Periodontal inflammation is related to increased serum calcitonin gene-related peptide (CGRP) levels in patients with chronic migraine

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Abstract

Background: Recently, a relationship was found between periodontitis and chronic migraine. Calcitonin gene-related peptide (CGRP) is a key element in migraine pathophysiology. However, no information exists of the potential association between periodontal inflammation and CGRP in chronic migraine. The aim of the study was, therefore, to investigate whether there is a link between periodontitis and peripheral levels of CGRP in a cohort of patients with chronic migraine.

Methods: We included 102 chronic migraineurs and 77 age- and sex-matched individuals free of headache/migraine. Full-mouth periodontal parameters were recorded and the periodontal inflamed surface area (PISA) was calculated to quantify the periodon- tal inflammatory status for each participant. Sociodemographic data and comorbidi- ties were assessed by means of a standard questionnaire. We collected blood samples and serum concentrations were done for CGRP, interleukin (IL)-6 and IL-10.

Results: In the chronic migraine group, patients with periodontitis had greater lev- els of serum CGRP (19.7 ± 6.5 versus 15.3 ± 6.2 pg/mL, $P < 0.0001$) and IL-6 (15.1 ± 9.2 versus 9.6 ± 6.3 pg/mL, $P < 0.0001$) while non-significant differences were observed with IL-10 (2.0 ± 1.0 versus 2.8 ± 1.5 pg/mL, $P = 0.675$) concentra- tions than those without periodontitis. PISA was independently associated with CGRP in patients with chronic migraine ($\beta = 0.003$; 95% confidence interval: 0.001 to 0.006, $P = 0.031$). PISA correlated positively with CGRP (r = 0.236; $P = 0.017$) and IL-6 (r = 0.262; $P = 0.008$) in chronic migraine.

Conclusions: Periodontal inflammation is associated with increased circulating levels of CGRP in chronic migraineurs. Elucidating the exact mechanisms through which periodontitis and CGRP are linked in these patients deserves further investigation.

KEYWORDS: calcitonin gene-related peptide, headache, inflammation, migraine disorders, periodontitis

INTRODUCTION

Migraine is a common neurovascular disorder characterized by severe headache often throbbing and unilateral.[1] Migraine attacks are usually accompanied with nausea, vomiting, pho- tophobia, and phonophobia.[1] Although most migraineurs have episodic migraine (<15 days of head pain/month), a subgroup of migraineurs suffers from chronic migraine (>15 days of head pain/month at least for 3 months).[2] The process by which a patient develops chronic migraine due to gradual increase of headache frequency over months or years is called chronification and yearly occurs in about 2.5% of patients with migraine.[3] Despite the fact that chronic migraine is not a fatal disease, it has a substantial impact on patient´s quality of life since it is considered as one of the most disabling diseases worldwide.[4]

Calcitonin gene-related peptide (CGRP) is a key element in migraine pathophysiology.[5] This vasoactive neuropeptide expressed in central and peripheral nervous systems modulates nociceptive input and mediates neurogenic inflammation through activation of the trigeminovascular system.[6] Indeed, CGRP is released inducing vasodilatation around cerebral vessels leading to migraine pain clinically seen as pulsating.[5] It has been suggested that repeated activation of trigeminovascular system might produce migraine chronification owing to central pain sensitization.[7] Levels of CGRP are increased in external jugular blood[8] during migraine attacks (ictal) and in peripheral blood[9] both ictal and interictal phase of migraine (headache-free periods).[9] Particularly, it has been shown that chronic migraineurs have interictal elevated circulating levels of CGRP.[10] For instance, onabotulinumtoxin A (OnabotA), a common treatment option for chronic migraine, is capable of reducing interictal CGRP serum levels[11] and it could be a predictor of good response to this therapy.[12,13]

Periodontitis, a highly prevalent disease[14] is associated with systemic inflammation and vascular changes[15] and has been recently associated with chronic migraine.[16] In this case-control study, patients with periodontitis were more likely to have chronic migraine than those without periodontitis (odds ratio [OR] = 2.4, 95% confidence interval [CI]:1.2 to 4.7).[16] In addition, few mechanistic reports suggested that periodontitis could increase

circulating levels of proinflammatory markers such as leptin[17] or procalcitonin[18] in chronic migraine patients, hence, hypothesizing a possible role of periodontitis as a chronifying factor of migraine.[19] Nevertheless, studies measuring the key neuropeptide CGRP to evaluate the impact of periodontitis on chronic migraine are still missing. Experimental studies showed that lipopolysaccharide, which is an endotoxin from Gram-negative bacteria such as those linked with periodontitis, is able to release CGRP and this process is mediated by proinflammatory cytokines.[20,21] Thus, besides its role on neurogenic inflammation, CGRP can be also involved in immune response after infectious stimulus.[22]

