▪ Headache Medicine 2023, 14(2): 89-96 ▪ p-ISSN 2178-7468, e-ISSN 2763-6178

Headache Medicine

▪ DOI: 10.48208/HeadacheMed.2023.18

Original

Calcitonin gene-related peptide (CGRP) concentrations outside migraine attacks in peripheral blood as a potential biomarker for chronic migraine

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Edited by:
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Abstract

Introduction
Chronic migraine is a debilitating condition that affects a significant portion of the population. Accurate diagnosis and treatment of chronic migraine remain a challenge due to the lack of objective biomarkers. Calcitonin gene-related peptide (CGRP) is a neuropeptide involved in the pathophysiology of migraine and has been proposed as a potential biomarker for migraine.

Objective
To determine whether CGRP could act as potential peripheral blood marker for chronic migraneurs.

Methods
We measured CGRP levels in peripheral blood samples collected from 142 participants with chronic or episodic migraine and 24 healthy controls during ictal periods, i.e., outside migraine attacks. We compared CGRP levels between the three groups and assessed the correlation between CGRP levels and clinical features of chronic migraine.

Results
Our results showed that CGRP levels were significantly higher in participants with chronic migraine, episodic migraine compared to healthy controls. When the CGRP levels were correlated between chronic migraine and healthy controls, a significant variation was observed (79±15.27 vs. 29.08±10.34, $p<0.05$), similarly episodic migraine and healthy controls also showed a significant variation (47.28±7.06 vs. 29.08±10.34, $p<0.01$). Our findings suggest that CGRP concentration in peripheral blood during ictal periods may serve as a potential biomarker for chronic migraine. The positive correlation between CGRP levels and clinical features of chronic migraine further supports the involvement of CGRP in the pathophysiology of migraine. This study highlights the potential utility of CGRP as a biomarker for chronic migraine and provides a foundation for further research in this area.

Conclusion
Our study provides evidence that CGRP levels in peripheral blood during ictal periods may serve as a potential biomarker for chronic migraine. Further studies are needed to validate these findings and to explore the clinical utility of CGRP as a biomarker for chronic migraine.

Keywords:
Migraine disorders
Chronic migraine disorders
Biomarkers
Calcitonin gene-related peptide
Neuropeptides
Blood specimen collection
Peripheral blood
Ictal period
Disease progression

Submitted: March 9, 2023
Accepted: May 16, 2023
Published online:

Introduction

Chronic migraine (CM) is a debilitating neurological disorder that affects a significant portion of the global population, with an estimated prevalence of 1.4-2.2%.[1] In India, the prevalence of migraine is approximately 10-12%.[2] CM is characterized by the occurrence of headaches on 15 or more days per month for at least three months, with at least eight of these headaches being migraines.[3] The condition is associated with a significant decrease in quality of life, high levels of disability, and increased healthcare costs.[4]

Currently, the diagnosis of CM is based on clinical criteria, which may not always be reliable, especially in patients with comorbidities or complex migraine phenotypes.[5] Therefore, there is a need for objective biomarkers that can aid in the diagnosis and management of CM. Calcitonin gene-related peptide (CGRP) is a neuropeptide that is involved in the pathophysiology of migraine.[6] Elevated levels of CGRP have been found in the peripheral blood during migraine attacks.[7] However, it is not clear if CGRP levels are also elevated outside of migraine attacks in patients with CM.

The aim of this study is to investigate CGRP concentration in the peripheral blood of patients with CM during and outside of migraine attacks, and to evaluate its potential as a biomarker for the diagnosis and management of CM. The findings of this study may contribute to the development of new diagnostic and therapeutic strategies for CM.

Methodology

Study Design

This is a prospective, observational study that was conducted at a tertiary care center. The study enrolled patients with CM who meet the International Classification of Headache Disorders (ICHD-3) criteria for CM.[3] The study was conducted in accordance with the Declaration of Helsinki and has been approved by the institutional review board.

Study Participants

The study included 96 cases with CM, 46 cases of episodic migraine (EM) and 24 healthy/normal controls (NC) were analysed and have had CM for at least six months. Patients with a history of other primary headache disorders, secondary headache disorders, or significant comorbidities were excluded from the study. Patients were from different areas of Kashmir state, both from urban and rural areas and the age group was from 18-65 yrs. Both male and female patients were recruited in this study. Patients were allowed to take prophylactic medication, but symptomatic medication was not allowed for at least 24 hours unless blood sample was taken.

