Clinical Oral Investigations
https://doi.org/10.1007/s00784-019-02950-9
ORIGINAL ARTICLE

Severe periodontitis is linked with increased peripheral levels of sTWEAK and PTX3 in chronic migraineurs

Yago Leira[1,2,3] & Pablo Ameijeira[2] & Clara Domínguez[4] & Esteban López-Arias[5] & Paulo Ávila-Gómez[5] & María Pérez-Mato[5] & Tomás Sobrino[5] & Francisco Campos[5] & Francesco D'Aiuto[1] & Rogelio Leira[4] & Juan Blanco[2,3]

Corresponding author: Yago Leira (y.leira@ucl.ac.uk)

[1]Periodontology Unit, UCL Eastman Dental Institute and Hospital, University College London, 256 Gray's Inn Road, London WC1X 8LD, UK
[2]Periodontology Unit, Faculty of Medicine and Odontology, University of Santiago de Compostela, Santiago de Compostela, Spain
[3]Medical-Surgical Dentistry (OMEQUI) Research Group, Health Research Institute of Santiago de Compostela (IDIS), Santiago de Compostela, Spain
[4]Department of Neurology, Headache Unit, University Clinical Hospital, Clinical Neurosciences Research Laboratory, Health Research Institute of Santiago de Compostela (IDIS), University of Santiago de Compostela, Santiago de Compostela, Spain
[5]Clinical Neurosciences Research Laboratory, Department of Neurology, Clinical University Hospital, Health Research Institute of Santiago de Compostela (IDIS), Santiago de Compostela, Spain

Rogelio Leira and Juan Blanco contributed equally to this work.

Abstract

Objectives: Periodontitis (PD) and chronic migraine (CM) have been recently linked, and inflammatory processes and vascular endothelial changes are hypothesized as potential mediators of this relationship. The aim of this cross-sectional analysis was to investigate the potential association of PD with vascular systemic inflammation and complement activation in patients with CM.

Materials and methods: Ninety-four chronic migraineurs underwent a full-mouth periodontal evaluation and a measure of PD activity and severity, namely the periodontal inflamed surface area (PISA) was calculated for each patient. We divided CM patients according to their periodontal status: mild PD (N = 14), moderate PD (N = 22), severe PD (N = 19), and non-PD (N = 39). Serum levels of C-reactive protein (CRP), pentraxin 3 (PTX3), soluble tumor necrosis factor-like weak inducer of apoptosis (sTWEAK), and complements C3 and C4 were measured outside of migraine attacks.

Results: We found that severe periodontal patients had significantly higher circulating levels of PTX3 and sTWEAK compared with those without PD (2475.3 ± 1646.8 pg/mL vs. 516.6 ± 1193.8 pg/mL, $P < 0.0001$ and 672.4 ± 118.2 pg/mL vs. 485.7 ± 112.2 pg/mL, $P < 0.0001$; respectively). For the remaining biomarkers, no significant differences were found between groups. Severe PD was independently associated with higher levels of PTX3 ($\beta = 1997.6$, $P < 0.0001$) and sTWEAK ($\beta = 187.1$, $P < 0.0001$) but not with CRP, C3, and C4. PISA positively correlated to PTX3 ($r = 0.475$, $P < 0.0001$) and sTWEAK ($r = 0.386$, $P < 0.0001$).

Conclusions: Based on these preliminary results, severe PD was linked with vascular systemic inflammation in patients with CM. However, further longitudinal studies should be performed to confirm these findings. Clinical relevance sTWEAK and PTX3 measured in serum could be used as biomarkers in the PD-CM link.

Keywords Periodontitis · Chronic migraine · Headache · Inflammation · Periodontal inflamed surface area

Introduction

Periodontitis (PD) is a chronic inflammatory infection affecting the gingiva. If remained untreated, periodontal tissue de- struction and bone loss might progress leading to tooth loss. Currently, 743 million people (10.8%) in the world are affected by severe forms of PD, being this disease, the sixth most common condition worldwide among adults [1]. Migraine is a common, disabling, and complex neurovascular disorder, which has a global estimated prevalence of 14.7% worldwide [2]. Based on frequency of headache attacks, migraine can be sub-classified into episodic mi- graine (EM) (< 15 days of headache/month) and chronic mi- graine (CM) (≥ 15 days of headache/month for at least 3 months with ≥ 8 days/month fulfilling migraine criteria) [3]. Among the general population, the prevalence of CM ranges from 1.4 to 2.2% [4]. The proportion of migraineurs with a CM pattern increases slightly with age. Annually, the progression rate from EM to CM is nearly 3% [5].