However, to the best of our knowledge, whether periodonti- tis could contribute to elevated levels of CGRP in chronic migraine patients has not yet been explored. Therefore, we aimed to investigate the relationship between periodontitis and CGRP in chronic migraine.

MATERIALS AND METHODS

In this case-control study with a cross-sectional biochemical analysis we included 102 patients with chronic migraine (mean age 47.0 ± 10.2; 98.0% females) and 77 age- and sex-matched healthy controls without neurological disor- ders (mean age 47.5 ± 8.9; 97.4% females). Neurological examination was performed by a senior neurologist (RL). Chronic migraine was defined according to the International Classification of Headache Disorders 3rd edition criteria.[2] Therefore, patients were diagnosed with chronic migraine if they presented headache occurring on ≥15 days per month for >3 months. In addition, we registered time of evolution of chronic migraine (in months), intensity of headache measured with the visual analogue scale, number of days with headaches per month, duration of migraine attacks (in hours), presence of aura and allodynia. Preventives and symptomatic drugs for migraine were also recorded along with analgesic overuse. Exclusion criteria were: 1) underage; 2) < 15 teeth (excluding 3o molars); 3) patients who had received periodontal therapy in the last year; 4) consumption of systemic antibiotics within 3 months before periodontal assessment; 5) severe systemic diseases; and 6) pregnancy or lactation. The study was performed in accordance with the Declaration of Helsinki of the World Medical Association (2008) and approved by the Ethics Committee of the Servizo Galego de Saúde (ID: 2016/079) as well as following the STROBE

(Strengthening the Reporting of Observational Studies in Epidemiology) guidelines.[23] Written informed consent was obtained from each patient or their relatives after full explanation of the periodontal assessment. Demographic and relevant medical information were registered by means of a structured questionnaire for both cases and controls. Periodontal examination protocol was recently reported.[16,24] In brief, two calibrated periodontists (YL and PA) masked to patient medical history recorded full- mouth periodontal measurements from each participant including probing depth (PD), attachment loss (AL), gingival recession (Rec), full-mouth plaque score (FMPS), and full-mouth bleeding score (FMBS).[25] The presence of periodontitis was established when ≥2 interproximal sites with AL ≥3 mm and ≥2 interproximal sites with PD ≥4 mm (not on the same tooth) or one site with PD ≥5 mm was present.[26] Patients were classified into three groups according to the severity of the disease into: 1) Mild periodontitis: those participants with ≥2 interproximal sites with AL ≥3 mm and ≥2 interproximal sites with PD ≥4 mm (not on the same tooth) or one site with PD ≥5 mm; 2) Moderate periodontitis: defined as ≥2 interproximal sites with AL ≥4 mm (not on the same tooth) or ≥2 interproximal sites with PD ≥5 mm, also not on the same tooth; and 3) Severe periodontitis: presence of ≥2 interproximal sites with AL ≥6 mm (not on the same tooth) and ≥1 interproximal site with PD ≥5 mm.[26] Additionally, we calculated a measure of periodontitis activity, the periodontal inflamed surface area (PISA), which reflects the surface area of bleeding pocket epithelium in mm^2.[27] PISA was calculated as follows: 1) With the mean clinical attachment level and Rec we obtained the periodontal epithelial surface area (PESA) for each tooth;[28] 2) The PESA value multiplied by the number of sites with BOP results in the PISA for a specific tooth; and 3) Full-mouth PISA is calculated for each participant (in mm^2) by the sum of the PISAs for each tooth.