Data Collection

Demographic and clinical data were collected from all patients, including age, sex, duration of CM, headache frequency, headache intensity, and use of prophylactic medications. Blood samples were collected from all patients during and outside of migraine attacks, and plasma levels of CGRP were measured using enzyme-linked immunosorbent assay (ELISA) kits. The timing of blood sample collection was based on the patient's headache diary.

Statistical Analysis

Descriptive statistics will be used to analyze the demographic and clinical data. The differences in CGRP levels during and outside of migraine attacks were analyzed using paired t-test. The relationship between CGRP levels and headache frequency, headache intensity, and use of prophylactic medications were analyzed using regression analysis.

Ethical Issues

In this study, ethical clearance was obtained from the institutional review board before the study was initiated. The study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki, including informed consent, respect for participant autonomy, and protection of participant confidentiality. All participants were given a detailed explanation of the study, including the procedures involved, potential risks and benefits, and their right to withdraw from the study at any time without any consequences. Written informed consent were obtained from all participants before enrollment in the study. Any potential adverse events were promptly reported to the IRB, and appropriate actions were taken to ensure the safety and well-being of the participants. Confidentiality of participant data was ensured throughout the study, with data being stored securely and access restricted to authorized personnel only.

Expected Outcomes

The study is expected to provide insights into the potential of CGRP as a biomarker for CM. If CGRP levels are found to be elevated outside of migraine attacks in patients with CM, it could indicate that CGRP is a useful biomarker for the diagnosis and management of CM. This could lead to the development of new diagnostic and therapeutic strategies for CM, leading to better outcomes for patients.

Results

Out of 166 recruited study participants, 96 had CM, 46 had EM, and 24 were healthy controls. The mean age of the three groups was 37.93±12.16 years for the CM, 38.61±11.12 years for those with EM, and 40.96±13.80 years for healthy controls. Most of the cases of chronic and episodic migraine were in the age group of 20-50 years. Most of our cases were female, comprising of 76% and 69.6% in CM and EM, respectively. Among the controls, 66.7% were female. There was no statistically significant difference in sex between the three groups. The majority of patients in all three groups came from urban locations. However, there was no significant difference in residence between the groups. In terms of marital status, married patients outnumbered unmarried ones in all three groups, but there was no significant difference between the groups. Most of our patients were educated in both chronic and episodic migraine (Table 1).

Table 1. Socio-demographic Characteristics of the studies participants

Characteristics	n	(%)	n	(%)
Sex	**Female**		**Male**	
Chronic Migraine	73	76.00%	23	24.00%
Episodic Migraine	32	69.60%	14	30.40%
Controls	16	66.70%	8	33.30%
Total	121	72.90%	45	27.10%
Dweller	**Rural**		**Urban**	
Chronic Migraine	35	36.50%	61	63.50%
Episodic Migraine	15	32.60%	31	67.40%
Controls	10	41.70%	14	58.30%
Total	60	36.10%	106	63.90%
Marital Status	**Unmarried**		**Married**	
Chronic Migraine	38	39.60%	58	60.40%
Episodic Migraine	13	28.30%	33	71.70%
Controls	6	25.00%	18	75.00%
Total	57	34.33%	109	65.66%
Education	**Uneducated**		**Educated**	
Chronic Migraine	27	28.10%	69	71.90%
Episodic Migraine	16	34.80%	30	65.20%
Controls	12	50.00%	12	50.00%
Total	55	33.10%	111	66.90%

We estimated blood CGRP concentrations in all three groups using ELISA. The CGRP levels in CM and EM were 79.13±15.27 pg/ml and 47.28±7.06 pg/ml, respectively. In contrast, controls had a CGRP level of 29.08±10.34 pg/ml (Table 2).

Table 2. Comparison of mean CGRP levels in different groups

Groups	CGRP Levels			
	Count (N)	Mean	Standard Deviation	P value
Chronic Migraine	96	79.13	15.27	<0.05
Episodic Migraine	46	47.28	7.06	
Controls	24	29.08	10.34	
Total	166	63.06	23.46	

Table 3 describes the multiple comparison on post hoc analysis. We found a statistically significant difference in the CGRP levels between CM and EM ($p<0.001$), with CM showing higher levels. Additionally, CM had significantly higher mean CGRP levels than controls ($p<0.001$). Compared to controls, EM had significantly higher mean CGRP levels ($p<0.001$). This difference was confirmed using both parametric and non-parametric tests.

