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Original Article

Correlation of symptoms of headache with inter-ictal plasma CGRP concentration in migraineurs

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ABSTRACT

Introduction: Diagnosis of primary headaches is based upon clinical symptoms. However, our knowledge regarding biochemical correlation of their symptoms is limited. This study tries to find out whether symptoms during migraine have any association with inter-ictal plasma CGRP concentration. **Methods:** Fifty migraineurs were included after screening for inclusion and exclusion criteria for this study. Their clinical history was taken in detail and recorded in a semi-structured performa. Blood was drawn from ante-cubital vein (at least after three days of last headache) and separated plasma was stored at -70C. CGRP was analysed with commercially available ELISA kit. Data was analyzed with the help of SPSS V11.0 for Windows. Chi-Square test, independent sample t test, one way ANOVA and multivariate regression analysis was used for statistical analysis. **Results:** Age of subjects (P=0.02), total duration of illness (P=0.04) and frequency of migraine episode per month (P=0.03) had significant association with inter-episodic plasma CGRP concentration. Other headache characteristics were not related to the plasma CGRP concentration except cranial autonomic symptoms where higher level was noticed in subjects with bilateral cranial autonomic symptoms with lateralized severity (F = 4.59; P=0.01). **Conclusion:** Laterality of cranial autonomic symptoms influences the inter-ictal plasma CGRP concentration in migraineurs.

Key Words: Migraine, cranial autonomic symptoms, CGRP, Inter-ictal period

INTRODUCTION

The primary headaches differ from each other on the basis of symptoms. According to the ICHD-2 criteria [1], a number of different headaches exist, yet our knowledge regarding patho-physiological basis, especially the biochemical correlation of their symptoms is limited. Young et al[2] proposed a modular headache theory which ascribed each headache symptom to a module (group of neurons). This theory proposed that sequential or simultaneous recruitment of different modules determines the final picture of headache. Activity of different modules may differ qualitatively, quantitatively as well as temporally not only during an episode of headache but also among different headache episodes thereby, generating phenomenological differences across episodes [2].

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Amid all biochemical changes associated with primary headaches, CGRP has gained maximum importance. This neuropeptide is released in circulation from trigeminal nucleus complex (TNC) during acute attacks of migraine [3-5]. However, studies assessing plasma CGRP concentration during and outside headache phase had provided conflicting results [3-8]. This could be attributed to a number of factors like- site of blood collection, biochemical method used for assessment of CGRP etc.

To our knowledge, only a few studies have tried to correlate migraine symptoms with plasma CGRP concentration, so far. Scalp pain (allodynia) and lacrimation were found to be related with plasma concentration of CGRP [9]. Intravenous infusion of CGRP was described to induce vasodilatation in lacrimal glands to initiate tearing [10]. Another evidence for involvement of CGRP in lacrimation came from reduction in tearing in rats pre-treated with capsaicin, as capsaicin was shown to selectively deplete CGRP-SP containing sensory fibres [11]. Considering all these evidences, plasma CGRP level are expected to be higher in migraineurs with these symptoms i.e., lacrimation and allodynia.

Pharmacological studies might throw further light on this issue. Scientific literature suggested that clinical symptoms determine the response to abortive therapy among migraineurs. Migraineurs with unilateral, pulsating pain of severe intensity with higher basal concentration of CGRP were more likely to respond to rizatriptan [12]. As we have already mentioned, like abortive therapy, migraineurs were found to show differential response to prophylactic therapies [13-14]. However, it is still not known why the response to therapy differ between migraineurs.

These studies were done during the headache phase. It is still not known whether some of these modules remain activated during inter-ictal period or not. It is also not known if the inter-ictal activation of some modules (especially related to CGRP release) can decide the final picture of headache during an acute attack. This knowledge can help us in proper selection of anti-migraine drugs. Though the concept of choosing a drug based upon symptomatology could be a novel idea in the field of headache medicine, this had already gained wider acceptance in the management of major depressive disorder. Newer guidelines suggested that the most effective molecule could be precisely identified based upon the symptomatology of depression [15].

