Hindawi
Acta Neurologica Scandinavica
Volume 2024, Article ID 2954374, 10 pages
https://doi.org/10.1155/2024/2954374

Review Article

The Role of Biomarkers Pituitary Adenylate Cyclase-Activating Polypeptide (PACAP) and Vasoactive Intestinal Peptide (VIP) in Chronic and Episodic Migraines: A Meta-Analysis

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Received 1 September 2023; Revised 24 November 2023; Accepted 13 December 2023; Published 6 January 2024

Academic Editor: Stephen D. m Silberstein

Background. Migraine is a neurological disorder that results in disability accumulation, and there are no blood-based markers for indicating migraine susceptibility. Here, we aimed to evaluate the possible biomarker role of neuropeptides, vasoactive intestinal peptide (VIP), and pituitary adenylate cyclase-activating polypeptide (PACAP) in chronic (CM) and episodic migraine (EM). *Method*. PubMed, medRxiv, and Google Scholar databases were searched up to the 7th of September 2022 using the search syntax to define all relevant articles addressing the plasma and serum levels of VIP and PACAP in migraineurs and controls. Concerning bias assessment, the risk of bias in nonrandomized studies-I (ROBINS-I) was assessed. Also, the standardized mean difference (SMD) was measured with a random effects model. *Results*. Five case-control studies with 503 migraine cases were included. CM patients had elevated VIP levels compared to controls (SMD = 0.44, 95% CI 0.25-0.62, $p < 0.00001$). In contrast, PACAP levels were lower in EM patients (SMD = −0.30, 95% CI -0.48 to -0.11, $p = 0.002$). Overall, migraine cases had higher VIP levels (SMD = 0.25, 95% CI 0.11-0.39, $p = 0.0006$) but lower PACAP levels (SMD = −0.16, 95% CI -0.30 to -0.03, $p = 0.020$) than controls. *Conclusion*. The results support the role of VIP and PACAP neuropeptides in migraine pathophysiology. CM patients have significantly higher serum VIP levels, while EM patients have lower serum PACAP levels compared to controls. Further studies should confirm these findings.

1. Introduction

Migraine is a neurological disorder characterized by episodic to chronic unilateral headaches. Episodic migraine (EM) is defined as experiencing 0 to 14 headache days per month while chronic migraine (CM) is experiencing 15 or more headache days per month. Both forms affect up to 14% of the population including 18% of women, encompassing episodic and chronic forms. Some migraines present with strong unilateral pulsating headaches accompanied by neurological symptoms such as aura, photophobia, nausea, and phonophobia that can persist for hours or days [1, 2]. Due to the coexistence of varying headache types and comorbidities, diagnosis of migraine, especially the chronic form, is challenging. Incorporating biochemical, neurophysiological, and radiological markers would improve the diagnostic accuracy of migraine [3].

Further insights into migraine headache pathophysiology are warranted. One contributing factor to the pain is the stimulation and sensitization of nociceptors surrounding extracranial and intracranial vessels and perivascular sensory afferents. Stimulation of trigeminal sensory fibers in the trigeminal nerve causes the release of vasoactive neuropeptides, particularly CGRP, VIP, and pituitary adenylate cyclase-activating polypeptide (PACAP), into the intracranial meninges (dura mater) resulting in neurogenic inflammation. A specific

brainstem reflex triggered during attacks results in vasoactive peptide (VIP and PACAP) release due to parasympathetic outflow. Upon release into the cephalic vascular system, these neurotransmitters cause pain by activating perivascular sensory afferents [4–6].

The VIP and PACAP neuropeptides belong to the secretin/glucagon/VIP family [7]. They have nearly identical affinity for VPAC1 and VPAC2 receptors coupled primarily to adenylyl cyclase. PAC1 receptors provide a high affinity, specific binding site for PACAP capable of initiating multiple intracellular signaling cascades. Among its diverse roles, PACAP significantly participates in nociception and relaxes vascular and respiratory smooth muscles [8]. The peptide appears colocalized with CGRP for simultaneous release and is highly expressed nervous system, including autonomic and sensory ganglia. Hence, PACAP and CGRP possess similar expression and regulatory mechanisms [9]. Additionally, clinical trials show that intravenous administration of PACAP can elicit headaches through vasodilation of the middle meningeal artery (MMA) in both migraineurs and control individuals [10]. Nociceptive pathways of the central nervous system and peripheral nervous system express highly PACAP and its receptor. There is also a correlation of plasma PACAP levels with the migraine phase, showing that migraineurs have higher plasma PACAP levels during migraine episodes and lower levels in the interictal plasma of migraineurs compared to healthy controls [1]. However, no research has been done to determine whether plasma PACAP levels may be used to distinguish between migraine and other primary headache diseases.

