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Pak J Med Res
Vol. 64, No. 4, 2025

Original Article

Serum Calcitonin Gene-Related Peptide Levels in Interictal period in Migraineurs versus Controls

Adina Qaiser[1], Anum Wahid[2], Maryam Shafaq[3], Muhammad Imran[3], Maria Muddassir[4], Faheem Hadi[5]
[1]Khawaja Safdar Medical College, Sialkot, [2]D.G.Khan Medical College, Dera Ghazi Khan, [3]Department of Physiology, Nishtar Medical University, Multan, [4]M. Islam Medical & Dental College, Gujranwala, [5]Faculty of Medicine and Allied Health Sciences, The Islamia University of Bahawalpur, Bahawalpur.

Abstract

Background: Migraine is a frequent neurological condition with a complex pathogenesis and neuro-physicians continue to face difficulties in diagnosing and treating migraines due to their clinical breadth and heterogeneity.
Objective: To determine the levels of serum calcitonin gene related peptide (CGRP) outside the attack (interictal) in migraineurs versus healthy controls.
Methods: This cross-sectional, analytical study was conducted in Centre of Research in Molecular Medicine, The University of Lahore, Pakistan from November 2023 to June 2024. A total of eighty cases were added in research. Forty were patients of common migraine (as per ICHD-3 criteria) and forty were healthy controls. Serum CGRP levels were estimated by enzyme linked immune-sorbent assay (ELISA) outside the attack in migraineurs and healthy controls. Shapiro-wilk test, Mann Whitney U and Chi square tests were used for statistical analysis. The ROC (receiver operating characteristic) curve and AUC (area under curve) were acquired to assess capacity of discrimination of CGRP levels for common migraine. The *p*-value <0.05 was considered statistically significant.
Results: Migraineurs had a median serum CGRP level of 99.6 (64.4–163.4) ng/L while for the healthy controls it was 14.8 (10.9–24.3) ng/L (*p* <0.001). ROC curve analysis had found cutoff serum CGRP level of 36.8 ng/L with positive predictive value (PPV) as 86.7%, negative predictive value (NPV) as 97.1%, sensitivity as 97.5%, specificity as 85% and accuracy as 91.3% for the diagnosis of common migraine.
Conclusion: Elevated serum CGRP levels outside the migraine attack can be used as a potential biomarker for the diagnosis of common migraine.
Key words: Calcitonin gene-related peptide, common migraine, migraine without aura.

Introduction

Migraine is very common neurological disorder with a composite pathophysiology. It was placed twenty-second in 1990 and fourteen in 2019 in terms of age group disability adjusted life years among the 369 illnesses listed in Global Burden of Disease study in 2019.[1] It is characterized by recurring episodes of varying severity migraine pain that last 4 to 72 hours. The pain is often unilateral, pulsating, worse by routine physical activity, alongwith photophobic, nausea and sometimes phonophobia.[2]

Clinical spectrum and heterogeneity in migraine remain a diagnostic and therapeutic challenge to the neuro-physicians. The linked aspects that must be examined in order to get deeper insights into migraine are genetics, epigenetics, and environmental factors. Generation of pain involves brainstem meninges, intracranial vasculature and trigemino-vascular system (TVS),[3] whose activation leads to release of some vasoactive neuropeptides such as CGRP, followed by cascade of events. These events include peripheral sensitization, central sensitization, nociceptive modulation and neurogenic inflammation.[4]

Corresponding Author:
Faheem Hadi
Faculty of Medicine and Allied Health Sciences
The Islamia University of Bahawalpur, Bahawalpur.
Email: faheem.hadi@iub.edu.pk
Received: 09 May 2025, **Accepted:** 15 December 2025
Published: 29 January 2026

Authors Contribution

AQ & AW conceptualized the project. MS & MI did the data collection. MI & FH performed the statistical analysis. MM did the literature search. Drafting, revision & writing of manuscript were done by AW & MI.

Table 1: Parameters of controls and patients included in study.

Parameters		Migraineurs		Healthy Controls	
		n	*%*	*n*	*%*
Gender	Male	7	17.4	21	52.5
	Female	33	82.6	19	47.5
Marital status	Non-married	18	45.0	28	70.0
	Married	22	55.0	12	30.0
Smoking	Non-Smoker	39	97.5	34	85.0
	Ex-smoker	1	2.5	1	2.5
	Current smoker	0	0.0	5	12.5
BMI	<18	29	72.5	35	87.5
	18-24.9	11	27.5	5	12.5
	>25	0	0.0	0	0.0

Research has shown that CGRP is the most powerful vasodilating peptide in the migraine pathogenesis.[5] CGRP is a neuropeptide of thirty-seven amino acids.[6] It is a key neurotransmitter produced during a migraine episode; blocking it directly aborts or even stops migraine headache.[7] It occurs in two variants in humans: alpha-CGRP, which is linked to migraine, is found in nerve cells of the dorsal root ganglia, TVS and vagal ganglia; whereas, beta-CGRP is found abundantly in enteric nervous system.[8] Measurement of CGRP levels is crucial to study pathophysiology of disease in patients and is considered vital component in diagnosing and finding treatment of disease. In this context the current study has been conducted to determine the levels of serum calcitonin gene related peptide (CGRP) outside the attack (interictal) in migraineurs versus healthy controls.