Earlier, we had shown that plasma CGRP concentration was different among migraineurs, tension type headache and control group during inter-ictal period [16]. Therefore, the present study was planned to correlate symptoms during acute pain with the plasma CGRP concentration during inter-ictal period among migraineurs.

METHOD

Fifty consecutive migraineurs were recruited from the headache clinic of a tertiary care teaching hospital. Approval of the institutional ethics committee was sought beforehand and a written informed consent was obtained from each participant. Diagnosis of migraine was based upon the International Classification of Headache Disorders (ICHD-2) criteria [1].

Exclusion criteria were: subjects with major neurological disorders, experiencing chronic daily headache (undiagnosed or mixed type), those consuming substance of abuse (except tobacco), those on prophylactic therapy for migraine longer than past three weeks, having another primary headache or psychiatric illness, and those consuming anti-oxidants for more than seven days.

Patient's history of headache was taken in detail, followed by the clinical examination (general physical examination, neurological examination, peri-cranial muscle tenderness using palpation and looking for the evidences of muscle parafunction). Parallel information regarding symptoms of headache was also gathered from a family member. History of headache included: duration of complaints, number of headaches per month on an average, time taken to attain maximum

severity after initiation of pain, predominant laterality and sidedness of pain, location of headache, radiation if any, quality of headache, average time spent in each episode, usual time of initiation of headache, factors that precipitated or relieved headache, premonitory symptoms (mainly yawning) and lastly, symptoms that are usually associated with migraine e.g. nausea and/or vomiting, intolerance to noise, photophobia, effect of exertion on headache, vertigo, allodynia etc. Depending upon the laterality of headache, patients were divided into the three categories: (a) bilateral headache- that included headache that was equally severe on both sides or bilateral but more severe on one side; (b) Unilateral headache- included patients experiencing pain only on one side of head or pain on one side was more frequent than on the other side or unilateral headache more frequent than bilateral and lastly, (c) bilateral headache more frequent than unilateral headache. Based on the side of headache, two groups were made: predominantly right-sided pain and predominantly left-sided pain.

Cranial autonomic symptoms (CAS)

Leading questions were asked regarding occurrence of conjunctival injection, tearing, rhinorrhoea or blocked nose, forehead sweating and puffiness around eye during an episode of heaadche. Relation of CASs with respect to laterality of headache was also asked for (ipsilateral CAS or bilateral CAS or bilateral CAS with more intensity towards side of severe pain).

Measurement of CGRP in plasma:

Details of the procedure had already been mentioned elsewhere [16]. In short, 3 ml fasting venous blood was collected, at least 3 days after the last episode of headache. Samples were collected in morning between 10.00 to 12.00 hours. After centrifuging them for three minutes at 2000 rpm, plasma was stored at -70C. Human á-CGRP ELISA kit (Peninsula Labs, LLC) was used to assess CGRP concentration in plasma. Absorbance was read at 450 nm using ELISA reader. Concentration in each sample was calculated by plotting the readings on a standard curve (ng/ml).

Statistical Analysis

Analysis was done using SPSS v 11.0 for Windows. Chi-square test was used for comparing the proportions. Independent sample 't' test was used to compare numerical values between two groups and one way ANOVA was run to compare numerical values among three groups. To assess association between headache characteristics and CGRP level, multivariate analysis was run.

RESULTS

Details regarding the sample characteristics were already published [16]. Multivariate analysis showed that inter-ictal plasma CGRP level had significant association with the age of subjects, total duration of illness and frequency of migraine episode per month (Table I). Among these, total duration of illness and frequency of migraine episodes had inverse relation with the plasma CGRP levels. However, inter-ictal plasma CGRP concentration could predict these dependent factors only to a limited extent ($R^2 = 0.233$).