CM patients likely experience more central sensitization and enhanced pain transmission compared to EM patients. Serum VIP and PACAP levels in CM may differ from EM given their critical involvement in pain transmission and central sensitization [11]. To our knowledge, no studies have assessed PACAP and VIP levels in the serum of EM and CM patients separately [12].

We aimed to determine the potential roles of VIP and PACAP neuropeptides as novel biomarkers in EM and CM. This first meta-analysis delineates their distinct roles in CM and EM migraine. Despite limited evidence currently available, we believe that this meta-analysis will promote further research into this area.

2. Methods

2.1. Literature Search. Two authors (AB, SS) searched the PubMed, Google Scholar, and medRxiv databases for related literature published up to the 7th of September 2022. Also, the references of reviews and included studies were examined to ensure relevant articles were included. The reviewers (SS and ST) searched the following syntax: ("vasoactive intestinal peptide" OR "VIP" OR "pituitary adenylate cyclase-activating polypeptide" OR "PACAP") AND ("migraine" OR "chronic" OR "episodic"). Also, the search syntax was adopted for each database.

2.2. Study Selection. Two authors (AB and ST) utilized the Preferred Reporting Items for Systematic Review and Meta-analysis (PRISMA) guideline [13] for the study selection. As a first screening step, included papers from databases were examined by considering the title and abstract. Afterward, articles were assessed through full-text screening. Therefore, eligible studies were selected after full-text screening. Discussions with author SS resolved any discrepancies that arose during the selection process. This systematic review protocol has not been registered in the PROSPERO.

2.3. Eligibility Requirements. The inclusion criteria were as follows:

(1) Case-control study design involving human subjects

(2) Cases were classified into either CM, EM, or both

(3) VIP and PACAP levels in both cases (CM and/or EM) and controls were measured

(4) VIP and PACAP levels were measured in plasma or serum samples

(5) VIP and PACAP levels were measured without acute attacks. VIP and PACAP were only considered if measured during the interictal or nonattack phase since, at this time, the brain is at normal status with the absence of neurovascular events and neuropeptide release. Furthermore, the significance of a biomarker lies in the absence of disease rather than during the disease

(6) The analytical assay used to measure VIP and PACAP was elaborated

We excluded records that reported VIP and PACAP levels during acute attacks. Furthermore, case reports, editorials, review articles, and abstracts were excluded.

2.4. Risk of Bias Assessment. Our bias assessment was based on the risk of bias in nonrandomized studies-I (ROBINS-I) tool [14] since the included studies did not involve randomized controlled trials. Two independent authors (YM and SS) conducted the risk of bias assessment process.

The ROBINS-I tool evaluates the risk of bias under six domains: (1) selection of comparison groups, (2) bias due to confounding, (3) ascertainment of exposure, (4) measurement of outcomes, (5) missing data, and (6) reporting of results. The risk of bias in a study can be classified as low if all domains possess low risk, moderate if at least one domain has moderate risk, serious if at least one domain possesses serious risk, and critical if at least one domain possesses a critical level of bias. The result of the risk of bias assessment did not involve the inclusion or exclusion of studies.

2.5. Data Extraction. Extraction of data was performed by two authors using the following data: (1) author and year of study, (2) country of study, (3) sample size, (4) gender of cases, (5) analytical assay, (6) findings of the study, (7) levels of VIP in cases and controls, and (8) levels of PACAP in cases and controls. In studies reporting the concentration of VIP and PACAP other than pg/mL, the values were extracted and converted to pg/mL. Furthermore, we

