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RESEARCH SUBMISSION

Differences in circulating alpha-calcitonin gene–related peptide levels in inflammatory bowel disease and its relation to migraine comorbidity: A cross-sectional study

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Abstract

Objective: To analyze the specificity of calcitonin gene–related peptide (CGRP) levels, we measured alpha-CGRP circulating levels in a large series of patients with a recent diagnosis of inflammatory bowel disease (IBD) who were interviewed regarding comorbid headache.

Background: Several studies have found an association between migraine and IBD.

Methods: In this cross-sectional study performed in an IBD clinic, morning serum alpha-CGRP levels were measured by enzyme-linked immunosorbent assay in 96 patients who were recently diagnosed with IBD and compared to those from 50 similar patients with chronic migraine (CM) and 50 healthy controls (HC).

Results: Alpha-CGRP levels were higher in patients with IBD (median [interquartile range] 56.9 [35.6–73.9] pg/mL) and patients with CM (53.0 [36.7–73.9] pg/mL) compared to HC (37.2 [30.0–51.8] pg/mL; $p=0.003$; $p=0.019$, respectively). Regarding IBD diagnostic subtypes, alpha-CGRP levels for ulcerative colitis (67.2 ± 49.3 pg/mL; 57.0 [35.6–73.4] pg/mL) and Crohn's disease (54.9 ± 27.5 pg/mL; 57.7 [29.1–76.1] pg/mL) were significantly higher than those of HC ($p=0.013$, $p=0.040$, respectively). Alpha-CGRP levels were further different in patients with IBD with migraine (70.9 [51.8–88.7] pg/mL) compared to HC ($p<0.001$), patients with IBD without headache (57.5 [33.3–73.8] pg/mL; $p=0.049$), and patients with IBD with tension-type headache but without migraine (41.7 [28.5–66.9] pg/mL; $p=0.004$), though alpha-CGRP levels in patients with IBD without migraine (53.7 [32.9–73.5] pg/mL) remained different over HC ($p=0.028$).

Conclusion: Together with CM, circulating alpha-CGRP levels are different in patients with IBD, perhaps reflecting a chronic inflammatory state. IBD is an example of how alpha-CGRP levels are not a totally specific migraine biomarker. However, alpha-CGRP

Abbreviations: CD, Crohn's disease; CGRP, calcitonin gene–related peptide; CM, chronic migraine; ELISA, enzyme-linked immunosorbent assay; HC, healthy controls; IBD, inflammatory bowel disease; UC, ulcerative colitis; U-IBD, unclassified inflammatory bowel disease.

levels were further increased in patients with IBD who have a history of migraine, which reinforces its role as a biomarker in migraine patients, always bearing in mind their comorbidities.

Plain Language Summary

Alpha-calcitonin gene–related peptide (CGRP) levels may be a potential migraine biomarker, but it is unclear if this is the case because changes in CGRP concentrations can also be present in other conditions. We measured morning serum alpha-CGRP levels in 96 patients with a recent inflammatory bowel disease (IBD) diagnosis, and compared them to 50 matched healthy participants and 50 matched patients with chronic migraine (CM). We found a significant increase in serum alpha-CGRP levels in both patients with IBD and CM compared to healthy controls, which we think may reflect chronic inflammation found in IBD; these results offer another example that alpha-CGRP concentrations are not totally specific for migraine.

KEYWORDS

alpha-calcitonin gene–related peptide, calcitonin gene–related peptide, chronic migraine, Crohn's disease, inflammatory bowel disease, ulcerative colitis

BACKGROUND

Migraine diagnosis is purely based on clinical criteria and, therefore, contains a subjective component. The availability of a validated migraine biomarker would allow an objective diagnosis and follow-up evaluation after treatment.[1] A variety of neuroimaging, neurophysiological, and biochemical measures have been tested as diagnostic or prognostic biomarkers in migraine,[2] but none seem to be consistent and fully specific. Having said that, calcitonin gene–related peptide (CGRP) has been the only neurotransmitter reliably shown to be released acutely during migraine attacks and interictally increased in patients with chronic migraine (CM) in several body fluids, including peripheral blood,[2–5] saliva,[6] tears,[7] and cerebrospinal fluid.[8] Increased CGRP levels have been shown to decrease and correlate with the clinical response after treatment with onabotulinumtoxinA[5,9] or CGRP monoclonal antibodies in CM.[10,11] Therefore, CGRP might be a specific and sensitive CM biomarker; however, such a role must be taken with caution as not every study has found increased CGRP levels in patients with migraine versus controls.[12] There are several doubts to be clarified in this regard. Some of them are methodological, such as the influence of the very short half-life of this neuropeptide or the heterogeneous enzyme-linked immunosorbent assay (ELISA) tests currently in the market and their specificity on measuring total, alpha-, or beta-CGRP.[13] Other doubts regard the specificity of the increased CGRP levels for migraine. Even though CGRP levels remain in the range of controls in chronic tension-type headache,[14] cervicogenic headache,[15] or fibromyalgia,[16] CGRP levels have been shown to be increased in other primary headaches, such as cluster headache[17] or hemicrania continua,[18] and in diseases like obesity[19] or COVID-19 infection,[20,21] in which inflammation plays a role. It would be convenient, then, to test the specificity of CGRP levels as a potential migraine biomarker, and mainly of the alpha-CGRP, as the isoform involved in migraine pathophysiology in conditions that are comorbid with migraine.