Methods

This cross sectional study was conducted in Centre of Research in Molecular Medicine, Institute of Molecular Biology and Biotechnology, the University of Lahore, Lahore from November 2023 to June 2024. The samples were collected from Department of Neurology, King Edward Medical University and Mayo Hospital, Lahore. The migraineurs (as per ICHD-3 criteria) of age 18-60 years of both genders, having $\geq$2 attacks/month of moderate to severe intensity, were included.[4] Cases of other types of headaches, new onset of headache with an underlying disease, headaches with focal neurological symptoms, headaches secondary to trauma, hypertension or ischemic heart disease, chronic liver/kidney diseases, drug allergies, pregnancy or lactation, menstrual irregularities or polycystic ovaries, hormone replacement therapy or malignancy, were excluded. Mean concentrations of calcitonin gene peptide levels in patients were and 252.7± 56.4 and in controls 130.1 ± 70.5.[9] Mean standard deviation (S.D) is 2.15 and variance is 4.6. Confidence interval is taken as 95. Power is taken as 80. The estimated sample size is 4 but for the better power of study we took a sample of 40 per group.

The migraineurs had to be headache- and analgesia-free for at least 72 hours. All cases underwent a general physical as well as neurological examination prior to the test. Blood was drawn from the right antecubital vein into EDTA-coated tubes. After allowing the blood to coagulate, it was centrifuged at 3000 revolutions per minute for duration of 15 minutes to separate the serum from blood. Serum was kept at -20^{o}C until assayed within 24 hours. Serum levels of CGRP were determined using a commercially available kit of ELISA, blindly with respect to the migraineurs and healthy controls. Study population's data was obtained on proforma after taking written informed consent from patients. The data was analyzed by using statistical package for social sciences (SPSS) version 23. Chi-square and Mann-Whitney U tests were utilized to find variances in data obtained from controls and patients. The receiver operating characteristic (ROC) curve and the AUC (area under curve) were acquired to assess capacity of discrimination capacity of CGRP levels. The *p*-value <0.05 was considered statistically significant.

Results

The data were tested for normality by Shapiro-wilk test, and it was observed that the distribution of serum CGRP was deviating from normality for migraineurs with p-value 0.017 and for healthy controls with *p*-value <0.001. Migraineurs had median age 30.0 (22.5–34.5) whereas healthy controls had 22.0 (18.0–26.5) years. The characteristics of the study population and the factors triggering common migraine are enlisted respectively as shown in Table-1 and Table-2.

It was noted that the serum CGRP levels were skewed for both migraineurs and healthy

controls. The migraineurs had a median serum CGRP level of 99.6 (64.4–163.4) ng/L while for the healthy controls it was 14.8 (10.9–24.3) ng/L. There were few cases in healthy controls with values 50.0 to 100.0 ng/L. This difference was clearly significant with *p*-value <0.001 (Figure-1).

Table 2: Factors Triggering Common Migraine.

Triggers	*Migraineurs*		*Healthy Controls*		*p-value*
	n	*%*	*n*	*%*	
Stress	*37*	*92.5*	*0*	*0.0*	*<0.001*
Travelling	*24*	*60.0*	*0*	*0.0*	*<0.001*
Sleep disturbance	*27*	*67.5*	*0*	*0.0*	*<0.001*
Skipping meal	*23*	*57.5*	*0*	*0.0*	*<0.001*
Hormonal changes	*7*	*17.5*	*0*	*0.0*	*0.006*
Loud noise	*32*	*80.0*	*0*	*0.0*	*<0.001*
Strong odor	*24*	*60.0*	*0*	*0.0*	*<0.001*
Physical exercise	*25*	*62.5*	*0*	*0.0*	*<0.001*
Weather changes	*14*	*35.0*	*0*	*0.0*	*<0.001*
Specific food	*8*	*20.0*	*0*	*0.0*	*0.003*

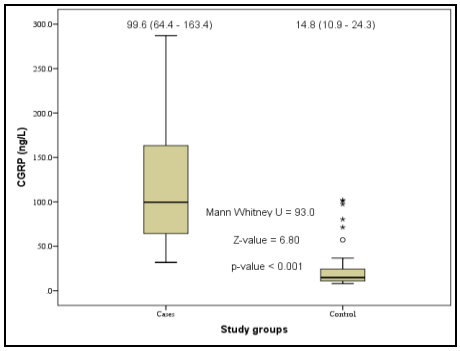

Figure 1: Distribution of CGRP and its comparison between two groups.