Laboratory tests

Fasted samples were obtained in the morning in a pain free period (at least 12 hours from the last migraine attack). Participants had not consumed anti-inflammatory or anal- gesic medication in the previous 72 hours. Briefly, 2 mL of venous blood was collected from the antecubital fossa by venepuncture using a 20-gauge needle with a 2-mL syringe. Blood samples were allowed to clot at room temperature and after 1 hour, serum was separated

from blood by cen- trifugation (15 minutes at 3,000 g) and 0.5 mL of extracted serum was immediately transferred to 1.5-mL aliquots. Each aliquot was stored at −80oC until the time of analysis. Serum levels of all biomarkers were measured by enzyme- linked immunosorbent assay (ELISA) technique following manufacturer's instructions. Interleukin (IL)-6 ELISA kit* minimum assay sensitivity was 1.6 pg/mL with an intra- and inter-assay coefficient of variation (CV) of 5.0% and 6.8%, respectively. IL-10 ELISA kit† minimum assay sensitivity was 2.0 pg/mL with an intra- and inter-assay CV of 4.1% and 6.2%, respectively. CGRP ELISA kit‡ minimum assay sensitivity was 5.3 pg/mL with an intra- and inter-assay CV of 5.9% and 7.4%, respectively. Determinations were done in the Clinical Neurosciences Research Laboratory, which was masked to clinical data. Clinical investigators were unaware of the laboratory results until the study had ended.

Statistical analysis

Mean values and standard deviation were calculated for con- tinuous variables, after the method of Kolmogorov-Smirnov was applied to confirm that the data were sampled from a nor- mal distribution. Categorical data were reported as percent- ages (%) and compared by X^2 test. Independent *t* test and one- way analysis of variance was used to compare the mean values of biomarkers among groups. Additionally, Bonferroni post- hoc tests for multiple comparisons between groups were used. Non-normally distributed variables were reported as median (interquartile range) and compared applying Mann–Whitney *U* test. Non-parametric correlation analyses between PISA and biomarkers among chronic migraine patients were per- formed using Spearman rank correlation coefficient. Linear regression analysis was done to test associations between peri- odontitis, PISA and CGRP levels. Similarly, we also evaluated the link between different grades of periodontitis and CGRP using a generalized linear model. All models were adjusted for potential confounding factors (i.e. age, sex, obesity, depres- sion, and low education level).[16] All tests were performed at a significance level of $\alpha = 0.05$. Statistical analyses were per- formed with data analysis software.§

RESULTS

Patients with chronic migraine had more frequently a history of depression (41.2% versus 9.1%, $P < 0.0001$), lower education level (48.0% versus 26.0%, $P = 0.003$), and obesity

(31.4% versus 5.2%, $P < 0.0001$) than controls (Table 1). The prevalence of periodontitis was higher in chronic migraineurs compared with the control group (58.5% versus 33.8%, $P = 0.001$). Indeed, continuous measures of the extent and severity of periodontitis were also significantly higher in chronic migraine than controls such as average of periodontal pockets with PD ≥6 mm (10.4 ± 15.9 versus 1.6 ± 4.1, $P < 0.0001$) and AL ≥5 mm (30.7 ± 27.5 versus 8.1 ± 10.0, $P < 0.0001$) (Table 1). In addition, increased levels of active periodontitis measured by the PISA method were more present in the chronic migraine group 436.9 (244.2, 698.7) mm^2 versus 43.2 (19.9, 204.4) mm^2 ($P < 0.0001$) (Fig. 1). Migraine-related characteristics are shown in Table 2.

Circulating levels of IL-6 and CGRP were significantly elevated in chronic migraine compared with controls (12.8 ± 8.6 versus 5.7 ± 5.4 pg/mL, $P < 0.0001$ and 17.9 ± 6.7 versus 6.8 ± 4.2 pg/mL, $P < 0.0001$; respectively). Conversely, chronic migraine patients had significantly lower IL-10 serum levels than non-migraine individuals (2.4 ± 1.3 versus 4.7 ± 3.4 pg/mL, $P < 0.0001$). Subgroup analysis according to periodontal status revealed that among patients with chronic migraine, those with periodontitis had significantly higher IL-6 serum levels compared with those without periodontitis (15.1 ± 9.2 versus 9.6 ± 6.3 pg/mL, $P < 0.0001$). Control participants with periodontitis also showed elevated levels of IL-6 compared with non- periodontitis controls (10.6 ± 6.5 versus 3.2 ± 2.0 pg/mL, $P < 0.0001$). Similarly, Figure 2 depicts that the presence periodontitis was linked with increased CGRP in both chronic migraine patients and con- trols. IL-10 concentrations, on contrary, were significantly increased in controls without periodontitis (5.3 ± 3.9 pg/mL) than those with periodontitis (3.6 ± 1.7 pg/mL, $P = 0.030$) as well as compared with chronic migraine patients both with (2.0 ± 1.0 pg/mL, $P < 0.001$) and without periodontitis (2.8 ± 1.5 pg/mL, $P < 0.001$).