Several epidemiological studies have found a significant comorbid association between migraine and inflammatory bowel disease (IBD).[22–26] IBD, including mainly Crohn's disease (CD) and ulcerative colitis (UC), involves chronic inflammation and disturbance of the gut immune system.[27] In IBD, the epithelial barrier is breached, allowing luminal microbiota to enter, stimulating a proinflammatory immune response. The mucosal injury, entry of luminal factors, and cytokine release overwhelm tissue protection and repair. The cause of IBD remains unknown, and its pathogenesis is complex, encompassing genetic and epigenetic factors, microbiota, and immunological abnormalities.[27]

The neuronal influence on the inflammatory state of the gastrointestinal tract in IBD is well known,[28,29] and gut immune cells express receptors for a range of neurotransmitters, including CGRP, substance P, vasoactive intestinal peptide, or nitric oxide synthase.[30,31] CGRP and its two isoforms (alpha and beta) have distinctive actions. While beta-CGRP is primarily released by primary afferent Dogiel type 2 neurons in the enteric nervous system, alpha-CGRP is liberated by neurons from the vagus nerve and the dorsal root ganglia in the spinal cord via splanchnic and pelvic nerves.[32] With respect to alpha-CGRP functions, they include vasodilation of submucosal vessels, stimulation of peristalsis and gastrointestinal propulsion, inflammatory cell migration from blood vessels, and increased cytokine liberation (Figure 1).[33,34]

In this study, we measured peripheral alpha-CGRP levels in patients with a recent diagnosis of IBD. Our aim was to analyze alpha-CGRP levels, taking into account the coexistence of headache and migraine, particularly in patients with IBD. IBD results were compared to those from two further groups: patients with CM and healthy controls (HC). We hypothesized that alpha-CGRP concentrations would show differences among the three groups and that migraine comorbidity could influence the peptide concentrations in patients with IBD.