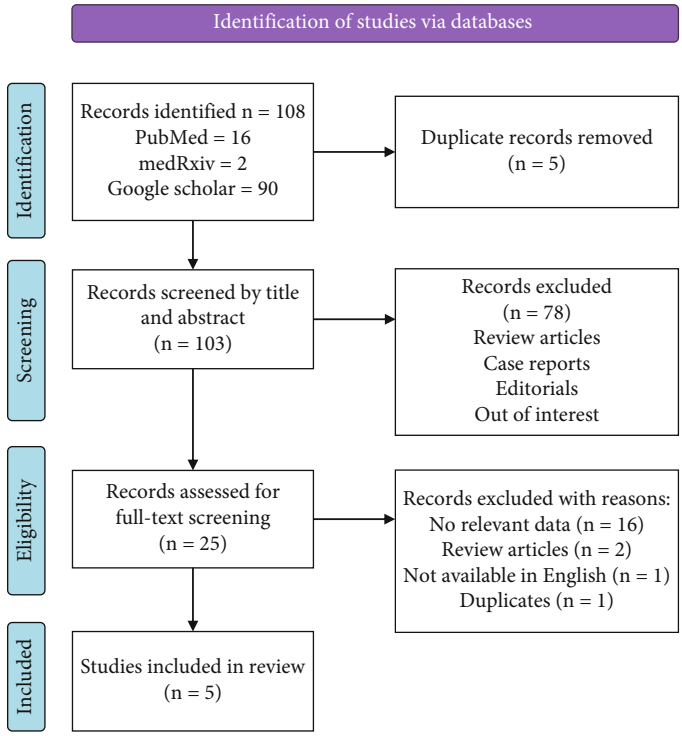

Figure 1: PRISMA flow diagram: the PRISMA diagram includes details of our search algorithm and study selection based on the inclusion and exclusion criteria. PRISMA: Preferred Reporting Items for Systematic Review and Meta-analyses.

converted the levels of PACAP and VIP as median (interquartile range) into mean (standard deviation) using Hozo et al.'s formula [15] since a few studies reported median (interquartile range).

2.6. Statistical Analysis. Meta-analysis of data was conducted by an author (SS) on VIP and PACAP levels in CM and/or EM patients and controls. This meta-analysis was conducted by the author AB using Review Manager 5.4.1 (Cochrane Collaboration) [2] from the extracted data. Four distinct analyses were performed, differences in VIP and PACAP levels between CM and controls, between EM and controls, and between CM and EM. In the end, the levels of VIP and PACAP in pooled (chronic+episodic) migraine versus controls were compared. The standardized mean difference (SMD) was selected as an expression of effect sizes. We used the I^2 statistics to assess heterogeneity within the data. We defined low, moderate, and high heterogeneity as values of I^2 of <25 percent, 25-50 percent, or >50 percent. The pooled SMD was estimated using either a fixed or random effects model based on the results of the heterogeneity analysis. To analyze data with a lower heterogeneity ($I^2 < 50\%$), a fixed effects model was utilized, while in cases of higher heterogeneity ($I^2 \leq 50\%$), a random effects model was conducted. The two-tailed chi-square test was used to test the hypothesis, and tau^2 was estimated using the DerSimonian and Laird method to verify the heterogeneity. An inverse-variance method was used to calculate pooled SMD, expressed as a 95% confidence interval (CI). For interpretation purposes, forest plots were created. Statistical significance was defined as a p value of 0.05. A funnel plot symmetry analysis and outlier analysis were used to detect publication bias.

3. Results

3.1. Descriptive Characteristics of the Studies. Our systematic search resulted in 108 studies. After screening of title, abstract, and full text, five case-control studies [3–7] fulfilled the inclusion criteria (Figure 1). Three studies were from Spain and one each from China and Iran. The studies enrolled a total number of 503 migraine patients and 242 controls. Except for the study by Cernuda-Morollón et al. [16], all studies classified the cases into CM and EM patients. The mean age of migraine patients in the studies ranged from 18 to 70. In all studies, female cases were more than male cases. In the study by Cernuda-Morollón et al. [17], all participants were female. All the studies estimated VIP and PACAP levels using enzyme-linked immunosorbent assay (ELISA) (Table 1).

3.2. Risk of Bias Assessment. Two studies had an overall low risk of bias, while the remaining had a moderate risk. For three studies, risks of bias were moderate concerning confounding and outcome measurement. No studies showed serious or critical risks of bias (Table 2 and Figure 2).

3.3. Comparison of VIP Levels: CM, EM, and Control Patients. CM patients had significantly higher VIP levels versus controls (SMD = 0.44, 95% CI 0.25 to 0.62, $p < 0.00001$) (Figure 3). However, there was no significant difference in

Table 1: Descriptive characteristics of the included studies.