Periodontitis as a categorical variable was independently associated with increased serum levels of CGRP in patients with chronic migraine, after adjusting for age, gender, depression, obesity and low education level ($\beta = 4.354$; 95% CI: 1.685 to 7.024, $P < 0.0001$). When severity of periodontitis was analyzed, mild ($\beta = 4.662$; 95% CI: 0.929–8.395, $P = 0.014$) and severe periodontitis ($\beta = 7.366$; 95% CI: 3.990 to 10.743, $P < 0.0001$) were associated with CGRP. In contrary, no significant association was found with moderate periodontitis ($\beta = 1.848$; 95% CI: −1.182 to 4.878, $P = 0.232$). PISA was also

significantly associated with CGRP independent of same confounders (β = 0.003; 95% CI: 0.001 to 0.006, P = 0.031). Indeed, PISA was positively correlated with serum levels of CGRP (r = 0.236; P = 0.017) and IL-6 (r = 0.262; P = 0.008) (Figs. 3A and 3B). A negative correlation was found with PISA and IL-10 concentrations in serum (r = −0.225; P = 0.023). IL-6 and CGRP were also correlated (r = 0.417; P < 0.0001).

DISCUSSION

We found that periodontal inflammation (i.e., PISA) was associated with increased circulating levels of CGRP in chronic migraineurs and this relationship could be mediated by enhanced systemic inflammation posed by periodontitis. To the best of our knowledge, this is the first report aiming to investigate the link between periodontitis and CGRP among chronic migraine patients.

Inflammation is present in migraine.[29] Previously, our group showed that chronic migraineurs had elevated IL-6 levels and lower IL-10 levels compared with individuals without headache.[13] We confirmed this observation in our cohort of participants, in which the chronic migraine group presented more IL-6 and less IL-10 in comparison with the control group. Recent evidence suggests that patients with periodontitis are more likely to have chronic migraine.[16] In our study, we corroborated that periodontitis is common in chronic migraine. Additionally, the median values of a measure of periodontal inflammation and disease activity, the PISA method, were significantly higher in chronic migraineurs than in controls. In fact, the periodontal ulcerated and inflamed epithelium was 4.3 cm^2 in the chronic migraine group versus 0.4 cm^2 in the control group. Based on this, it is speculated that the biological plausibility behind the potential association between periodontitis and chronic migraine is mainly due to the transient entrance of inflammatory mediators into the bloodstream such as leptin or procalcitonin[17,18] that could travel to cerebral blood ves- sels exacerbating migraine attacks, which in turn could lead to the chronification process of the disease. In the present study we observed that chronic migraineurs with periodontitis had significantly higher serum levels of IL-compared with those without periodontitis and that there was a linear positive correlation between PISA and IL-6, thus, confirming again the link between periodontitis, systemic inflammation, and chronic migraine. On contrary and as expected owing to its anti-inflammatory nature, IL-10