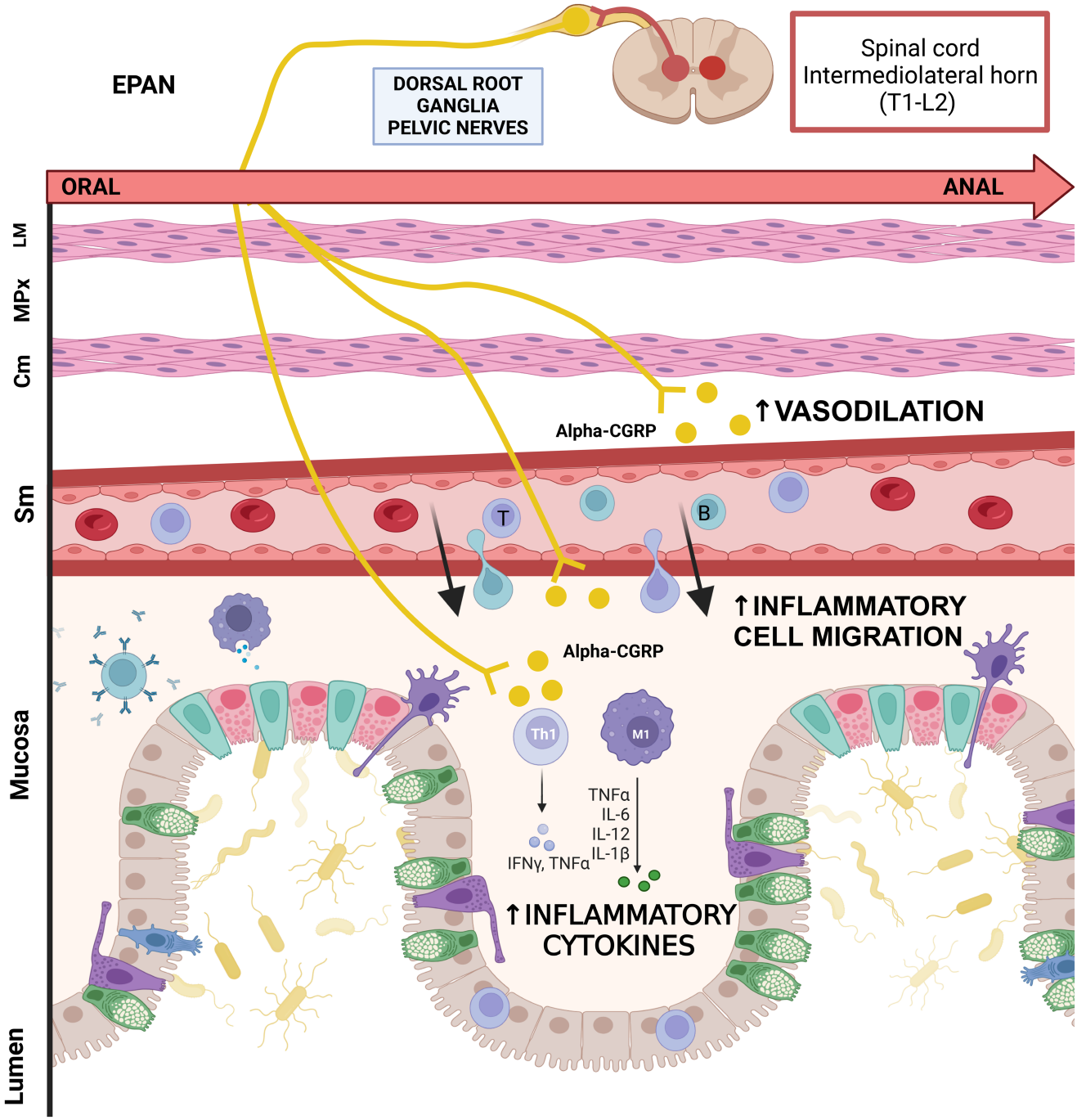

FIGURE 1 Gastrointestinal tract innervation with emphasis on efferent neuronal pathways involved in CGRP release. Alpha-CGRP is liberated by sensory EPANs, located in DGR and supplying the gut via splanchnic nerves and pelvic nerves. Alpha-CGRP release increases gastrointestinal anal propulsion and vasodilation, while promoting inflammatory cell migration and proinflammatory cytokine liberation. See Holzer and Holzer-Petsche[33] and Tan et al.[34] for additional information. Created with BioRender.com. B, B lymphocytes; CGRP, calcitonin gene–related peptide; Cm, circular muscle layer; DC, dendritic cell; DGR, dorsal root ganglia; EPANs, extrinsic primary afferent neurons; IFNγ, interferon gamma; IL, interleukin; LM, longitudinal muscle layer; MPx, myenteric plexus; Sm, submucosal layer; T, T lymphocytes; TNFα, tumor necrosis factor alpha. [Color figure can be viewed at wileyonlinelibrary.com]

METHODS

Study participants

Participants aged >17 years with a recent diagnosis of IBD and classified according to the Montreal Classification[35] were recruited from our IBD Unit from January 2021 to March 2023. To be included, patients with IBD had to have a new, recent (<1 year) IBD diagnosis. Patients on biologics or immunosuppressants were excluded. We allowed only treatment with mesalazine or corticosteroids. All patients with IBD who referred to a history of headache were interviewed by a neurologist from our Headache Unit, who diagnosed

their headache according to current criteria.[36] We registered autoimmune comorbidities and/or extraintestinal manifestations and cardiovascular risk factors. We considered as a significant cardiovascular risk factor the presence of active smoking, arterial hypertension, dyslipemia, diabetes mellitus, body mass index >30 kg/m^2, and cardiovascular events.

Data from patients with IBD were compared to those coming from two groups: CM patients and HC. First, a group of comparable patients who met current CM criteria[36] was recruited in our Headache Unit. They were all taking oral preventive drugs for migraine, but no patient with CM was on anti-CGRP injectable or oral medications. Samples obtained in CM cases who were migraine free and had not taken any acute medication in the previous 24 h of blood extraction were analyzed. Second, as HC, we recruited, specifically for this work, healthy individuals with similar age and sex distribution as the IBD group (medical students, residents, nurses, or physicians from our hospital, or patients' relatives) with subjective absence of headache, without a history of migraine or IBD, and who were not taking any medication for the previous 24 h of blood extraction.

Exclusion criteria for all groups were being pregnant or breast feeding, excessive use of alcohol, and serious, active somatic, or psychiatric diseases. Recruitment was performed simultaneously for the three groups. The study received institutional review board approval by the Ethics Committee of Cantabria (28/2020). All participants gave written informed consent.