Acute-phase reactants (APRs) such as C-reactive protein (CRP) and pentraxin 3 (PTX3) that are overexpressed in periodontal patients are able to activate the complement through the classical pathway. Increased local activation of comple- ment products (e.g., C3 and C4) in the periodontal tissues increases the intensity of the local inflammatory response, resulting in enhanced vascular permeability and vasodilatation and recruitment of inflammatory cells, which in turn will lead to excessive release of reactive oxygen species and interleukins (ILs) and other pro-inflammatory mediators such as IL- 1β, IL-8, or tumor necrosis factor-alpha (TNF-α) [6]. Some of these locally produced inflammatory markers can disseminate into the blood circulation exerting systemic effects in distant organs, thus, contributing to the overall body inflammatory burden. For example, systemic elevated levels of PTX3 and soluble fragment of tumor necrosis factor-like weak inducer of apoptosis (sTWEAK) were linked with PD in patients with small vessel ischemic stroke [7]. In migraine, it has been hypothesized that some inflammatory mediators released during migraine attacks could have an effect in activation and sensitization of peripheral nociceptors [8]. In fact, several studies showed a hyperinflammatory response during migraine with increased levels of CRP, ILs, TNF-α, or PTX3 in the systemic circulation of these patients [9–12]. Furthermore, we demon- strated that PTX3 and sTWEAK are elevated in CM patients and that PTX3 could be a predictor of good response to

onabotulinumtoxin A (OnabotA), which is widely used for the treatment of CM [13]. Recently, our group showed that patients with PD were more likely to have CM than those without PD [14]. We also reported that in CM patients, PD was associated with increased circulating levels of inflammatory markers such as leptin and procalcitonin [15, 16]. However, no information is available regarding the potential link between PD and peripheral blood biomarkers of vascular inflammation as well as complement activation in CM patients. Our aim was, therefore, to investigate the relationship between PD and serum levels of PTX3, sTWEAK, CRP, C3, and C4 in a cohort of patients diagnosed with CM.

Material and methods

Study population

In this cross-sectional study, we included 94 patients with CM (mean age 47.2 ± 10.6; 97.9% females). Neurological exami- nation was carried out by a senior neurologist (RL). CM was defined according to the International Classification of Headache Disorders 3rd edition criteria [3]. Hence, patients were considered to have CM if they presented headache oc- curring on 15 or more days per month for more than 3 months. Additionally, we registered time of evolution of CM (in months), intensity of headache measured with the visual ana- logue scale, number of days with headaches per month, duration of migraine attacks (in hours), and presence of aura and allodynia. Preventives and symptomatic drugs for migraine were also recorded along with analgesic overuse.

Exclusion criteria were (i) < 18 years of age; (ii) < 15 teeth (excluding third molars); (iii) patients who had received periodontal treatment in the previous 12 months; (iv) use of sys- temic antibiotics, within 3 months prior to periodontal assessment; (v) severe systemic diseases; and (vi) pregnancy or lac- tation. 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). Informed consent was ob- tained from each patient or their relatives after full explanation of the periodontal examination. Demographic and relevant medical information were registered by means of a structured questionnaire [14].