circulating concentrations were lower in chronic migraine than in the control group. Furthermore, patients with both conditions (chronic migraine and periodontitis) had also lower levels of IL-10 in comparison with those with chronic migraine and without periodontitis. It has been shown that CGRP has a causal role in migraine attacks[5] and studies showed that this biomarker could be a good predictor of efficacy to prevent migraine attacks in chronic migraineurs.[11,12] Release of peripheral CGRP causes vasodilation and alters the trigeminovascular system microenvironment resulting in more CGRP releases and, therefore, increasing the odds of having more frequent migraine attacks. Evidence from human experiments demonstrated that intravenous injections of CGRP could trigger migraine-like attacks in migraineurs but not in healthy individuals.[5,30] In addition, trials using selective non-peptide CGRP receptor antagonists provided more proof of the causative role of CGRP in migraine pathogenesis.[31] Firstly, we observed that serum levels of CGRP were significantly higher in the chronic migraine group than those without chronic migraine. These findings are in accordance with previous studies where CGRP is consid- ered an important biomarker of chronic migraine.[10] However, negative results were also published where interictal CGRP levels in peripheral blood did not differ between chronic migraineurs and controls.[32] Secondly, those patients with chronic migraine and periodontitis presented increased CGRP levels compared with individuals with chronic migraine and a healthy periodontium. Thirdly, among the control group, periodontal patients had higher levels of serum CGRP in com- parison with those without periodontitis. Contrary to these results, Haririan and co-workers observed no differences in CGRP concentrations measured in both serum and saliva of patients with periodontitis and without periodontitis.[33] However, another study showed that periodontally healthy sites showed increased gingival crevicular fluid concentrations of CGRP compared with sites with gingivitis or periodontitis (no detection of CGRP).[34] A key finding from our study is that periodontitis and in particular the PISA were significantly associated with elevated peripheral levels of CGRP in patients with chronic migraine. This could be explained by the vasodilatory effect produced by periodontal inflammation that might increase the net rate of CGRP removal from the gums and elevate CGRP systemically, thus, promoting migraine chronification due to an increase in the number of headaches. Nevertheless, it

could also be feasible that CGRP initiates the inflammatory process in the gingiva after microbial challenge in periodontally susceptible individuals. Hence, further studies assessing the risk of chronic migraineurs for having periodontitis are warranted. Another possibility is that the association observed between periodontal inflammation and CGRP could be indeed mediated by a systemic inflammatory response.

In our sample of chronic migraineurs, we found a linear correlation between PISA, IL-6 and CGRP. This is supported by experimental data, which showed that lipopolysaccharide from bacteria similar to those involved in the pathogenesis of periodontitis produced cytokines that facilitated CGRP release and sensitized the terminals via regulation of the peripheral releasing function of primary sensory afferents.[20] Due to the cross-sectional nature of our investigation, we cannot rule out the hypothesis that CGRP stimulates IL-6 production. It has been shown that CGRP is able to produce proinflammatory cytokines from lymphocytes and macrophages after lipopolysaccharide infection.[21,35] It would be of great interest whether an enhanced inflammatory response contributes to release of CGRP, or is CGRP one of the contributors to increased systemic inflammation in patients with chronic migraine.

We recognize some limitations regarding the present study. Although the aforementioned cross-sectional design does not allow us to analyze causality, our results are in line with previous studies where periodontitis acts as a systemic inflammatory stressor in chronic migraine patients. Some medica- tions (e.g., non-steroidal anti-inflammatory drugs [NSAIDs], OnabotA, or triptans) as well as conditions like obesity can either decrease or increase CGRP circulating levels. However, CGRP concentrations were still significantly higher in chronic migraine than controls. In our regression models, after adjusting for obesity we found a significant association between periodontitis and CGRP in chronic migraine. Similarly, a number of factors related to either reduction or increase of gingival bleeding/inflammation such as tobacco consumption and medications (e.g., NSAIDs, OnabotA, or antihyper- tensives) together with diseases such as diabetes, obesity, or hypertension can influence the PISA scores.

CONCLUSIONS

Our results indicate that periodontal inflammation is linked with higher serum levels of CGRP in patients with chronic migraine and this association could be mediated indeed by a

systemic inflammatory state due to periodontitis. Longitudinal data are needed to confirm our findings. Experimental studies are also warranted to investigate the biological mechanisms underlying the relationship between periodontitis, CGRP, systemic inflammation, and chronic migraine.

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Table 1. Baseline characteristics and periodontal conditions.