Table 3. Multiple Comparisons on post hoc analysis (95% Confidence Interval)

Dependent Variable: CGRP levels						
Migraine type	Comparison Groups	Mean Difference	Std. Error	P value	Lower bound	Upper bound
Chronic	Episodic Migraine	31.849*	2.302	<.001	26.16	37.54
	Controls	50.048*	2.930	<.001	42.81	57.29
Episodic	Chronic Migraine	-31.849*	2.302	<.001	-37.54	-26.16
	Controls	18.198*	3.233	<.001	10.21	26.18

In addition, we compared CGRP levels in patients with migraine with aura and without aura in both the chronic and episodic migraine groups. However, we found no statistically significant difference in CGRP levels between the subgroups ($p>0.05$) (Table 4).

Table 4. CGRP levels in two migraine types

		CGRP levels		
Migraine type		Mean	Standard Deviation	P value
Chronic	No aura	79	12.59	0.062
	Aura	79	20.45	
Episodic	No aura	49	6.93	
	Aura	44	6.64	

Table 5 and Figure 1 show the comparison of mean CGRP levels. For those with or without aura, "No aura" group had a sample size of 98, a mean of 69.5, and a standard deviation of 18.04, and "Aura" group with a sample size

of 44, a mean of 67.26, and a standard deviation of 23.75. The p-value of 0.538 indicates that there was no significant difference in mean between the two sub-groups. Between the "Sexes," "Females" with a sample size of 121, a mean of 64.99, and a standard deviation of 23.048, and "Males" with a sample size of 45, a mean of 57.9, and a standard deviation of 24.04. The p-value of 0.095 indicates that there was no significant difference in mean between the two sub-groups. Participants had "Dwellings," in "Rural" with a sample size of 60, a mean of 63.13, and a standard deviation of 23.352, and "Urban" with a sample size of 106, a mean of 63.03, and a standard deviation of 23.633. The p-value of 0.65 indicates that there was no significant difference in mean between the two sub-groups. Also, while comparison of "Education," showed that "Uneducated" with a sample size of 55, a mean of 59.74, and a standard deviation of 27.93, and "Educated" with a sample size of 111, a mean of 64.71, and a standard deviation of 20.843. The p-value of 0.19 indicates that there was a significant difference in mean between the two sub-groups.

Table 5. Comparison of mean CGRP levels in relation to demographic characteristics

Variables	Sub-Variables	N	Mean	Std. Deviation	P Value
Aura	No aura	98	69.5	18.037	0.538
	Aura	44	67.26	23.746	
Gender	Female	121	64.99	23.048	2.095
	Male	45	57.9	24.04	3.584
Dwelling	Rural	60	63.13	23.352	3.015
	Urban	106	63.03	23.633	2.295
Education	Uneducated	55	59.74	27.93	3.766
	Educated	111	64.71	20.843	1.978

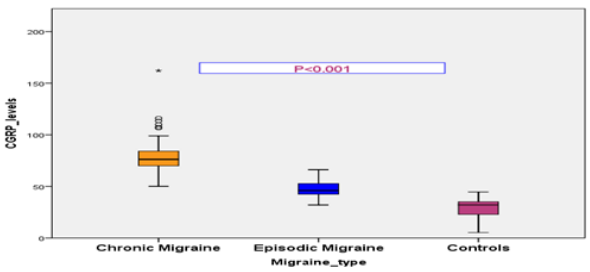

Figure 1. Box plot showing CGRP concentration in three groups.

Discussion

Migraine is a common and disabling neurological disorder characterized by episodic attacks of headache and associated symptoms. When the migraine progresses to a chronic form (e.g., CM), the frequency of migraine attack increases, and head pain can persist even between attacks. The strategy of treatment differs between EM and CM. Till now the diagnosis of migraine is based on the patients' description of symptoms. Although the International Headache Society offers well-structured diagnostic criteria for migraine and its subtypes, it is often challenged in the clinic by the language barrier (e.g. deafness or cognitively impaired patient), recall bias, and instability of patient-reported headache frequency. Therefore, researchers have been seeking a biomarker which can aid the diagnosis and follow-up of migraine. During the last decade the role of increased CGRP in external jugular venous blood during migraine attacks is one of the most cited findings in the headache literature. In the present study, therefore we analyzed the role of CGRP in the pathogenesis of migraine, as migraine is a gradual process from episodic to chronic. In Kashmir previously no such study regarding the analysis of the role of CGRP in migraine has been conducted in the patients. We employed a case-control study design in Kashmir patients with chronic and episodic migraine.