To check levels of CGRP as biomarker for common migraine, the ROC curve and the AUC were obtained. The cutoff serum CGRP level of 36.8 ng/L was found with sensitivity of 97.5% and specificity of 85.0% (Figure-2).

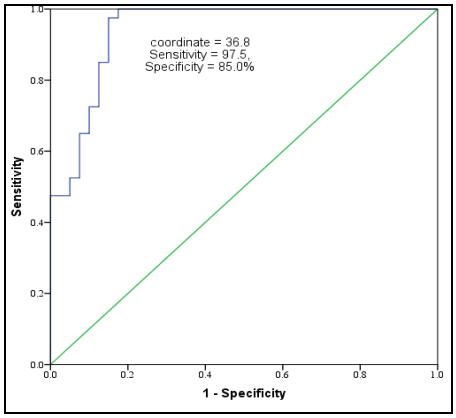

Table 2: ROC curve to determine the cutoff for CGRP levels.

There were 39 (97.5%) patients of common migraine who had CGRP level above the cutoff of 36.8 ng/L while 34 (85.0%) of the healthy controls had CGRP levels below the cutoff as shown in Table-3**.** The area under AUC was 0.94 (*p* <0.001).

Table 3: Distribution of cases as per cutoff of CGRP determined by ROC curve.

Serum CGRP (ng/L)	*Migraineurs*		*Healthy Controls*		*Total*	
	n	*%*	*n*	*%*	*N*	*%*
> 36.8	39	97.5	6	15.0	45	56.2
≤ 36.8	1	2.5	34	85.0	35	43.8
Total	40	100.0	40	100.0	80	100.0

The diagnostic measures were determined i.e., positive predictive value, negative predictive value, sensitivity, specificity and accuracy, as per cutoff of CGRP as shown in Table-4**.**

Table 4: Diagnostic measures as per cutoff of CGRP.

Measure	*Value*	*95% Confidence*
Sensitivity	97.5	(92.7 - 102.3)
Specificity	85.0	(73.9 - 96.1)
PPV	86.7	(76.8 - 96.6)
NPV	97.1	(91.5 - 102.7)
Accuracy	91.3	(85.1 - 97.4)

Discussion

This study was performed to investigate role of CGRP for diagnosis of common migraine in our local population. We found that serum concentration of CGRP outside the migraine attacks in migraineurs were higher than in healthy controls (*p* <0.001). The current study also proposes that serum CGRP level of more than 36.8 ng/L is strongly suggestive of common migraine having sensitivity of 97.5% and specificity of 85%.

CGRP has been linked to neurogenic inflammatory response of trigeminal nerve fibers and dura mater vasodilation in the pathogenesis of migraine. [10,11] The existing evidence has shown that CGRP is the most vasodilating peptide, in the migraine pathogenesis.[8] Its levels were raised in ictal (during attack of migraine) and interictal (outside attack of migraine) patients. Moreover, it was found that its levels were decreased after abortive as well as prophylactic treatment in migraine.[12,13]

Ashina *et al* [14] found CGRP levels were significantly high in migraineurs with and without aura than in controls (*p* =0.005). Moreover, cases of migraine patients (without aura) had raised CGRP levels as compared to controls (*p* =0.001). These

findings are corroborative with our findings. Also, no significant correlation of levels of CGRP with age of pateints, duration of migration history and frequency of migraine attacks, was found.

Similarly, Cernuda-Morollón E *et al*[15] reported raised levels of CGRP in patients of chronic migraine and Migraineurs with aura as compared to controls, patients of episodic migraine and migraineurs without aura, also evidenced by differences in specificity and sensitivity among groups. However, it was also found that some variables such as age, vascular risk factors, analgesic overuse, history of triptan usage or an alternate kind of prophylaxis, did not affect levels of CGRP in patients.

Raised peripheral CGRP levels is also considered as sign of TVS activation because size of related molecule is quite larger and cannot cross the blood-brain barrier. It is emphasized that the CGRP has a key role in sensitization of pain circuits which lead to development of migraine. CGRP concentration measurements depends on the specific kit used and the isoform detected. A recent literature review and analysis by Gárate G *et al* [16] showed levels of alpha-CGRP was found in range from 28.2 to 54.4 pg/mL and levels of beta-CGRP from 2.4 to 6.4 pg/mL.