Code	Author year	Study country	Sample size (cases, control)	Age of cases mean (SD)	Gender of cases (male : female)	Analytical assay/ sample used	Key findings
1	Han et al. 2015 [31]	China	Migraine: 133 Chronic: 38 Episodic: 95 Controls: 50	40.86 (11.97)	50 : 11	ELISA plasma	Significantly lower PACAP level in both chronic migraine ($p < 0.001$) and episodic migraine ($p = 0.001$).
2	Morollon "1" et al. 2014 [16]	Spain	Migraine: 81 Chronic: 81 Controls: 33	46.2 (11.0)	1 : 24	ELISA plasma	There was a significantly higher VIP level in chronic migraine than in healthy controls ($p < 0.001$).
3	Morollon "2" et al. 2016 [17]	Spain	Migraine: 121 Chronic: 86 Episodic: 35 Controls: 32	Chronic migraine: 42.8 (13.4) Episodic migraine: 43.9 (13.4)	0 : 121	ELISA serum	There was a significantly higher VIP and PACAP in chronic migraine ($p = 0.027$) and no difference in episodic migraine and controls ($p = 0.093$). Statistically nonsignificant differences of VIP and PACAP between chronic and episodic migraines ($p = 0.371$).
4	Pereda et al. 2020 [3]	Spain	Migraine: 199 Chronic: 101 Episodic: 98 Controls: 97	41 (10)	1 : 9	ELISA serum	VIP and PACAP were significantly elevated in chronic and episodic migraine compared to controls.
5	Togha et al. 2021 [25]	Iran	Migraine: 59 Chronic: 36 Episodic: 23 Controls: 30	38.5	18 : 71	ELISA serum	VIP and PACAP were significantly higher ($p = 0.027$ and 0.043, respectively) in episodic migraine, while there were no significant differences in the case of chronic migraine.

ELISA: enzyme-linked immunosorbent assay; PACAT: pituitary adenylate cyclase-activating polypeptide; VIP: vasoactive intestinal peptide.

Table 2: Result of risk of bias assessment of the included studies.

Study	Bias domains						
	Selection of comparison groups	Bias due to confounding	Ascertainment of exposure	Measurement of outcomes	Missing data	Reporting of results	Overall risk of bias
Han et al. [31]	Low	Low	Low	Low	Low	Low	Low
Morollon "1" et al. [16]	Moderate (only women were assigned controls)	Low	Low	Moderate (no measurement for episodic migraine)	Low	Low	Moderate
Morollon "2" et al. [17]	Low	Moderate (all patients were females)	Low	Low	Low	Low	Moderate
Pereda et al. [3]	Low	Low	Low	Low	Low	Low	Low
Togha et al. [25]	Low	Moderate (patients were under treatment with onabotulinum toxin type A)	Low	Low	Low	Low	Moderate

VIP levels between EM patients and controls (SMD = −0.01, 95% CI −0.23 to 0.21, $p = 0.910$) (Figure 3). Irrespective of migraine subtype, pooled analysis revealed significantly higher VIP levels in migraine cases (SMD = 0.25, 95% CI 0.11 to 0.39, $p < 0.001$) (Figure 3). The asymmetric funnel plot indicated potential publication bias (Supplementary Figure 1). No significant differences in VIP levels were observed between CM and EM patients (SMD = 0.22, 95% CI −0.25 to 0.69, $p = 0.350$) (Figure 4). The symmetric funnel plot revealed no publication bias (Supplementary Figure 2).

3.4. Comparison of PACAP Levels: CM, EM, and Controls. There were no significant differences in PACAP levels between CM patients and controls (SMD = −0.03, 95% CI −0.22 to 0.16, $p = 0.770$) (Figure 5). However, PACAP levels were significantly lower in EM patients versus controls (SMD = −0.30, 95% CI −0.48 to −0.11, $p = 0.002$) (Figure 5). Overall, migraine cases had significantly lower PACAP levels (SMD = −0.16, 95% CI -0.30 to -0.03, $p = 0.020$) (Figure 5). The asymmetric funnel plot revealed potential publication bias from three outliers (Supplementary Figure 3). Moreover, no significant differences in PACAP levels emerged between CM and EM patients (SMD = −0.18, 95% CI −0.78 to 0.41, $p = 0.55$) (Figure 6). The symmetric funnel plot showed an absence of significant publication bias (Supplementary Figure 4).

4. Discussion

This meta-analysis compared the interictal plasma concentrations of VIP and PACAP plasma levels between migraineurs and age-matched healthy controls. Overall, VIP and PACAP levels significantly differed between migraine cases and controls but not always in the same direction. A significant increase in VIP was observed among migraineurs, while PACAP levels showed a significant decrease. Key findings were that serum VIP levels were markedly higher in chronic migraineurs while PACAP concentrations were significantly lowered in episodic migraineurs versus controls. This underscores their potential involvement in chronic and episodic migraine pathophysiology, respectively. Our meta-analysis revealed no significant differences in VIP or PACAP levels between CM and EM cases.