When we analyzed the effect of laterality of headache, we found that subjects with bilateral headache had highest CGRP levels (1.36 ± 0.72) followed by subjects with bilateral headache but more severe on one side (1 ± 0.17). Subjects with strictly unilateral headache had lowest values (0.98 + 0.14). It must be noted here that the difference among three groups was statistically not significant (F=2.56; P=0.06). Similarly, sidedness of the headache did not affect the results, too (F=1.12; P=0.31).

CGRP concentration did not have any impact on the quality of pain, although highest levels were found in subjects experiencing pressing quality pain during onset of headache (F=0.93; P=0.40). Subjects with pressing kind of pain at the zenith of headache had lowest values (F=0.59; P=0.55). Interestingly, subjects

with pulsating pain during headache onset had lowest values.

The plasma concentration of CGRP was not related to any of the disease related qualitative variable (table 2), in other words, baseline levels of plasma CGRP were not able to predict any of the clinical symptoms.

Further, plasma CGRP concentration was higher in subjects with bilateral cranial autonomic symptoms that were more severe towards one side (F = 4.59; P=0.01). It must be noted that in present study, none of the subject had strictly unilateral cranial autonomic symptoms.

DISCUSSION

This study had shown that the inter-ictal CGRP concentration in plasma was related to some of the clinical manifestations. To best of our knowledge, none of the studies in the past had touched the objectives of this study and

Table 1. Effect of disease related factors on serum CGRP among migarineurs using multivariate analysis

Variables	Unstanderdized coefficient		β	t	P	95% CI	
	B	SE				Lower	Upper
(Constant)	0.991	0.250		3.96	.000	0.487	1.495
Age (yrs)	0.02	0.009	0.37	2.31	0.02	0.003	0.038
Total Duration Illness (yrs)	-0.04	0.020	-0.31	-2.05	0.04	-0.083	-0.001
Frequency / month	-0.01	0.008	-0.31	-2.23	0.03	-0.034	-0.002
Duration of episode (hrs)	-0.002	0.003	-0.09	-0.68	0.50	-0.009	0.004
Time to reach max severity (hrs)	-0.02	0.021	-0.19	-1.40	0.16	-0.072	0.013

Dependent variable: CGRP

Table 2. Effect of headache variables on plasma CGRP levels among migraineurs

S. N.	Variable	N	Mean	SD	t	P	SED	95% CI Low	95% CI Upper
1.	*Course of illness*								
	Stable	12	0.93	0.16	-1.65	0.10	0.16	-0.60	0.50
	Progressive	38	1.20	0.55					
2.	History of Aggravating factors								
2.1	*Head bending*								
	Yes	35	1.21	0.57	1.76	0.08	0.15	-0.03	0.57
	No	15	0.95	0.19					
2.2	*Physical exercise*								
	Yes	21	1.07	0.27	-0.76	0.45	0.14	-0.4	0.18
	No	29	1.18	0.61					
3.	History of Premonitory symptoms								
3.1	*Yawning*								
	Present	15	0.95	0.15	-1.71	0.09	0.15	-0.56	0.04
	Absent	35	1.21	0.57					
4.	History of Associated symptoms								
4.1	*Blurred vision*								
	Present	28	1.24	0.63	1.76	0.08	0.14	-0.03	0.53
	Absent	22	1.00	0.18					
4.2	*Allodynia*								
	Present	35	1.19	0.58	1.1	0.27	0.15	-0.14	0.48
	Absent	15	1.01	0.20					
4.3	*Orthostatic hypotension*								
	Present	27	1.23	0.61	1.51	0.13	0.14	0.07	0.49
	Absent	23	1.02	0.29					
5.	History of Cranial autonomic symptoms								
5.1	*Conjunctival injection*								
	Present	19	1.02	0.30	-1.25	0.21	0.14	-0.47	0.11
	Absent	31	1.20	0.58					
5.2	*Lacrimation*								
	Present	27	1.25	0.65	1.8	0.07	0.13	-0.02	0.53
	Absent	23	1.00	0.17					
5.3	*Peri-orbital swelling*								
	Present	19	1.07	0.28	-0.74	0.46	0.14	-0.40	0.18
	Absent	31	1.18	0.60					
5.4	*Nasal symptoms*								
	Present	6	1.99	0.99	2.37	0.63	0.40	-0.07	2.02
	Absent	44	1.02	0.23					
6.	Examination findings								
6.1	*Pericranial tenderness*								
	Present	27	1.25	0.61	1.81	0.07	0.13	-0.02	0.53
	Absent	23	1.00	0.29					
6.2	*Muscle parafunction*								
	Present	10	1.00	0.17	-0.96	0.33	0.17	-0.53	0.18
	Absent	40	1.17	0.55					