In our study of 166 cases, 96 cases of CM, 46 cases of EM and 24 healthy/normal controls were analysed for the possible role of the CGRP on migraine. Patients were recruited from the Department of Neurology, Sheri Kashmir Institute of Medical Sciences. Age and sex matched controls were also included in the study. Patients were from different areas of Kashmir state, both from urban and rural areas and the age group was from 18-65 yrs. Both male and female patients were recruited in this study. Patients were allowed to take prophylactic medication, but symptomatic medication was not allowed for at least 24hrs unless blood sample was taken. We observed a significant increase in CGRP levels in the peripheral blood of CM patients during ictal period, as compared to EM. When the CGRP levels were correlated between chronic migraine and healthy controls, a significant variation was observed, similarly episodic migraine and healthy controls also showed a significant variation. Our findings clearly indicate that there is a gradual increase in the CGRP levels from healthy controls to EM and to CM patients. In our study patients with different age group were studied and their CGRP levels were measured, there was no difference in CGRP levels in the age group. Similarly CGRP levels were measured in both sex groups, there were no significant differences between the sexes. Subsequently, patients were compared between urban and rural areas and their CGRP levels were assessed, again there was no difference between the two. Also in our study there was no difference in CGRP

levels of chronic or episodic migraine patients with aura or without aura. A landmark study of Eva Cernuda et al.[8] who reported the role of CGRP in migraine patients and in their study showed that there is a significant increase in CGRP levels in ictal period of CM patients as compared with the EM, which was in accordance with our patients. Also, in their study there was no significant difference in CGRP levels when compared with the age, gender, and dweller group, which was again in accordance with our study. Our results are no different from many other studies carried out on analysis of CGRP in episodic and chronic migraine. However, in the study by Eva Cernuda et al.[8] there was a significant difference in CGRP levels when compared with the patients of chronic or episodic migraine with or without aura. This was supported by previous studies by Herekar et al.[9] and Alzoubi et al.[10] where CGRP levels were higher in chronic or episodic migraine patients with aura than without aura. In our study there was no significant variation or increase in CGRP levels of chronic or episodic migraine patients with or without aura. This variability may be due to its different genetic makeup of different populations. The valley of Kashmir is an ethnic population, different from that of the others, with respect of genetic makeup, social and cultural milieu.

A study conducted by Hansen et al.[11] suggested that CGRP level remains elevated in migraineurs outside headache period as compared to controls. Another study conducted by Eva Cernuda et al.[8] in 2013 also showed Increased CGRP level measured in peripheral blood outside migraine attacks and in the absence of symptomatic medication could be a biomarker helping in the diagnosis of CM. Fusayasu et al.[12] proved that the ictal level of CGRP was not increased in the plasma only, but also in the saliva of the patients with migraine. Since, migraine is primarily a cephalic neurovascular disorder, it was expected that CGRP concentration may be higher in the jugular blood than the peripheral blood. Studies have also suggested that CGRP is increased in the internal as well as external jugular venous blood during headache. The role of CGRP as a biomarker was further validated by Jansen-Olesen et al.[13] who reported that CGRP in the external jugular blood and peripheral blood was significantly different on non-headache days, but not on the headache days in patients. Conversely, Hansen et al.[14] proved that CGRP levels were nearly the same in both external jugular and anti-cubital veins either in the ictal or interictal periods. Cernuda et al.[15] reported that CGRP concentrations were low in the NC and EM groups, while subjects with a high (>100 pg/mL) CGRP level were present only in the CM group. However, a study conducted by Tvedskov et al.[16] did not find increased CGRP levels in the plasma during inter-episodic period as reported by our study and the previous studies on the role of CGRP as a biomarker in migraine. The inconsistency in the results could be because the time between sampling and centrifugation was too long and considering the short half-life of CGRP, would predict little neuropeptide left in the sample. According to Cernuda et al.[15] migraine with aura was associated with higher serum CGRP concentration in women with CM. While nearly half of CM patients had aura in their study, the prevalence of migraine with aura was less than one fifth in our CM patients. This is not surprising because Asians have less prevalence of migraine with aura, although the prevalence of migraine is overall similar across countries.[16-18] This might explain the inconsistency in part between our and their study results. However, a one study conducted in South Korea did not find any significant association of CGRP levels with different types of migraine.[19] They found no increase in serum CGRP concentration in patients with CM and, it did not support CGRP as a biomarker of CM.