VARIABLES	Chronic migraineurs (n=102)	Controls (n=77)	*P*-value
Hypertension, n (%)	12 (11.8%)	5 (6.5%)	0.234
Diabetes mellitus, n (%)	1 (1.0%)	2 (2.6%)	0.404
Hypercholesterolemia, n (%)	14 (13.7%)	8 (10.4%)	0.501
Bruxism, n (%)	12 (11.8%)	9 (11.7%)	0.987
Depression, n (%)	42 (41.2%)	7 (9.1%)	<0.0001
Current smoker, n (%)	17 (16.7%)	11 (14.3%)	0.664
Stress, n (%)	22 (21.6%)	12 (15.6%)	0.312
Fibromyalgia, n (%)	12 (11.8%)	4 (5.2%)	0.127
Obesity, n (%) [a]	32 (31.4%)	4 (5.2%)	<0.0001
Low education level, n (%)	49 (48.0%)	20 (26.0%)	0.003
FMPS (%)	38.2±22.2	20.9±2.8	<0.0001
FMBS (%)	52.3±26.8	22.1±6.9	<0.0001
Severity of periodontitis, n (%)			<0.0001
Mild	14 (13.7%)	13 (16.9%)	
Moderate	26 (25.5%)	12 (15.6%)	
Severe	21 (20.6%)	1 (1.3%)	
PD measures			
Mean PD (mm)	3.1±0.5	2.4±0.4	<0.0001
Number of sites/mouth PD ≥6 mm	10.4±15.9	1.6±4.1	<0.0001
Rec (mm)	0.5±0.4	0.2±0.1	<0.0001
AL measures			
Mean AL (mm)	3.6±0.8	2.7±0.5	<0.0001
Number of sites/mouth AL ≥5 mm	30.7±27.5	8.1±10.0	<0.0001
Number of present teeth	24.5±3.1	26.0±1.5	<0.0001

[a]Body mass index ≥ 30 kg/m^2.

Table 2. Summary of migraine information and treatment among chronic migraine patients (N=102).

VARIABLES	
Time of chronic migraine evolution (months)	25.8±13.8
Intensity of headache	8.2±1.4
Number days with headache/month	18.8±5.7
Aura, n (%)	41 (40.2%)
Allodynia, n (%)	58 (63.7%)
Duration of migraine attack	
< 12 h	7 (6.9%)
12-24 h	20 (19.6%)
>24 h	75 (73.5%)
Analgesic overuse, n (%)	20 (21.3%)
Preventive treatment in the last 3 months, n (%)	
Topiramate	28 (27.7%)
β-blockers	39 (38.2%)
Amitriptyline	41 (40.2%)
Flunarizine	18 (17.6%)
Onabotulinumtoxin A	70 (68.6%)
Antihypertensives	16 (15.7%)

Figure 1. PISA values in patients with and without chronic migraine.

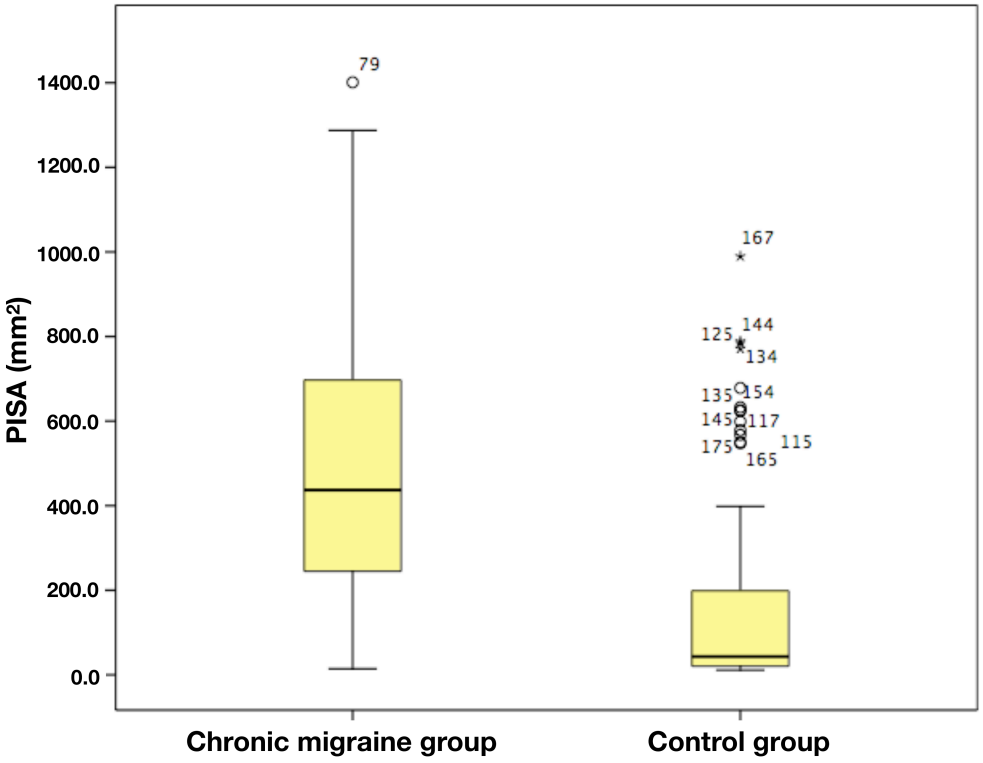


Figure 2. CGRP serum levels of patients with and without chronic migraine according to periodontal status (expressed as mean and SD).