Periodontal assessment

Periodontal examination protocol was recently reported [14, 17]. In brief, two calibrated periodontists (YL and PA) carried out full-mouth periodontal measurements from each

participant including probing pocket depth (PPD), clinical attachment level (CAL), gingival recession (Rec), and bleeding on probing (BoP) [18]. Patients were classified into three groups according to the severity of the disease: (a) Mild PD: those participants with ≥ 2 interproximal sites with CAL ≥ 3 mm and ≥ 2 interproximal sites with PPD ≥ 4 mm (not on the same tooth) or 1 site with PPD ≥ 5 mm; b) Moderate PD: defined as ≥ 2 interproximal sites with CAL ≥ 4 mm (not on the same tooth) or ≥ 2 interproximal sites with PPD ≥ 5 mm, also not on the same tooth; c) Severe PD: presence of ≥ 2 interproximal sites with CAL ≥ 6 mm (not on the same tooth) and ≥ 1 interproximal site with PPD ≥ 5 mm [19]. Additionally, we calcu- lated a measure of PD activity, the periodontal inflamed surface area (PISA), which reflects the surface area of bleeding pocket epithelium in mm^2 [20]. PISA was calculated as follows: (i) with the mean CAL and Rec, we obtained the periodontal epithelial surface area (PESA) for each tooth [21]; (ii) the PESA value multiplied by the number of sites with BoP results in the PISA for a specific tooth; (iii) 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 h from the last migraine attack). Subjects had not consumed anti-inflammatory or analgesic medication in the previous 72 h. In brief, 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 h, serum was separated from blood by centrifugation (15 min. at 3000 g) and 0.5 mL of extracted serum was immediately transferred to 1.5-mL aliquots. Each aliquot was stored at − 80 °C until the time of analysis. Serum levels of high-sensitive CRP (hs- CRP) were measured using an immunodiagnostic IMMULITE® 2000 Systems (Siemens Healthcare Diagnostics, Malvern, PA, USA); minimum assay sensitivity was 0.02 mg/dL. The enzyme-linked immunosorbent assay (ELISA) technique was used to assess serum concentrations of PTX3 and sTWEAK following manufacturer instructions. A PTX3 ELISA kit (Rockland antibodies & assays, Limerik PA, USA) minimum assay sensitivity was 40 pg/mL with an intra- and inter-assay coefficient of variation (CV) of < 8% and < 10%, respectively. A sTWEAK ELISA kit (Thermo Scientific, Massachusetts, USA) minimum assay sensitivity

was 10 pg/mL with an intra- and inter-assay CV of < 6% and < 7%, respectively. Complements C3 and C4 serum levels were measured with C3 and C4 Flex® reagent cartridge using the Dimension Vista® System (Siemens, Healthcare GmbH, Marburg, Germany), minimum assay sensitivity 4.00 mg/dL and 1.50 mg/dL, respectively. Determinations were performed in an independent laboratory blinded to clinical data. Clinical investigators were unaware of the laboratory results until the study had ended.

Statistics

All data analyses were performed with IBM SPSS Statistics 20.0 software for Mac (SPSS Inc., Chicago, IL, USA). Mean values and standard deviation (mean ± SD) were calculated for continuous variables, after the method of Kolmogorov-Smirnov was applied to confirm that the data were sampled from a normal distribution. Categorical data were reported as percentages (%) and compared by X^2 test. One-way analysis of variance (ANOVA) was used to compare the mean values among different groups of PD severity. Bonferroni post hoc tests for multiple compari- sons between groups were used. Non-normally distributed variables were reported as median [P_{25}, P_{75}] and compared applying the Kruskal-Wallis H test. Parametric correlation analyses between PISA and significant biomarkers among CM patients were performed using Pearson's correlation coefficient. Generalized linear models were performed to test associations between PD severity and biomarkers. Linear regression models were also created to assess the relationship of continuous measures of PD and biomarkers. All models were adjusted for potential confounding factors (i.e., age, sex, obesity, depression, low education level, and smoking). All tests were performed at a significance level of α=0.05.

Results

Characteristics of the population according to periodontal status

Baseline characteristics of 94 CM patients according to sever- ity of PD are shown in Table 1. In general, periodontal patients were older and had more frequently a history of hypertension, hypercholesterolemia, bruxism, stress, asthma, fibromyalgia, obesity, and lower educational level. However, only preva- lence of depression reached statistical significance (68.4% vs. 28.2%, P = 0.036). Similarly, we observed differences in terms of neurological variables between the PD groups and non-PD patients although were not statistically significant. With regard to migraine treatment, PD patients tended to take more

drugs, even though only opioid consumption was sig- nificantly different between groups ($P = 0.024$). As expected, PISA values increased from periodontal health to disease ($P < 0.0001$). Indeed, severe PD patients showed significantly higher PISA values than those without PD (958.9 ± 457.3 mm^2 vs. 390.2 ± 332.8 mm^2, $P < 0.0001$).