The previous studies related to abnormal hemodynamics in interictal phase of migraine patients [17,18] together with current study findings of elevated serum CGRP levels outside the attack, are suggestive of increase in pressures of vessels of both cephalic and extracephalic areas. It is also suggested that raised CGRP levels in headache-free periods may be linked with excessive release of CGRP from sensory nerve terminals due to dysregulation in vascular control. These features may be the reasoning for susceptibility of migraine attacks in some people than the others.

Limitations include small number of participants in our study so the results may not be generalizable. Moreover, there was missed data on migraineurs that should have been obtained such as duration of disease since diagnosis, intensity of attack during ictal phase and family history of patients etc.

Conclusion

The serum concentration of CGRP in interictal period in migraineurs were elevated as compared to healthy controls. This proposes that serum CGRP levels can be considered as an important biomarker for diagnosis of common migraine. Similar study with a larger sample size may have been considered to acquire longitudinal data and more robust findings.

Funding: None.

Availability of Data: The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethical Approval: The Ethical Review Committee of The University of Lahore, Lahore approved the study dated 10th August, 2023.

Conflict of Interest: None declared.

References

1. Safiri S, Pourfathi H, Eagan A, Mansournia MA, Khodayari MT, Sullman MJ et al. Global, regional, and national burden of migraine in 204 countries and territories, 1990 to 2019. Pain 2022; 163(2): 293-309.
2. Edvinsson L. The CGRP pathway in migraine as a viable target for therapies. Headache 2018; 58(Suppl 1): 33-47.
3. Goadsby PJ, Holland PR. An Update: Pathophysiology of Migraine. Neurol Clin 2019; 37(4): 651-71.
4. Pietrobon D, Moskowitz MA. Pathophysiology of migraine. Annu Rev Physiol 2013; 75: 365-91.
5. Wattiez AS, Sowers LP, Russo AF. Calcitonin gene-related peptide (CGRP): role in migraine pathophysiology and therapeutic targeting. Expert Opin Ther Targets 2020; 24(2): 91-100.
6. Iyengar S, Ossipov MH, Johnson KW. The role of calcitonin gene–related peptide in peripheral and central pain mechanisms including migraine. Pain 2017; 158(4): 543-59.
7. Bohm PE, Stancampiano FF, Rozen TD. Migraine headache: updates and future developments. Mayo Clin Proc 2018; 93(11): 1648-53.
8. Giamberardino MA, Affaitati G, Costantini R, Cipollone F, Martelletti P. Calcitonin gene-related peptide receptor as a novel target for the management of people with episodic migraine: current evidence and safety profile of erenumab. JPain Res 2017; 2751-60.
9. Etefagh HH, Shahmiri SS, Melali H, Sayadi M, Ansari H, Shahzamani A, Deyhimi MS. Bariatric surgery in migraine patients: CGRP level and weight loss. Obes Surg 2022; 32(11): 3635-40.
10. Durham P, Papapetropoulos S. Biomarkers associated with migraine and their potential role in migraine management. Headache 2013; 53(8): 1262-77.
11. Kincses ZT, Veréb D, Faragó P, Tóth E, Kocsis K, Kincses B et al. Are migraine with and without aura really different entities? Front Neurol 2019; 10: 982.
12. Iyengar S, Johnson KW, Ossipov MH, Aurora SK. CGRP and the trigeminal system in migraine. Headache 2019; 59(5): 659-81.

13. Kamm K. CGRP and migraine: what have we learned from measuring CGRP in migraine patients so far? Front Neurol 2022; 13: 930383.
14. Ashina M, Bendtsen L, Jensen R, Schifter S, Olesen J. Evidence for increased plasma levels of calcitonin gene-related peptide in migraine outside of attacks. Pain 2000; 86(1-2): 133-8.
15. Cernuda-Morollón E, Larrosa D, Ramón C, Vega J, Martínez-Camblor P, Pascual J. Interictal increase of CGRP levels in peripheral blood as a biomarker for chronic migraine. J Neurol 2013; 81(14): 1191-6.
16. Gárate G, Pascual J, Pascual-Mato M, Madera J, Martín MMS, González-Quintanilla V. Untangling the mess of CGRP levels as a migraine biomarker: an in-depth literature review and analysis of our experimental experience. Headache 2024; 25(1): 69.
17. Liu Y, Sun A, Zhang Y, Wang B, Kuang X, Dai F, Wang H, Ding J, Wang X. Increased cerebral hemodynamics during the interictal period of migraine and the association with migraine features. Clin Radiol 2025; 85: 106918.
18. Hansen, J. M., Schankin, C. J. Cerebral hemodynamics in the different phases of migraine and cluster headache. J. Cereb. Blood Flow Metab 2019; 39(4): 595-609.