hence, the following discussion will largely focus upon the explanation of our hypothesis.

Analysis showed that inter-ictal plasma CGRP concentration had decreased with the longer duration of illness and higher frequency of migraine episodes i.e., as we moved towards chronic illness. Direct comparison of episodic and chronic migraine groups also revealed similar results in present study (1.14 ng/ml versus 0.94 ng/ml, respectively). Theoretically, CGRP levels should decrease with the progression of the episode owing to the depletion from the peripheral endings of trigeminal neurons. This was confirmed experimentally in the past and can explain our results [17].

We had previously shown that longer duration of illness and longer individual episodes as well, produce clinical symptoms of sensitization of the trigemino-parasympathetic reflex, i.e., cranial autonomic symptoms (CAS) [18]. These symptoms in migraineurs might be important as unilateral CAS were found to be associated with higher CGRP level and better response to triptan therapy [12]. Contrarily, in present study, CGRP level was higher in subjects in whom cranial autonomic symptoms were bilateral but more severe on one side. In theory, the concentration is supposed to be higher in subjects with bilateral CAS. Two explanations might be able to throw some light on this contradictory finding- first, as we have already mentioned, lower inter-ictal level might be secondary to the large amount of release (as with bilateral CAS) and consequent of CGRP depletion or secondly, these cases could be closer to the tension type headache on the spectrum and thus they had lesser involvement of CGRP. To confirm the finding, we need a study examining plasma CGRP concentration with the difference in severity as well as laterality of CAS. However, we could not assess it due to the retrospective design of the study. Therefore, it is clearly an area for future research.

Goadsby et al [6] reported higher levels in MA than in MO during spontaneous headache. Development of aura depends upon the cortical spreading depression and it was found that migraine without aura subjects either did not develop cortical spreading depression at all, or it was silent in them [19,20]. Moreover, Piper et al [21] correlated the CGRP concentration with the development of cortical spreading depression and found that it did not affect the CGRP concentration in jugular venous blood. This could be one reason why many of the previous studies [3,19] and the present study as well, failed to find any effect of aura on the CGRP concentration.

Throbbing quality of pain and its increment with the head movement denote the peripheral sensitization [22]. These findings were supported by the facts that migraineurs with pulsating pain had higher level of CGRP in the blood [12]. This is in sharp contrast to results of present study, where higher levels were reported by subjects with history of pressing pain rather than pulsating pain. However, due to design of the present study (pain quality was noted historically), vis-a-vis comparison was difficult, still results suggested that at least inter-episodic CGRP levels were independent of pain quality.

Central sensitization had been considered responsible for allodynia and pericranial tenderness among migrainuers. Despite the history of allodynia in around 70% subjects and presence of pericranial tenderness at the time of sample collection in nearly half of migraineurs, CGRP levels were not different. Probably, during central sensitization CGRP release remained confined to the trigeminal nucleus caudalis and it failed to reach the peripheral blood. Thus, the CGRP concentration was comparable not only during inter-ictal period, but even during the headache phase [23].

In short, these findings suggested that inter-episodic CGRP concentration was influenced only by the laterality of CAS, among all symptoms of migraine. However, this study had some limitations- firstly, sample size was small relative to prevalence of illness and retrospective data collection. We propose that prospective studies on a larger population would be able to throw more light on this issue.

In conclusion, only the laterality of cranial autonomic symptoms correlated with the basal levels of plasma CGRP in migraineurs.

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