Comparisons of biomarker levels between groups

Circulating levels of PTX3 and sTWEAK were significantly elevated in severe periodontal patients compared with those without PD (2475.3 ± 1646.8 pg/mL vs. 516.6 ± 1193.8 pg/mL, $P < 0.0001$ and 672.4 ± 118.2 pg/mL vs. 485.7 ± 112.2 pg/mL, $P < 0.0001$; respectively). No significant differ- ences were observed between groups regarding hs-CRP, C3, and C4 (Table 2).

Association between PD and its clinical parameters and increased levels of biomarkers

After adjusting for all relevant confounders, severe PD was independently associated with increased serum levels of PTX3 ($\beta = 1997.6$; 95% CI 1219.2 to 2776.0, $P < 0.0001$) and sTWEAK ($\beta = 187.1$; 95%CI 118.2 to 256.0, $P < 0.0001$) in our cohort of CM patients Table 3). Similarly, continuous measures of PD such as PPD and CAL were also significantly associated with both biomarkers (Table 4). In fact, PISA showed a pos- itive correlation with both PTX3 ($r = 0.475$, $P < 0.0001$; Fig. 1a) and sTWEAK ($r = 0.386$, $P < 0.0001$; Fig. 1b). No significant associations were found of PD and its clinical parameters with hs-CRP, C3, and C4 (Table 3 and Table 4).

Discussion

In the present study, we sought to investigate the association between PD and vascular systemic inflammation in CM. Our results showed that severe PD was linked with increased cir- culating levels of PTX3 and sTWEAK in a group of patients diagnosed with CM. However, no significant associations were observed regarding serum levels of CRP, C3, and C4.

Due to its capability to produce a permanent state of low grade inflammation, PD is considered to increase the risk for developing several neurological diseases such as ischemic stroke, Alzheimer's disease, or multiple sclerosis [22–24]. In particular, we recently found that patients with PD had 2.4- fold-increased risk for having CM [14]. In the present analysis we confirmed findings previously observed [14–16], in which PD is common in patients with CM (55 individuals diagnosed with PD, 58.5% of the whole

sample). It has been hypothe- sized that during periodontal inflammation, pro-inflammatory mediators that are produced locally within the gingiva are disseminated systemically due to endotoxemia and, therefore, could be involved in migraine chronification by increasing neurogenic inflammation [25]. In our study, we found an independent association of severe PD and clinical parameters of PD with increased circulating levels of PTX3 and sTWEAK in chronic migraineurs. These results are in accordance with previous reports in the literature in which enhanced systemic inflammation measured by serum levels of leptin and procalcitonin was linked with PD among CM patients [15, 16].

PTXs are members of the superfamily of APRs and can be divided into short and long PTXs. Short constituents include CRP, which is synthesized in the liver mostly upon IL-6 stim- ulation. On contrary, long constituent PTX3 is produced by neutrophils, fibroblast dendritic cells, macrophages, epithelial cells, and endothelial cells, in response to pro-inflammatory signals such as bacterial products, TNF-α, IL-1β, and by toll-like receptor engagement [26, 27]. Our results confirmed pre- vious studies carried out in chronic periodontal patients, which showed a positive relationship between periodontal clinical parameters (i.e., PPD or CAL) and PTX3 levels both locally and systemically [28–30]. Furthermore, cross-sectional clinical data found an increase in PTX concentrations in periodontally affected sites compared with those sites without PD [29]. Similarly, we observed that the PISA method, which is a mea- sure of PD activity and severity, was correlated with levels elevated of PTX3 in peripheral blood. PTX3 has been studied as a marker of vascular endothelial dysfunction. Experimental data suggests that exogenous administration of PTX3 significantly blunted nitric oxide production through the matrix metalloproteinase (MMP)-1 and P-selectin pathway leading to morphological alterations of endothelial cells [31]. Clinical ev- idence demonstrated that plasma PTX3 but not CRP was associated with flow-mediated dilatation (FMD) (a direct measure of endothelial function); thus, shown among patients with coronary artery disease, PTX3 might be a more potent predictor of endothelial dysfunction compared with CRP [32]. These results were supported with other studies performed in different populations of patients such as chronic kidney disease (CKD) or obstructive sleep apnea syndrome [33, 34]. In migraine, PTX3 is not only overexpressed [12] but also could be a new biomarker for the selection of CM treatment because it is asso- ciated with good response to OnabotA [13], giving this mole- cule