Blood extraction and serum processing

Patients were asked to keep a minimum fasting time of 12 h prior to blood extraction. Blood samples from antecubital veins were obtained in our outpatient clinics in the morning (9:00 a.m.–12:00 p.m.) as a part of the research study. After extraction, the samples were left to clot for 10 min, then centrifuged at 3500 rpm and 4°C for 10 min, immediately transferred into sterile tubes, and stored at −80°C. Samples were frozen within 30 min, and none remained cryopreserved for more than 6 months before being analyzed.

Laboratory procedures

Alpha-CGRP levels were determined using a commercial ELISA specific for alpha-CGRP (Abbexa) following the manufacturer's instructions. For the last step of the ELISA process, in which manufacturers give a window of time specifying that the user must determine the optimum, we incubated the plates with the substrate for 20 min after internal validation. All samples were measured in duplicate, and all measurements met the quality criteria set by manufacturers, having an intra-assay coefficient of variation below 8%. A standard curve was generated for every single incubation, and they were calculated using a four-parameter logistic regression with r^2 >0.999. Besides the standards, to ensure the results' reproducibility, we included in each ELISA plate at least 10 previously assayed samples and obtained an inter-assay variability of <10%.

Statistical analysis

Categorical variables are reported as percentages, whereas continuous variables are displayed as mean ± standard deviation for normally distributed data and as median with interquartile range for non-normally distributed data unless stated differently in the text. The normality assumption of quantitative variables has been checked using the D'Agostino & Pearson test. Significance between groups of continuous variables following normal distribution was assessed by independent samples *t*-test. For non-normally distributed data, the Mann–Whitney U test was performed. For multiple group comparisons, we performed the Kruskal–Wallis test followed by Dunn's test.

The *p* values presented are for two-tailed testing, and we considered a $p < 0.05$ to prove statistical significance. All analyses were performed using GraphPad Prism version 9.4.1 (GraphPad Software).

Based on our data from previous studies measuring alpha-CGRP in patients with COVID-19 and patients with CM,[11,20,21] we calculated that with an expected difference between means of 33% between the IBD and the HC groups, an estimated concentration for this latter group of 40 ± 21 pg/mL, an enrollment ratio of 1:1, alpha equal to 0.05, and a power of 80%, we had to include a minimum of 41 individuals per group.

RESULTS

Baseline patient characteristics

We asked 106 patients with IBD to participate in the study; none of them declined the invitation. Ten patients with IBD were excluded: eight patients had an IBD diagnosis longer than 1 year upon review of clinical history, one had an alternative diagnosis after surgery (ischemic ileitis), and one blood sample was lost during the laboratory procedures. Among the CM cases, seven were excluded due to a migraine attack and/or having taken acute medication the previous 24 h (Figure 2). In the end, we included 96 IBD cases (age 47 ± 17 years, range 18–82 years; 60 [62.5%] women), 50 patients with CM (age 47 ± 11 years, range 18–82 years; 38 [76.0%] women), and 50 HC (age 48 ± 16 years, range 23–77 years; 31 [62.0%] women).

There were no missing data for the included participants. Among patients with IBD, 47 (49.0%) met diagnostic criteria for UC, 43 (44.8%) for CD, and 6 (6.2%) for unclassified-IBD (U-IBD). The mean time from diagnosis to blood extraction was 74.9 ± 64.5 days (median: 55.5 days; range: 0–250 days). Regarding treatments, 72 (75%) patients were treated with either corticosteroids, mesalazine, or a combination. Fourteen patients (14.6%) had at least one associated autoimmune disorder or extraintestinal manifestation of IBD. Forty-five patients (46.9%) had one vascular risk factor (Table 1). A total of 47 (49.0%) patients with IBD admitted a history of headache.

