache and PedMIDAS before and after the treatment with each drug ($P > 0.05$). Tumor necrosis factor α, interleukin-1β, and interleukin-6 levels after treatment were significantly lower than before treatment ($P < 0.05$), but leptin levels were not significantly different ($P > 0.05$). After treatment, levels tumor necrosis factor α, interleukin-1β, and interleukin-6 levels did not differ between treatment and control group ($P > 0.01$).

No correlations were found between these cytokines and headache frequency, duration, severity, or PedMIDAS score before or after the treatment ($P > 0.05$).

Comparison of Drug Groups

The level of improvement for frequency, duration, and severity of headache, the PedMIDAS scores, and levels of tumor necrosis factor α, interleukin-1β, interleukin-6, and leptin was compared among the four drug groups (Table 2).

Cyproheptadine proved to be the most effective drug in its capacity to reduce duration (62.2% improvement) ($P < 0.05$). Amitriptyline was better than flunarizine in reducing the duration (33% improvement vs 16.3%) ($P < 0.05$).

Cyproheptadine was most effective in alleviating headache severity (61.5% improvement), followed by propranolol (54.3%) ($P < 0.05$).

Amitriptyline was most effective in reducing headache frequency (80.1% improvement), followed by propranolol (78.8%) and flunarizine (71.5%) ($P < 0.05$).

None of the drugs was superior in terms of percentage change in levels of tumor necrosis factor α, interleukin-1β, or interleukin-6 ($P > 0.05$). Thus, all the drugs had similar effect on decreasing cytokine levels.

Comparison of cytokine levels in patients who had a 50% reduction in headaches and in patients who did not revealed no differences in the rates of reduction of cytokine level ($P > 0.05$).

Cyproheptadine and flunarizine showed the highest change in leptin levels after treatment (120% and 90%, respectively), compared with the other two drugs ($P < 0.05$). However, there was no significant percentage change when cyproheptadine and flunarizine were compared with each other ($P > 0.05$).

Discussion

Migraine is a common disorder, characterized by recurrent episodes of headache and associated symptoms [25]. In general practice, cyproheptadine, amitriptyline, propranolol, and flunarizine are the agents commonly prescribed in children with migraine [3,4,7,8]. There are few controlled pediatric trials to justify this practice, and these have largely yielded conflicting data [20-24,29-31].

In the present study, all four drugs not only decreased the frequency and duration (in accord with previous investigations), but also significantly decreased the severity of headache and the PedMIDAS score. The most effective drug in terms of frequency reduction was amitriptyline (80.1%), and the most effective drug in terms of reducing duration (62%) and severity (61.5%) was cyproheptadine.

On the other hand, to develop effective treatment options it is important to understand the underlying mechanisms of migraine [4]. The presumably multifactorial pathogenesis of migraine remains unclear, but there is much evidence for association with immune system modification, and a role for cytokines has been suggested [13-16]. Cytokines are important mediators of the immune and inflammatory pathways and their receptors are widely expressed in the central nervous system [14,15]; however, few studies examined cytokine levels in patients with migraine [13,15].

In animal studies, injection of tumor necrosis factor α induces a dose-dependent hyperalgesia by activation or sensitization of the nerve terminals [16,17,32]. This hyperalgesia is also mediated via an induced release of interleukin-1β [17,32]. Pretreatment with cyproheptadine (which is an antihistaminic and antiserotonergic) inhibited the brain vascular permeability induced by forced swimming in rats, suggesting that both histamine and serotonin may be involved in blood-brain permeability in rodents [32].

Proinflammatory cytokines such as tumor necrosis factor α, interleukin-1β, interleukin-6, and anti-inflammatory cytokines such as interleukin-10, interleukin-4, interleukin-3, and interleukin-2 have been reported to play a significant role in the modulation of pain threshold and they could contribute to trigeminal nerve fibers sensitization [11,12,17,30,31,33,34]. Several studies have demonstrated a fluctuation of circulating cytokine levels in primary headaches [16,17], although with conflicting results. Decreased levels of interleukin-4, interleukin-6, and interleukin-2 were observed in some studies [14,35,36], but in other studies increased levels of tumor necrosis factor α, interleukin-

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Table 2. Percentage change in clinical response and serum cytokines and leptin levels in children before and after treatment with one of four drugs for migraine*