important therapeutic implications in chronic migraineurs.

No association was found between PD and its clinical parameters and CRP in our CM patients. Although it is sug- gested that CRP circulating levels are increased in periodontal patients [35], migraine results are controversial [36–41]. Our results are similar to previous studies where PD was not linked with hs-CRP in chronic migraineurs [14, 15]. Nevertheless, it is worth mentioning that severe periodontal patients had two- fold serum hs-CRP serum levels compared with those without PD (0.4 ± 0.9 mg/dL vs. 0.2 ± 0.3 mg/dL). Another finding from our study is the lack of between complement activation markers (i.e., C3 and C4) and PD among our cohort of patients. C3a and C5a can initiate the local inflammatory re- sponse similar to the one occurring in PD, and several com- plement proteins also are able to activate endothelial and phagocytic cells [6]. Even though a few case series observed that complement activation through the classical pathway could occur in migraineurs showing a decrease of C4 and C5 at headache onset as well as C3 breakdown prior to migraine attack [42, 43], further clinical data demonstrated that complement factors might not be involved in migraine pathophysiology [44–46].

sTWEAK belongs to the TNF superfamily and is expressed in several tissues such as the heart, lung, and brain as well as endothelial and smooth muscle cells [47]. Gingival tissues affected by PD overexpress TWEAK compared with healthy periodontal tissues [48]. In our sample of patients, an association was found between severe PD and sTWEAK. What is more, we observed that a clinical measure of periodontal in- flammation (i.e., PISA) was positively correlated with increased serum levels of sTWEAK, and other periodontal pa- rameters such as PPD and CAL confirmed the positive link between PD and sTWEAK. In vitro studies showed that TWEAK has the potential to induce IL-1β, endothelial adhesion molecules [intracellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1)] in human gingival fibroblasts [49, 50]. TWEAK is indeed linked with vascular endothelial dysfunction. In vitro evidence showed that TWEAK is able to induce E-selectin and ICAM-1 on the cell surface of human umbilical vein endothelial cells [51] and in patients with CKD, sTWEAK correlates with FMD [52]. Regarding migraine, our group showed higher levels of peripheral sTWEAK in migraineurs compares with controls although failed to demonstrate a potential role as predictor of good response to OnabotA [13]. However, sTWEAK is related to increased permeability of the blood–brain

barrier (BBB), which is a critical event in the development and progression of neuroinflammatory diseases [53]. For example, sTWEAK modulates the production and activation of MMP-9, thus, being involved in BBB disruption in human cerebral microvascular endothelial cell cultures [54]. We showed increased production of MMP-9 during migraine attacks [55]. Therefore, it is feasible that sTWEAK might also participate in BBB disruption via MMP-9. Further work in this area is needed to explore this hypothesis.

Several shortcomings have to be acknowledged related to our study. The cross-sectional design does not allow demon- strating causality. Secondly, some medications (e.g., antihypertensives or NSAIDs) and smoking habit may influence PISA values. However, none of these variables significantly differ between groups of PD severity and tobacco consumption was adjusted in all regression models. Thirdly, systemic inflammatory biomarkers measured in the present investigation could be increased in the peripheral blood due to other inflammatory conditions related to CM such as obesity [56]. Nevertheless, the association between severe PD and elevated concentrations of PTX3 and sTWEAK remained significant after adjusting for obesity. Finally, we have to be cautious when interpreting our results owing to the low number of patients with severe PD. In spite of having 19 patients with severe PD in our sample (20.2%), it is almost double the number of subjects that we would expect from the general population (10.8%).