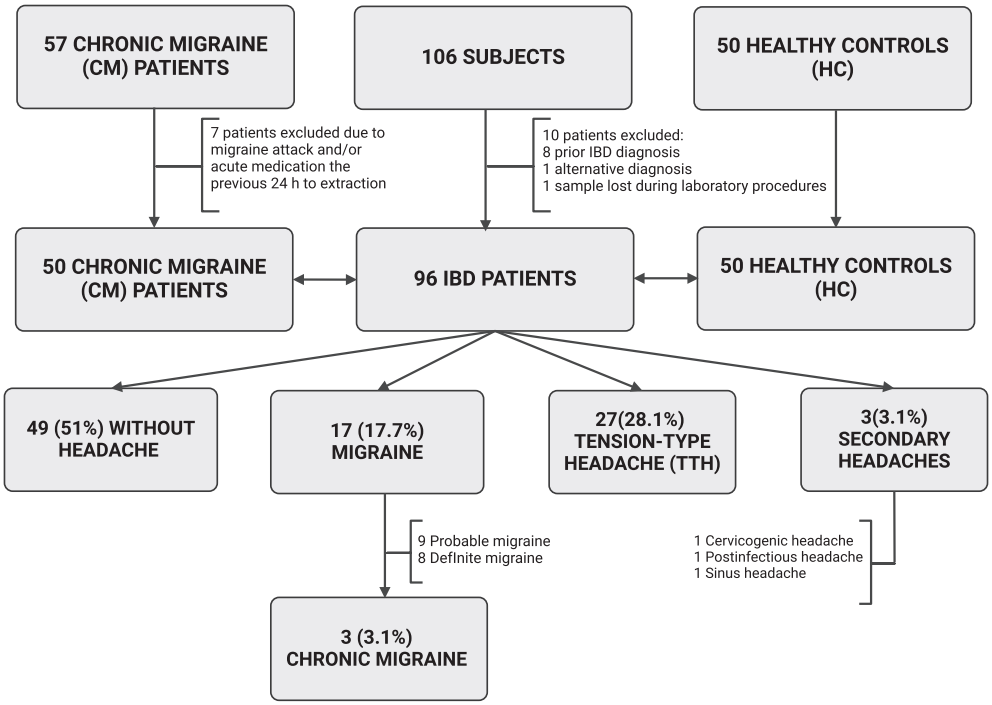

FIGURE 2 Flow diagram of participants, controls, and patients included and excluded in the study. IBD, irritable bowel disease.

Seventeen (17.7%) met migraine criteria (9 [9.4%] probable migraine and 8 [8.3%] definite migraine). Three (3.1%) patients with IBD met CM criteria (Figure 2). Only one patient with IBD and migraine was on preventive treatment (metoprolol). Out of the 30 remaining with headache, 27 (28.1%) met tension-type headache diagnostic criteria, and 3 (3.1%) had secondary headaches.

Alpha-CGRP levels

As part of the primary analysis of the data, we compared alpha-CGRP circulating levels in patients with IBD (61.3 ± 39.7 pg/mL; 56.9 [35.6–71.8] pg/mL) and those obtained from patients with CM (59.4 ± 32.6 pg/mL;53.0 [36.7–73.9] pg/mL), with the concentrations measured from HC (45.9 ± 28.0; 37.2 [30.0–51.8] pg/mL) showing significant differences ($p = 0.003$ and $p = 0.019$, respectively; Figure 3A). As a secondary analysis, we compared alpha-CGRP concentrations according to the IBD diagnostic subtype. Alpha-CGRP levels for UC (67.2 ± 49.3 pg/mL; 57.0 [35.6–73.4] pg/mL) and CD (54.9 ± 27.5 pg/mL; 57.7 [29.1–76.1] pg/mL) were significantly different to those of HC ($p = 0.013$ and $p = 0.040$, respectively), while alpha-CGRP for U-IBD remained only numerically higher (60.1 ± 20.7 pg/mL, 57.3 [43.0–77.9] pg/mL, $p = 0.255$; Figure 3B).

Also, as part of the primary analysis, we conducted a pre-planned multiple-group comparison of our IBD group once classified as migraine, tension-type headache, or absence of headache, and HC. Alpha-CGRP levels were statistically different in those patients with IBD who met migraine criteria (73.0 ± 27.9 pg/mL; 70.9 [51.8–88.7] pg/mL) versus patients with IBD without migraine (58.8 ± 41.4 pg/mL; 53.7 [32.9–73.5] pg/mL; $p = 0.046$), versus patients with IBD with tension-type headache (52.7 ± 40.7 pg/mL; 41.7 [28.5–66.9] pg/mL; $p = 0.004$), as well as versus those who did not have headache (59.5 ± 35.3 pg/mL; 57.5 [33.3–73.8] pg/mL; $p = 0.049$). Moreover, alpha-CGRP levels in IBD subgroups with and without migraine remained significantly different compared to HC ($p < 0.001$ and $p = 0.028$, respectively; Figure 4). Post hoc analysis revealed that three patients with IBD met CM criteria; their alpha-CGRP levels being 74.5 ± 7.3 pg/mL.

Influence of clinical factors in alpha-CGRP levels

This is a secondary analysis performed post hoc of the previously analyzed data to check whether different comorbidities related to CGRP could influence the differences found in our IBD group from the primary analysis. No significant differences arose when patients in the IBD group were classified by presence/absence of cardiovascular risk factors (yes: 54.7 ± 35.6 pg/mL; 55.0 [24.0–73.82] vs. no: 66.7 ± 42.4 pg/mL; 58.3 [37.5–75.2] $p = 0.298$), autoimmune comorbidities (yes: 57.9 ± 18.5 pg/mL; 62.5 [38.4–73.8] pg/mL vs. no: 61.9 ± 42.4 pg/mL; 55.0 [34.0–75.4] pg/mL; $p = 0.728$), active mesalazine treatment (yes: 61.3 ± 40.5 pg/mL; 58.4 [35.5–73.8] pg/mL vs. no: 61.4 ± 38.9 pg/mL; 55.0 [38.0–77.6] pg/mL; $p = 0.821$), or active corticosteroid treatment (yes: 60.3 ± 43.1 pg/mL; 54.9 [35.3–73.9] pg/mL vs. no: 61.5 ± 39.2 pg/mL; 58.2 [35.5–74.5] pg/mL; $p = 0.760$).