Trigeminovascular activation elicits the primary migraine pain through stimulation and sensitization of nociceptors surrounding extracranial and intracranial vessels. Activation of perivascular sensory afferents by parasympathetic outflow to cephalic vasculature can also contribute [17–20]. Parasympathetic activation clearly participates in migraine pathophysiology [21–23]. With trigeminal-vascular system (TVS) stimulation, CGRP release from trigeminal nerve terminals is accompanied by VIP and PACAP release from the trigeminal-facial arch efferent arm [3]. Trigeminal activation resulting in repetitive pain episodes sensitizes and dysfunctions pain pathways [24].

Several substances, including acetylcholine, PACAP, and VIP, are released during parasympathetic innervation of the cerebral circulation [17]. The trigeminal ganglion expresses

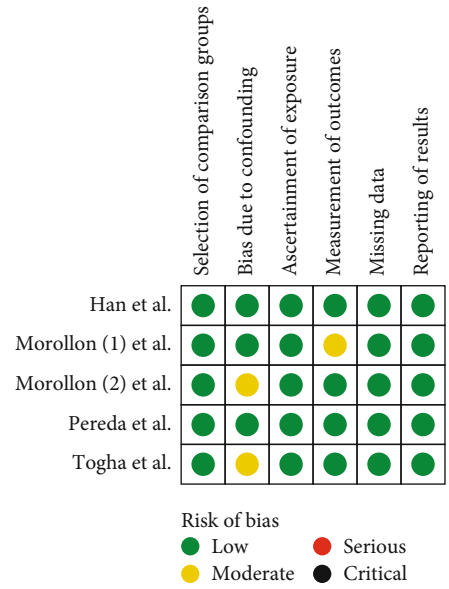

Figure 2: Risk of bias summary of the included studies.

a nonselective cation channel called transient receptor potential cation channel subfamily V member 1 (TRPV1) to release several neuropeptides that contribute to central sensitization, including CGRP, VIP, PACAP, and substance P [25]. As a sensory, sympathetic, and parasympathetic neuropeptide, PACAP-38 is released by nerve endings in the dura mater and other brain parts [26]. Glucagon, secretin, and gastrin inhibitory peptides are members of the structural superfamily of peptides that includes VIP [23]. Several studies on VIP and PACAP in migraine have been conducted in humans. Patients with migraine without aura suffer migraine-like headaches following the administration of PACAP38 to healthy volunteers [27]. Control subjects experience only a short-lasting headache, and migraine patients without aura do not experience any migraine attacks as a result of VIP [28, 29]. Patients with CM often suffer from mild cranial autonomic parasympathetic symptoms. Cranial autonomic parasympathetic symptoms like lacrimation, rhinorrhea, and eyelid edema occur in 27–73% of migraineurs [30]. Extensive parasympathetic innervation of the meningeal vasculature likely contributes to the migraine pathogenesis based on these and other relevant factors [23]. More frequent and prolonged attacks in CM may involve dysfunctional PACAP metabolism that promotes migraine pathophysiology [31]. Both sensory and parasympathetic TVS arms appear activated in CM given CGRP and VIP levels twice as high as controls [16].

PACAP-38 increases intracellular cyclic adenosine monophosphate (cAMP) levels by modulating vessels and nerve fibers. Evidence suggests that increased cAMP levels activate and sensitize trigeminal neurons [32, 33]. Unlike VIP, interictal PACAP concentration measured in peripheral blood does not reflect parasympathetic activation in the CM [17]. Furthermore, PACAP, in contrast to CGRP and VIP, does not appear to be a useful biomarker to measure TVS activity from the cranial parasympathetic arm [17]. PACAP infusion generates migraine and induces