We can conclude, within the limitations inherent to our study, that severe PD was associated with increased circulat- ing levels of PTX3 and sTWEAK in patients with CM. Future studies with larger sample sizes are needed to confirm our preliminary results. In addition, trials investigating the poten- tial role of PD in the chronification process of migraine are also warranted.

Acknowledgements: YL holds a Senior Clinical Research Fellowship supported by the UCL Biomedical Research Centre. FC (CP14/00154) and TS (CP12/03121-CPII17/00027) are recipients of a research contract from Miguel Servet Program of Institute of Health Carlos III, Spain. We thank Professor José Castillo for his helpful comments.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest: The authors declare that they have no conflict of interest.

Funding: This study was partially supported by grants from the Spanish Ministry of Economy and Competitiveness – Institute of Health Carlos III (PI15/01578). The funders had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Ethical Approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent: Informed consent was obtained from all individual participants included in the study.

REFERENCES

1. Kassebaum NJ, Bernabé E, Dahiya M, Murray CJ, Marcenes W (2014) Global burden of severe periodontitis in 1990-2010: a systematic review and meta-regression. J Dent Res 93:1045-1053.
2. Vos T, Flaxman AD, Naghavi M et al (2012) Years lived with disability (YLDs) for 1160 sequelae of 289 diseases and injuries 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet 380:2163-2196.
3. Headache Classification Committee of the International Headache Society (IHS) (2013) The International Classification of Headache Disorders, 3rd edition (beta version). Cephalalgia 33:629-808.
4. Natoli JL, Manack A, Dean B et al (2010) Global prevalence of chronic migraine: a systematic review. Cephalalgia 30:599-609.
5. Bigal ME, Serrano D, Buse D, Scher A, Steart WF, Lipton RB (2008) Acute migraine medications and evolution from episodic to chronic migraine: a longitudinal population-based study. Headache 48:1157-1168.
6. Damgaard C, Holmstrup P, Van Dyke TE, Nielsen CH (2015) The complement system and its role in the pathogenesis of periodontitis: current concepts. J Periodontol 50:283-293.
7. Leira Y, Rodríguez-Yáñez M, Arias S et al (2018) Periodontitis is associated with systemic inflammation and vascular endothelial dysfunction in lacunar infarct patients. J Periodontol. https://doi.org/10.1002/JPER.18-0560.
8. Waeber C, Moskowitz MA (2005) Migraine as an inflammatory disorder. Neurology 64:9-15.
9. Vanmolkot FH, de Hoon JN (2007) Increased C-reactive protein in young adults with migraine. Cephalalgia 27:843-846.
10. Perini F, D'Andrea G, Galloni E et al (2005) Plasma cytokine levels in migraineurs and controls. Headache 45:926-931.
11. Martami F, Razeghi Jahromi S, Togha M et al (2018) The serum level of inflammatory markers in chronic and episodic migraine: a case-control study. Neurol Sci. https://doi.org/10.1007/s10072-018-3493-0.