DISCUSSION

In this work, we found a significant difference in circulating alpha-CGRP levels in patients with a recent diagnosis of IBD compared to

TABLE 1 Main characteristics of IBD patients.

	Crohn's disease	Ulcerative colitis	Unclassified IBD
Total, *n*	43	47	6
Sex, women (%)	27 (62.8)	32 (68.1)	1 (16.7)
Age, years	50 ± 18 (21–82)	45 ± 18 (18–74)	45 ± 10 (27–56)
Days from diagnosis to serum extraction	757 ± 664, 0–250	87 ± 68 0–250	107 ± 69, 0–193
Cardiovascular risk factors (%)			
≥1	26 (60.5)	13 (27.7)	5 (83.3)
Active smoking	17 (39.5)	5 (10.6)	2 (33.3)
Arterial hypertension	8 (18.6)	2 (4.3)	1 (16.7)
Dyslipemia	8 (18.6)	6 (12.8)	1 (16.7)
Diabetes mellitus	2 (4.6)	0	1 (16.7)
Body mass index, kg/m^2	25.6 (17.2–40.1)	23.5 (17.5–32.3)	26.5 (21.9–31.5)
BMI >30	5 (11.6)	2 (4.3)	2 (4.3)
Immune disorders (%)			
≥1	7 (16.3)	7 (12.8)	0
Rheumatoid arthritis	1 (2.3)	0	
Multiple sclerosis	1 (2.3)	0	
Intrinsic asthma	1 (2.3)	2 (4.3)	
Spondylitis	3 (7)	0	
Uveitis	1 (2.3)	0	
Celiac disease	1 (2.3)	0	
Psoriasis	0	2 (4.3)	
Erythema nodosum	0	1 (2.1)	
Dermatitis herpetiformis	0	1 (2.1)	
Urticaria-vasculitis	0	1 (2.1)	
Treatment (%)			
Any	30 (69.8)	36 (75.6)	6 (100)
Mesalazine	21 (48.8)	33 (70.2)	6 (100)
Corticosteroids	10 (23.3)	7 (14.9)	1 (16.7)
Headache without migraine (%)	14 (32.6)	14 (29.8)	2 (33.3)
Tension-type headache (%)	13 (30.2)	12 (25.5)	2 (33.3)
Secondary headaches (%)	1 (2.3)	2 (4.3)	
Migraine, total (%)	7 (16.3)	10 (21.3)	0
Probable	2 (4.6)	7 (14.9)	
Definite	5 (11.6)	3 (6.4)	
≥15 episodes a month	2 (4.6)	1 (2.1)	

Abbreviations: BMI, body mass index; IBD, Inflammatory bowel disease.

HC. The increase seen in patients with IBD was similar to that found here and in other works in CM[2–5,11] and was uniform for all diagnostic categories of IBD, including CD, UC, and U-IBD. To the best of our knowledge, this is the first study testing the levels of this neuropeptide in IBD and its varieties.

IBD is considered a chronic systemic inflammatory disease, and inflammation has also been proposed to play a role in migraine initiation and chronification via an increased expression of proinflammatory cytokines, including interleukins 1β, 6, and 8, and tumor necrosis factor alpha,[37] whose release is a known consequence of the activation of the trigemino-vascular system, the final mechanism responsible for migraine pain. In fact, besides CM, a few studies have shown an increase in CGRP levels in other inflammatory conditions, both acute, as in COVID-19 disease,[20,21] or chronic, such as obesity.[19] As occurred in patients who had COVID-19 with pneumonia, serum alpha-CGRP levels were significantly higher in those patients with IBD who also met migraine criteria compared to HC, but also in those individuals who had headache, but did not fulfill migraine criteria.[21] These data show that circulating alpha-CGRP levels are not completely specific for migraine, as they can be increased in other