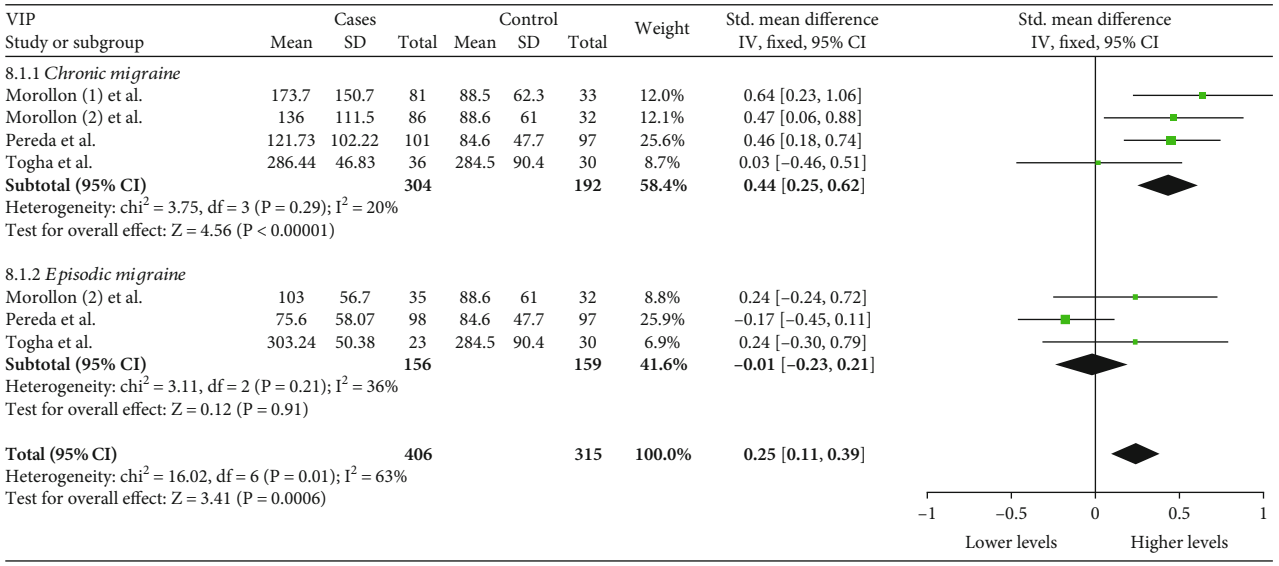

Figure 3: The forest plot shows the VIP levels in chronic, episodic, and overall migraines. VIP mean and SD is expressed in terms of pg/mL. VIP: vasoactive intestinal peptide.

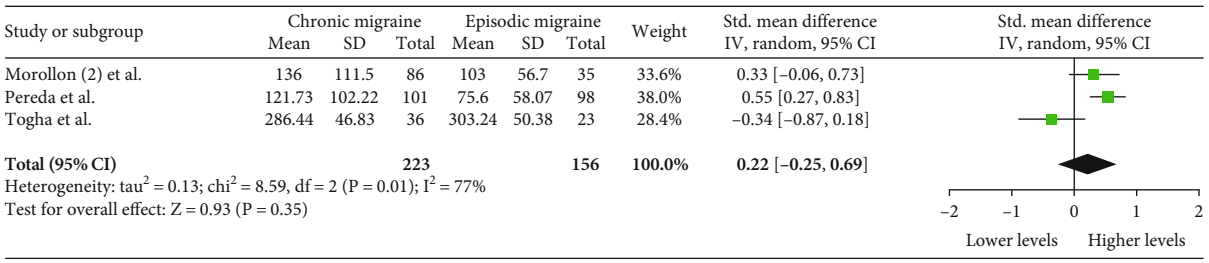

Figure 4: Forest plot showing the levels of VIP in CM and EM patients. VIP mean and SD is expressed in terms of pg/mL. VIP: vasoactive intestinal peptide.