12. Ceylan M, Bayraktutan OF, Becel S, Atis Ö, Yalcin A, Kotan D (2016) Serum levels of pentraxin-3 and other inflammatory biomarkers in migraine: association with migraine characteristics. Cephalalgia 36:518-525.
13. Domínguez C, Vieites-Prado A, Pérez-Mato M et al (2018) CGRP and PTX3 as predictors of efficacy of Onabotulinumtoxin type A in chronic migraine: an observational study. Headache 58:78-87.
14. Ameijeira P, Leira Y, Domínguez C, Leira R, Blanco J (2019) Association between periodontitis and chronic migraine: a case-control study. Odontology 107:90-95.
15. Leira Y, Ameijeira P, Domínguez C, Leira R, Blanco J (2017) The role of leptin as a biomarker in the relationship between periodontitis and chronic migraine. J Clin Periodontol 44:1208-1214.
16. Leira Y, Ameijeira P, Domínguez C, Leira R, Blanco J (2018) High serum procalcitonin levels in patients with periodontitis and chronic migraine. J Periodontol 89:1069-1074.
17. Leira Y, Rodríguez-Yañez M, Arias S et al (2019) Periodontitis as a risk indicator and predictor of poor outcome for lacunar infarct. J Clin Periodontol 46:20-30.
18. Ainamo J, Bay I (1975) Problems and proposals for recording gingivitis and plaque. Int Dent J 25:229-235.
19. Eke PI, Page RC, Wei L, Thornton-Evans G, Genco RJ (2012) Update of the case definitions for population-based surveillance of periodontitis. J Periodontol 83:1449-1454.
20. Nesse W, Abbas F, van der Ploeg I, Spijkervet FK, Dijkstra PU, Vissink A (2008) Periodontal inflamed surface area: quantifying inflammatory burden. J Clin Periodontol 35:668-673.
21. Hujoel PP, White BA, García RI, Listgarten MA (2001) The dentogingival epithelial surface area revisited. J Periodontal Res 36:48-55.
22. Leira Y, Seoane J, Blanco M et al (2017) Association between periodontitis and ischemic stroke: a systematic review and meta-analysis. Eur J Epidemiol 32:43-53.
23. Leira Y, Domínguez C, Seoane J et al (2017) Is periodontal disease associated with Alzheimer´s disease? A systematic review with meta-analysis. Neuroepidemiology 48:21-31.
24. Sheu JJ, Lin HC (2013) Association between multiple sclerosis and chronic periodontitis: a population-based pilot study. Eur J Neurol 20:1053-1059.

25. Ameijeira P, Leira Y, Blanco J, Leira R (2017) Periodontal disease as a potential factor of migraine chronification. Med Hypotheses 102:94-98.
26. Mantovani A, Garlanda C, Doni A, Bottazi B (2008) Pentraxins in innate immunity: form C-reactive protein to the long pentraxin PTX3. J Clin Immunol 28:1-13.
27. Vilahur G, Badimon L (2015) Biological actions of pentraxins. Vascul Pharmacol 73:38-44.
28. Pradeep AR, Kathariya R, Raghavendra NM, Sharma A (2011) Levels of pentraxin-3 in gingival crevicular fluid and plasma in periodontal health and disease. J Periodontol 82:734-741.
29. Fujita Y, Ito H, Sekino S, Numabe Y (2012) Correlations between pentraxin 3 or cytokine levels in gingival crevicular and clinical parameters of chronic periodontitis. Odontology 100:215-221.
30. Temelli B, Yetkin Ay Z, Savaş HB et al (2018) Circulation levels of acute phase proteins pentraxin 3 and serum amyloid A in atherosclerosis have correlations with periodontal inflamed surface area. J Appl Oral Sci 26:1-9.
31. Carrizo A, Lenzi P, Procaccini C et al (2015) Pentraxin 3 induces vascular endothelial dysfunction through a P-selectin/matrix metalloproteinase-1 pathway. Circulation 131:1495-1505.
32. Yasunaga T, Ikeda S, Koga S et al (2014) Plasma pentraxin 3 is a more potent predictor of endothelial dysfunction than high-sensitive C-reactive protein. Int Heart J 55:160-164.
33. Suliman ME, Yilmaz MI, Carrero JJ et al (2008) Novel links between the long pentraxin 3, endothelial dysfunction, and albuminuria in early and advanced chronic kidney disease. Clin J Am Soc Nephrol 3:976-985.
34. Kanbay A, Kaya E, Büyükoğlan et al (2015) Correlation between pentraxin-3 and endothelial dysfunction in obstructive sleep apnea syndrome. Ann Thorac Med 10:199-203.
35. Paraskevas S, Huizinga JD, Loos BG (2008) A systematic review and meta-analyses on C-reactive protein in relation to periodontitis. J Clin Periodontol 35:277-290.
36. Silva FA, Rueda-Clausen CF, Silva SY et al (2007) Endothelial function in patients with migraine during the interictal period. Headache 47:45-51.
37. Guldiken B, Guldiken S, Demir M et al (2011) Soluble CD40 ligand and prolactin levels in migraine patients during interictal period. J Headache Pain 12:355-360.