Calcitonin gene related peptide is a molecule associated with the pathogenesis of migraine. It is the peptide transmitter in perivascular sensory trigeminal nerve fibres. These are liberated in the perivascular area and from there it diffuses in the venous blood where it is found in increased amount in trigeminal activation. CGRP plays a causative role to induce vascular headache. Calcitonin gene-related peptide (CGRP) is a 37 amino acid neuropeptide which was discovered some 30 years ago.[20] It is derived from the gene encoding calcitonin and comprises two isoforms, alpha and beta CGRP.[21] CGRP is widely distributed in the nervous system, and particularly densely at anatomical sites known to be integral to the migraine process.[22] Within the central nervous system, these sites include the hypothalamus, cerebellum and brain stem.[23] Immuno cyto-chemistry studies have shown that up to half of the trigeminal neurons produce CGRP within the trigeminal system, at various sites including the trigeminal ganglion, nerve endings and in higher order neurons and glia.[24] Centrally, CGRP is therefore involved in nociceptive transmission through second and third order neurons, and pain modulation in the brainstem, whereas peripherally it mediates vasodilatation through smooth muscle receptors.[25] Biomarker studies using CGRP has been challenged because of its short half-life in venous blood. In earlier studies using plasma samples, investigators made a supreme effort to reduce the time from sampling to freezing.[26, 27] We still do not have a good understanding of the pathophysiology of CM or of the exact mechanisms that lead to transformation from EM to CM. It is well established, however, that activation of the trigeminovascular system has a crucial role and

leads to afferent and efferent release of neuropeptides, especially CGRP. This facilitates a peripheral inflammatory and vasodilating response and causes activation of second-order neurons involved in pain transmission. In most vessels, CGRP causes endothelium-and nitric oxide independent vasodilation through a direct action on the smooth muscle cells mediated both by cyclic adenosine monophosphate and activation of adenosine triphosphate–dependent K1 channels.[16,28] Persistent release of CGRP is thought to induce sensitization of central trigeminal neurons triggering a signaling pathway mediated by brain-derived neurotrophic factor leading to increased expression of a gene encoding the P2X receptors.[29, 30] These peptidergic central neurons in dorsal horn and trigeminal nucleus caudalis use L glutamate as their primary neurotransmitter. CGRP, acting via a unique receptor complex, increases neurotransmitter release at these levels, which would lead to central sensitization underlying chronic pain states such as CM.[31, 32]

Our study further emphasizes on the role of CGRP in chronic migraine patients. The most significant observation of our study was CGRP levels were significantly higher in chronic migraine patients in comparison to Episodic and healthy controls. The gradual increase in the CGRP from healthy controls to episodic migraine to chronic migraine shows with the severity of the disease CGRP levels also get enhanced. Also, the insignificant association of CGRP with other clinical variables lays further evidence that the role of CGRP in the pathogenesis of chronic migraine is of vital importance in the management of chronic migraine patients.

Strengths & Limitations of the Study

Sample size: Sample size of the study was adequate, it may be representative of the larger population, and the findings may be generalizable to all people with chronic migraine. This is one of the strengths of the study.

Selection bias: There might have been a risk of selection bias if the study participants were not have been selected randomly or if the inclusion criteria have been too narrow. This could have limited the generalizability of the results. In our study, the inclusion criterions were too broad.

Confounding variables: There may be other factors that could have influenced CGRP levels, such as medication use or comorbid conditions. These factors were controlled for, before the sample collection for CGRP.

Limited assessment of CGRP levels: CGRP levels may fluctuate depending on the time of day or other physiological factors. Therefore, assessing CGRP levels at a single time point may not accurately reflect the overall CGRP levels outside of migraine attacks. This can be a limitation for our study.

Lack of longitudinal data: The study design may only allow for a cross-sectional analysis of the data, which may not provide information on the changes in CGRP levels over time.

Conclusion

Our study further supports the role of CGRP in distinguishing migraine from non-migraine patient, which in future could become an important milestone for developing anti CGRP antibodies in the management of migraine. However, the role of CGRP needs to be further studied on a larger cohort of patients across different populations.

Funding: None
Conflict of Interest: None
Author Contribution: SHB, collection of data; RA, study design and guide; ZAS, estimation of CGRP; AR writing text and analysis.

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