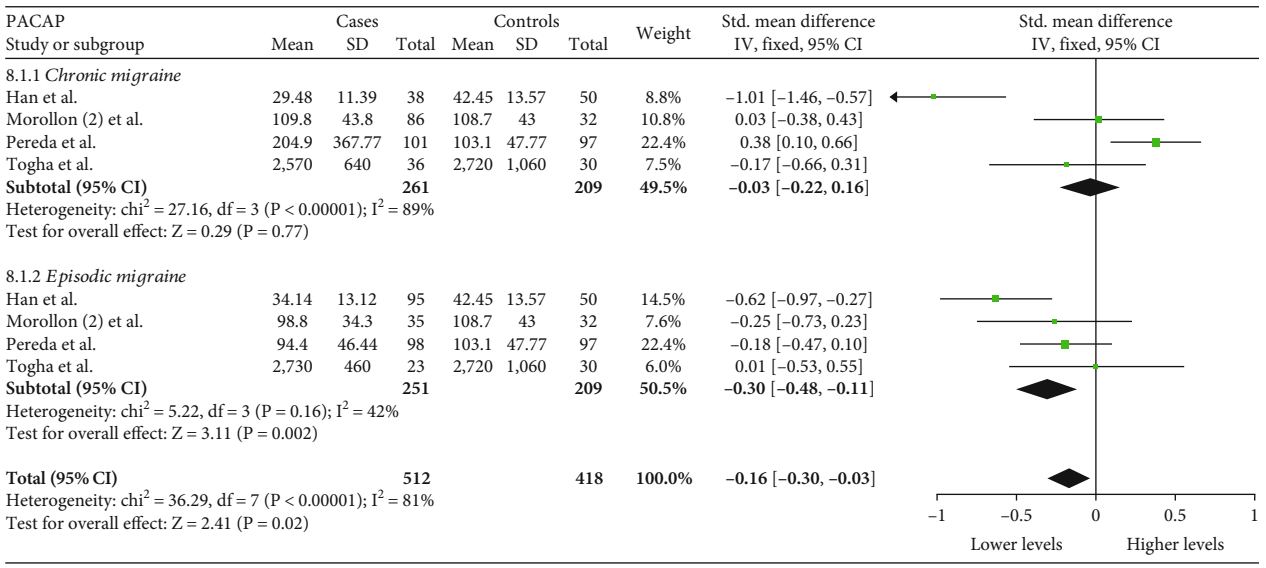

Figure 5: Forest plot showing the levels of PACAP in chronic, episodic, and overall migraine. PACAP mean and SD is expressed in terms of pg/mL. PACAT: pituitary adenylate cyclase-activating polypeptide.

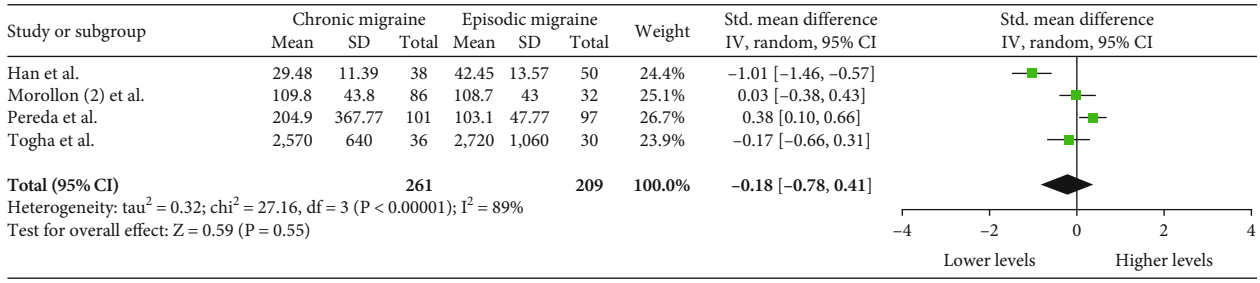

Figure 6: Forest plot showing the levels of PACAP in CM and EM patients. PACAP mean and SD is expressed in terms of pg/mL. PACAT: pituitary adenylate cyclase-activating polypeptide.

vasodilatation and headache in migraineurs, whereas VIP, a more potent vasodilator, does not [17, 29, 34]. Also, Chan et al. showed that as a migraine pathogenesis agent, VIP and PACAP might be less relevant for direct vasodilating effects than for their central effects [35].

VIP can be found throughout the parasympathetic nerve fibers of the temporal artery and middle cerebral artery [5]. This meta-analysis found that VIP was significantly higher overall in particular among CM patients. VIP serum levels were not significantly different between the EM and CM groups. Consequently, VIP was the only model that produced substantial accuracy regarding CM classification. CM diagnosis could be assisted by an increased interictal VIP level measured in peripheral blood, though it cannot clearly differentiate EM from CM [23]. Cernuda-Morollón et al. showed that CM population levels of VIP and CGRP were significantly higher than controls [16]. Also, Cernuda-Morollón et al. found that a significant increase in VIP levels was observed in CM compared to healthy controls. Contrary to our findings, they found that VIP levels were significantly higher in CM patients than in EM patients [17]. In a study, a subgroup of migraineurs with pronounced autonomic symptoms showed elevated VIP levels in the cranial circulation [36]. A study believed that while interictal serum CGRP and VIP were higher in CM than either EM or healthy controls (HC), they did not help detect migraine categories [3]. While we found no significant difference in VIP level between EM and controls, Togha et al. showed that the VIP serum levels in the EM group were significantly higher than those in the control group [25]. It is believed that parasympathetic activation can lead to sensitization and repeated stimulation of afferent nociceptors, contributing to the transformation of EM into CM. VIP is also thought to contribute to migraine chronification [37]. Patients with migraine had higher serum VIP levels with increased parasympathetic activation during migraine attacks and even in the interictal period when they had episodic and chronic migraines [16, 23, 38]. Furthermore, according to Bellamy et al., subjects with migraine experienced significantly higher salivary levels of CGRP and VIP between attacks when compared to controls [39].