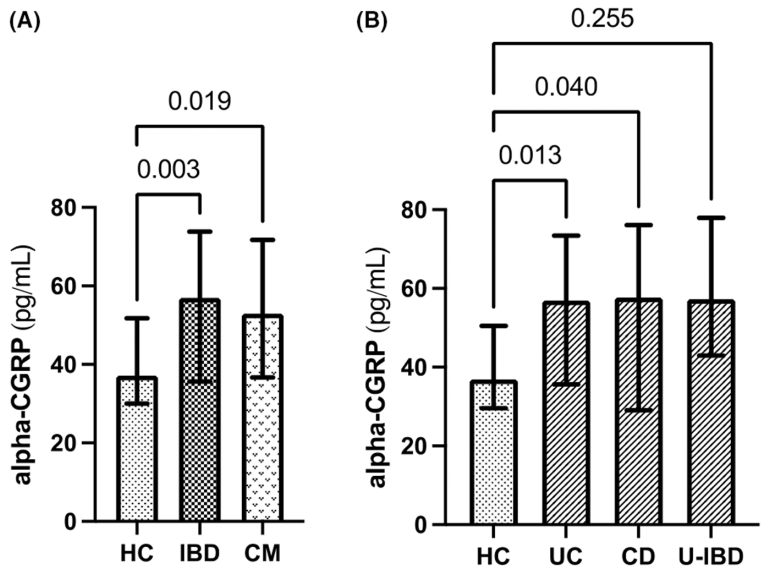

FIGURE 3 Circulating levels of alpha-CGRP in: (A) HC, IBD, and CM patients. (B) HC and IBD patients classified by diagnostic categories. Comparisons are established using the Kruskal–Wallis test followed by Dunn's test and *p* values are displayed on top of the lines connecting compared groups. All data are presented as median with interquartile range. CD, Crohn's disease; CGRP, calcitonin gene–related peptide; CM, chronic migraine; HC, healthy controls; IBD, inflammatory bowel disease; UC, ulcerative colitis; U-IBD, undefined inflammatory bowel disease.

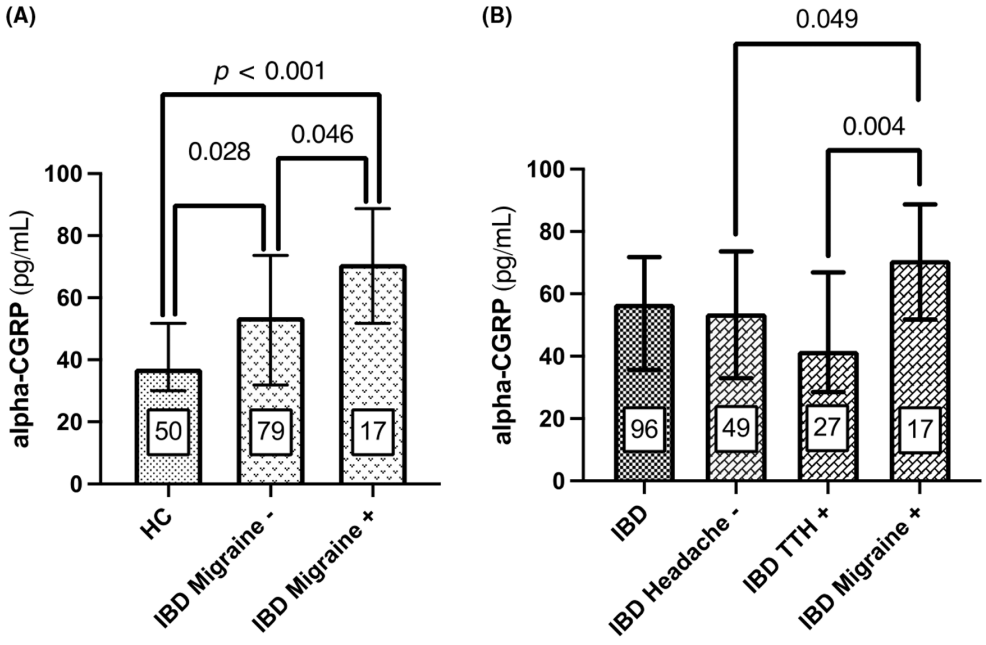

FIGURE 4 Circulating levels of alpha-CGRP in HC and IBD and its subgroups. (A) HC and IBD patients classified by presence of migraine. (B) IBD patients and classified by presence of migraine, presence of tension-type headache or absence of headache. Number of patients per group is displayed inside its corresponding box. Comparisons displayed are established using the Kruskal–Wallis test followed by Dunn's test and *p* values are displayed on top of the lines connecting compared groups. All data are presented as median with interquartile range range. CGRP, calcitonin gene–related peptide; HC, healthy controls; IBD, inflammatory bowel disease; IBD Headache −, inflammatory bowel disease patients without headache; IBD Migraine −, inflammatory bowel disease patients without migraine; IBD Migraine +, inflammatory bowel disease patients with migraine; IBD TTH +, inflammatory bowel disease patients with tension-type headache.