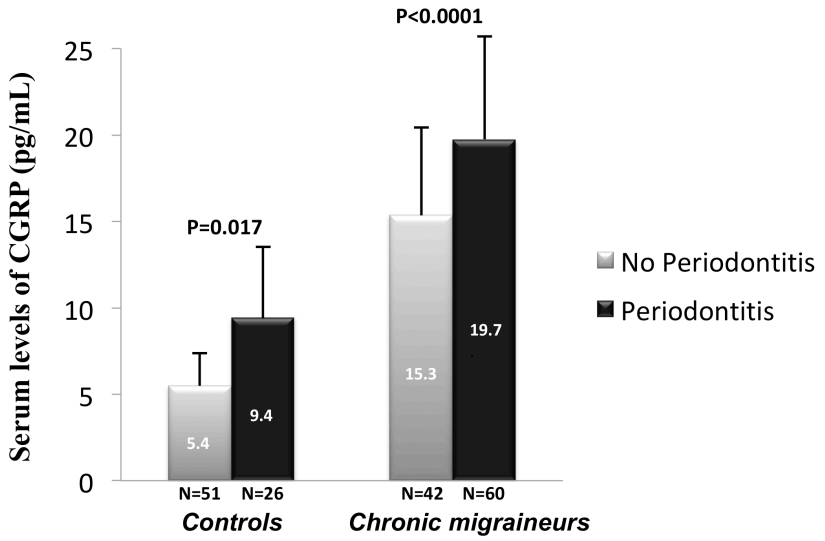
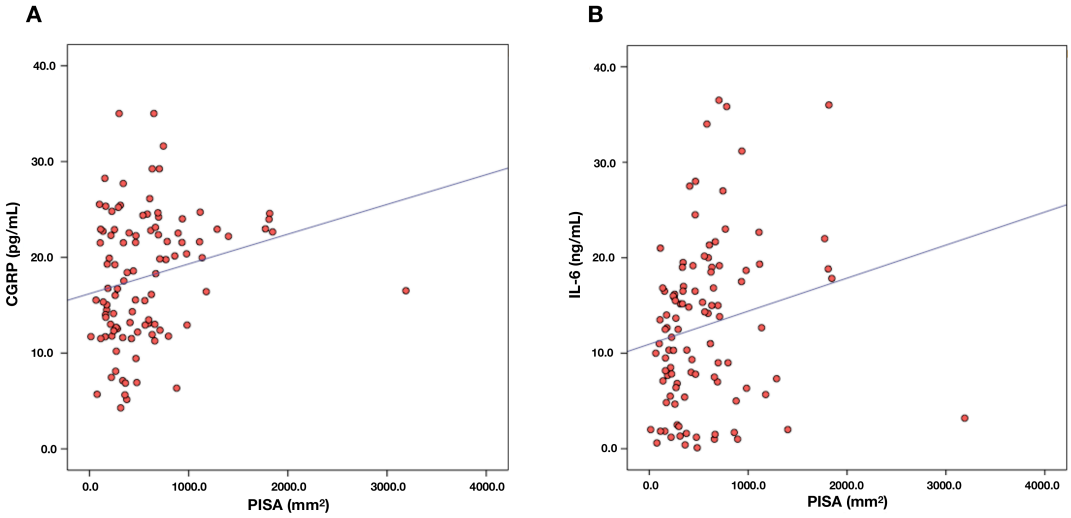


Figure 3. Correlation between PISA and: **A)** CGRP (pg/mL); **B)** IL-6 (ng/mL).



PRODUCT AND COMPANIES

[¶] BioLegend, San Diego, California, USA

[#] BioLegend, San Diego, California, USA

[**] Cloud-Clone, Katy, Texas, USA

[††] IBM SPSS Statistics 20.0 software for Mac, SPSS Inc., Chicago, IL, USA.