Variable	Percentage Change		*P* value†
	Mean ± S.D.	Median (range)	
Frequency			0.010
Cyproheptadine	78.8 ± 8.8	83.3 (60.0-91.7)	
Amitriptyline	80.1 ± 9.8	80.0 (60.0-92.9)	
Propranolol	68.2 ± 19.7	71.4 (33.3-86.7)	
Flunarizine	71.5 ± 10.3	66.7 (60.0-85.7)	
Duration			0.000
Cyproheptadine	62.2 ± 7.3	63.6 (50.0-71.4)	
Amitriptyline	33.3 ± 29.2	33.3 (0.0-76.9)	
Propranolol	16.3 ± 17.9	15.5 (0.0-66.7)	
Flunarizine	9.9 ± 17.1	0.0 (0.0-50.0)	
Severity			0.001
Cyproheptadine	61.5 ± 19.0	71.4 (25.0-80.0)	
Amitriptyline	36.5 ± 20.4	28.6 (0.0-66.7)	
Propranolol	54.3 ± 18.7	56.3 (14.3-77.8)	
Flunarizine	45.8 ± 13.6	37.5 (28.6-70.0)	
PedMIDAS score			0.002
Cyproheptadine	82.0 ± 6.8	80.0 (73.5-93.4)	
Amitriptyline	88.7 ± 6.2	91.5 (75.0-95.8)	
Propranolol	90.8 ± 2.2	90.8 (86.5-94.4)	
Flunarizine	89.9 ± 4.1	88.0 (83.3-95.7)	
Tumor necrosis factor α			0.421
Ayproheptadine	13.0 ± 13.3	6.2 (0.0-39.3)	
Amitriptyline	13.9 ± 12.4	12.1 (0.0-40.9)	
Propranolol	8.6 ± 10.1	6.6 (6.8-31.5)	
Flunarizine	8.2 ± 10.9	5.5 (0.0-40.9)	
Interleukin 1β			0.840
Cyproheptadine	11.4 ± 20.5	0.0 (0.0-62.7)	
Amitriptyline	6.0 ± 17.6	0.0 (0.0-80.9)	
Propranolol	11.5 ± 24.1	0.0 (0.0-93.2)	
Flunarizine	17.2 ± 29.4	0.0 (0.0-95.5)	
Interleukin 6			0.117
Cyproheptadine	26.6 ± 29.7	19.1 (0.0-74.6)	
Amitriptyline	11.4 ± 22.3	0.0 (3.8-78.9)	
Propranolol	30.8 ± 35.9	14.2 (0.0-89.0)	
Flunarizine	17.5 ± 23.2	0.0 (0.0-62.9)	
Leptin			0.000
Cyproheptadine	−120.3 ± 120.4	−113.2 (−395-2.78)	
Amitriptyline	−28.8 ± 77.0	−6.5 (−290.1-64.0)	
Propranolol	6.1 ± 32.0	0.0 (−85.8-67.6)	
Flunarizine	−91.8 ± 112.3	−44.0 (−358.8-24.5)	

* Sample size $N = 77$ (cyproheptadine, $n = 17$; amitriptyline, $n = 21$; propranolol, $n = 20$; flunarizine, $n = 19$). Cyproheptadine was prescribed only for children under 12 years of age; the other three drugs, only for children older than 12.

† Kruskal-Wallis test.

Abbreviation:
S.D. = Standard deviation

1β, interleukin-5, and interleukin-4 were observed [37]. Other researchers have found no difference in tumor necrosis factor α, interferon γ, and interleukin-10 serum levels in patients with migraine, when evaluated on the basis of standard values [38,39]. In the present study, increased levels of interleukin-6, tumor necrosis factor α, and interleukin 1β levels, compared with control levels, before treatment supported the hypothesis that there is an increased basal cytokine level in patients with migraine and that it leads to susceptibility to pain and inflammation. Moreover, both polymorphism in the gene for tumor necrosis factor α and mast cell stabilization deficiency may be associated with a susceptibility to migraine [13,17,37,40-46].

There are a limited number of studies on the relation between cytokine levels and prophylactic drugs in patients with migraine, and these include only adult migraine cases, offering no data for migraine in childhood [16,17]. In an earlier study, tumor necrosis factor α levels have been found to decrease after 2 months of amitriptyline therapy [47]. Cyproheptadine was also shown to inhibit mast cell activation, as did the heterocyclic histamine-receptor antagonist hydroxyzine, which also inhibited neurogenic inflammation [48]. These additional properties of hydroxyzine, along with its weak anxiolytic action, may explain why it is useful in treatment of pain [32,48,49].

Recently, Rozen and Swidan [50] reported an elevation of tumor necrosis factor α levels in cerebrospinal fluid of adult patients with daily persistent headache, chronic migraine, and posttraumatic headache, and almost all the patients showed minimal to no improvement during aggressive inpatient treatment. Thus, the authors suggested that persistent elevation of cerebrospinal fluid tumor necrosis factor α levels might be one of the causes of treatment refractory chronic migraine [50]. Similarly, in the present study significantly lower levels were observed for tumor necrosis factor α, interleukin-1β, and interleukin 6 after a 4-month therapy with cyproheptadine, amitriptyline, propranolol, or flunarizine. Nonetheless, none of the drugs were superior to the others in terms of reducing cytokines levels. Furthermore, no differences were found for the cytokine results of patients with 50% reduction in headaches compared with those of patients with no reduction. Although there was significant decrease in cytokine levels after treatment, tumor necrosis factor α levels (one of the contributing mechanisms in migraine mentioned by Rozen and Swidan [50]) persisted.

High leptin levels also cause an increase in pain sensitivity in mice, and ibuprofen treatment leads to decreases in serum leptin concentrations [18,51]. In the present study, cyproheptadine and flunarizine increased leptin levels, which is in accord with findings of a study on patients with migraine by Berilgen et al. [52]. They argued that both drugs might cause leptin resistance, possibly by different mechanisms, and thereby lead to increased serum leptin levels [52]. Thus, in the present study, both cyproheptadine and flunarizine may have led to increases in leptin resistance and to increased appetite (although perhaps via direct effects on cytokines in migraine). Further studies are needed to elucidate the effects of leptin on headache.

Conclusion

Simultaneous evaluation of all these data suggests that increased plasma levels of cytokines might be related with pathophysiologic dysfunctions in migraine. Furthermore,

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cytokine levels can be used to evaluate clinical response. The role and relationship among biomarkers, cytokines, and leptin levels and clinical responses to the prophylactic drugs have not previously been demonstrated to be valid indicators in pediatric migraine. The present study represents a novel trial in this aspect. In the future, larger controlled clinical trials are needed to evaluate levels not only of cytokines but also of leptin, galanin, and ghrelin for clinical response to prophylactic drugs in children with migraine.

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