inflammatory conditions. Even so, the data reinforce the value of alpha-CGRP levels as a migraine biomarker, considering that patients who have IBD with migraine (and in particular those with IBD plus CM) showed the highest CGRP levels. In addition, our data concur with the described comorbidity between migraine and IBD.[22–26] They are an example of the bidirectional relationship between the gastrointestinal and nervous systems known as the "gut-brain axis," which has been involved, besides IBD, in the pathophysiology of

migraine and other comorbid gastrointestinal disorders, such as celiac disease, irritable bowel syndrome, or Helicobacter infection.[38,39] The mechanisms of how the brain and gut interact in patients with migraine are not entirely clear, but changes in the microbiota and/or in the biochemistry of neuronal innervation on the gastrointestinal tract could be implicated. Microbiota prevents systemic inflammation and plays a key role in nociception by preparing host adaptation to stress factors,[40] while the neuronal influence on the inflammatory status of the gastrointestinal tract is well demonstrated involving a variety of neurotransmitters,[38] such as serotonin, and of pain-producing neuropeptides, including CGRP. CGRP has been shown to exert a key role in both migraine and gut mucosa homeostasis: infusion of CGRP induces migraine pain and diarrhea[41] (the hallmark symptom of IBD), and the increase of alpha-CGRP shown here could explain some of the gastrointestinal manifestations also happening in patients with migraine. This increase in alpha-CGRP in IBD could also be interpreted as a protective, anti-inflammatory action on the gastrointestinal mucosa,[42] an aspect that should be taken into account and clinically analyzed when administering long-term CGRP antagonists to patients with migraine who also experience IBD.

One limitation of the study is the influence of concomitant IBD medication and activity. Three quarters of patients with IBD were using either corticosteroids or mesalazine, mainly initiated after the first clinical evaluation or endoscopic procedure due to clinical activity, and we were able to recruit only 28 patients with no treatment. Corticosteroids have been shown to decrease CGRP release.[43] Although we did not find significant differences between the subgroups with or without treatment, the number of patients does not allow us to draw definitive conclusions. However, the fact that we still found an increase in alpha-CGRP levels despite symptomatic anti-inflammatory treatment, which is able to decrease CGRP levels, supports our main finding that alpha-CGRP is increased in patients with IBD.

As the recruitment was carried out in specialized IBD and headache clinics, we cannot be absolutely sure of the external validity of our results. One further limitation here could be that we do not know with certainty to what extent the serum alpha-CGRP levels reflect the enteric or trigemino-vascular release of this peptide. At least in migraine, experimental data clearly indicate that circulating levels do reflect the release of alpha-CGRP by an activated trigemino-vascular system,[44] but there can be a contribution from neurons in the dorsal root ganglia supplying the vascular system (Figure 1).[32] This increase in circulating CGRP levels in migraine is selective for the alpha-CGRP as beta-CGRP have been shown to be within HC limits.[11] Strengths of the study include the high number of IBD cases included here; the fact that samples were obtained at the beginning of the disease, avoiding the potential influence of drugs and surgical procedures employed in IBD; and the use of an ELISA test that was alpha-CGRP specific.

CONCLUSIONS

In summary, together with CM, circulating alpha-CGRP levels were higher in patients with IBD than in HC, perhaps reflecting the chronic inflammation linked to IBD. Therefore, IBD is a further example that alpha-CGRP levels are not a totally specific biomarker for migraine. Alpha-CGRP levels were further increased in patients with IBD who had a concomitant history of migraine, which supports alpha-CGRP's role as a migraine biomarker in patients with headache who fulfill the criteria for migraine.

AUTHOR CONTRIBUTIONS

Marta Pascual-Mato: Data curation; investigation; methodology; writing – original draft; writing – review and editing. **Gabriel Gárate:** Data curation; formal analysis; investigation; methodology; writing – original draft; writing – review and editing. **Vicente González-Quintanilla:** Data curation; writing – review and editing. **Jorge Madera-Fernández:** Data curation; writing – review and editing. **Beatriz Castro:** Data curation; writing – review and editing. **María José García:** Data curation; writing – review and editing. **Javier Crespo:** Conceptualization; writing – review and editing. **Montserrat Rivero:** Conceptualization; writing – review and editing. **Julio Pascual:** Conceptualization; data curation; formal analysis; funding acquisition; supervision; writing – original draft; writing – review and editing.

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CONFLICT OF INTEREST STATEMENT

Marta Pascual-Mato, Gabriel Gárate, Vicente González-Quintanilla, Jorge Madera-Fernández, Beatriz Castro, María José García, Javier Crespo, Montserrat Rivero, and **Julio Pascual** declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data, laboratory methods, and study materials are available to other researchers upon reasonable request.

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