The trigeminal-facial arch releases PACAP to induce vasodilation similarly to VIP [25]. Our meta-analysis revealed significantly reduced PACAP levels overall, especially among EM patients, relative to controls. No significant differences arose between CM and EM patients concerning PACAP serum levels either. Hence, PACAP levels could substantially aid EM diagnosis. PACAP seems to exert multifaceted roles in migraine pathogenesis including TVS activation and intracranial vasodilation [31]. To explain lowered interictal PACAP-38 concentrations in migraineurs, researchers posit suboptimal brain energy levels, mitochondrial abnormalities, neuronal Mg2+ imbalances, and PACAP-releasing circuit deterioration [31]. The infusion of PACAP could increase the superficial temporal artery diameter and decrease the middle cerebral artery mean blood flow velocity [27]. Different studies have been done to reveal the link between plasma PACAP levels and the migraine phase and have demonstrated different results. Two studies have examined interictal peripheral levels of PACAP or its component PACAP-38 in migraineurs. Both studies found low levels of interictal PACAP in migraine patients. Tuka et al. used radioimmunoassays demonstrating lower interictal serum PACAP-38 levels in EM patients without attacks. Ictal PACAP-38 levels were higher than interictal levels among migraineurs overall [26]. Liu et al. found higher CGRP and PACAP-38 levels during ictal/interictal phases in migraineurs versus controls [40]. Our findings revealed decreased interictal serum PACAP in EM but no significant difference between CM patients and controls [8], as opposed to the findings of Han et al. Conversely, the causal study by Han et al. showed significantly lower plasma PACAP levels in both the EM and CM groups than healthy controls [31]. Some evidence indicates decreased PACAP levels following migraine treatment with sumatriptan [41, 42]. Studies have found that interictal PACAP levels negatively correlate with migraine disease duration [26, 31]. Despite our results, Pérez-Pereda et al. showed significantly higher serum PACAP levels in CM that better distinguished them from EM cases and controls [3].

Comparisons of VIP and PACAP levels between CM and EM patients revealed no significant differences in our meta-analysis. However, larger confirmatory studies should verify or refute this finding based on comparisons showing significant differences between migraine subgroups and controls.

4.1. Limitations and Strengths. In this study, we encountered some limitations. First, only a few studies met our inclusion criteria, and to avoid heterogeneity and unwise comparisons, a few studies have been removed. The number of participants, both cases and controls, was less. Only female

participants were included in one of the studies that we included. Lastly, the potential methodological bias in each included study could not be ruled out. Our findings implicate that VIP and PACAP can be biomarkers in migraines but vary according to the disease subtype. While VIP can be used in the case of chronic migraine, the role of PACAP lies only in the case of episodic migraine. Five studies included in the meta-analysis used ELISA assays manufactured by Chinese companies that had not been validated. This limitation was not avoidable because the number of studies was limited.

As the first meta-analysis of VIP/PACAP levels in migraine, our study revealed several significant findings. Physicians may benefit by measuring VIP/PACAP levels to diagnose EM or CM headaches.

5. Conclusion

The findings of this study support the hypothesis that VIP and PACAP neuropeptides play a role in migraine. The VIP serum level is significantly higher in CM patients than in the control group. Compared with the control group, the serum level of PACAP in EM patients increased considerably. A comparison of VIP and PACAP serum levels in the EM and CM groups does not show significant differences. This meta-analysis demonstrated the value of VIP and PACAT serum markers in diagnosing CM and EM headaches, which will assist physicians in diagnosing these disorders. There is a need to standardize sample handling and determination for these neuropeptides. Further replication of our findings on the PACAP and VIP roles in EM and CM is required.

Data Availability

All the required information is in the manuscript itself.

Disclosure

Small portion of the manuscript was presented at the 8th International Conference on Neurology and Brain Disorders.

Conflicts of Interest

Authors have no conflict of interest to declare.

Supplementary Materials

Supplementary figure 1: funnel plot of the studies evaluating VIP in chronic/episodic migraine and controls. Supplementary figure 2: funnel plot of the studies evaluating VIP in chronic versus episodic migraine. Supplementary figure 3: funnel plot of the studies evaluating PACAP in chronic/episodic migraine and controls. Supplementary figure 4: funnel plot of the studies evaluating PACAP in chronic versus episodic migraine. *(Supplementary Materials)*

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