38. Rockett FC, Perla Ada S, Perry ID, Chaves ML (2013) Cardiovascular disease risk in women with migraine. J Headache Pain 14:1-9.
39. Fava A, Pirritano D, Consoli D et al (2014) Chronic migraine in women is associated with insulin resistance: a cross-sectional study. Eur J Neurol 21:267-272.
40. Welch KM, Brandes AW, Salermo L, Brandes JL (2006) C-reactive protein may be increased in migraine patients who present with complex clinical features. Headache 46:197-199.
41. Vanmolkot FH, de Hoon JN (2007) Increased C-reactive protein in young adult patients with migraine. Cephalalgia 27:843-846.
42. Lord GD, Duckworth JW, Charlesworth JA (1977) Complement activation in migraine Lancet 1:781-782.
43. Lord GD, Duckworth JW (1977) Immunoglobulin and complement studies in migraine. Headache 17:163-168.
44. Behan WM, Behan PO, Durward WF (1981). Complement studies in migraine. Headache 21:55-57.
45. Moore TL, Ryan RE Jr, Pohl DA, Roodman ST, Ryan RE Sr (1980) Immunoglobulin, complement, and immune complex levels during a migraine attack. Headache 20:9-12.
46. Kemper RH, Meijiller WJ, Korf J, Ter Horst GJ (2001) Migraine and function of the immune system: a meta-analysis of clinical literature published between 1966 and 1999. Cephalalgia 21:549-557.
47. Chicheportiche Y, Bourdon PR, Xu H et al (1997) TWEAK, a new secreted ligand in the tumor necrosis factor family that weakly induces apoptosis. J Biol Chem 272:32401-410.
48. Kataria NG, Bartold PM, Dharmapatni AA, Atkins GJ, Holding CA, Haynes DR (2010) Expression of tumor necrosis factor-like weak inducer of apoptosis (TWEAK) and its receptor, fibroblast growth factor-inducible 14 protein (Fn14), in healthy tissues and in tissues affected by periodontitis. J Periodontal Res 45:564-573.
49. Hosokawa Y, Hosokawa I, Ozaki K, Nakae H, Matsuo T (2006) Proinflammatory effects of tumor necrosis factor-like weak inducer of apoptosis (TWEAK) on human gingival fibroblasts. Clin Exp Immunol 146:540-549.

50. Hosokawa Y, Hosokawa I, Shindo S, Ozaki K, Nakae H, Matsuo T (2012) Tumor necrosis factor-like weak inducer of apoptosis increases CC chemokine ligand 20 production in interleukin 1β-stimulated human gingival fibroblasts. Hum Immunol 73:470-473.
51. Harada N, Nakayama M, Nakano H, Fukuchi Y, Yagita H, Okumura K (2002) Pro-inflammatory effect of TWEAK/Fn14 interaction on human umbilical vein endothelial cells. Biochem Biophys Res Commun 299:488-493.
52. Yilmaz M, Carrero JJ, Ortiz A et al (2009) Soluble TWEAK plasma levels as a novel biomarker of endothelial function in patients with chronic kidney disease. Clin J Am Soc Nephrol 4:1716-1723.
53. Boulamery A, Deplat-Jégo S (2017) Regulation of neuroinflammation: what role for the tumor necrosis factor-like weak inducer of apoptosis/Fn14 pathway? Front Immunol 8:1-7.
54. Stephan D, Sbai O, Wen J et al (2013) TWEAK/Fn14 pathway modulates properties of human microvascular endothelial cell model of blood brain barrier. J Neuroinflammation 10:1-14.
55. Leira R, Sobrino T, Rodríguez-Yáñez M, Blanco M, Arias S, Castillo J (2007) MMP-9 immunoreactivity in acute migraine. Headache 47:698-702.
56. Bigal ME, Lipton RB, Holland PR, Goadsby PJ (2007) Obesity, migraine, and chronic migraine: possible mechanisms of interaction. Neurology 68:1851-1861.

AUTHORS CONTRIBUTION STATEMENT

To qualify for authorship, we indicate the contribution of each author to this manuscript:

Leira, Y: design, conceptualization of the study, acquisition of clinical data, analysis, interpretation of the data, drafting, revising the manuscript for intellectual content and final approval of the version to be published.

Ameijeira, P; Domínguez C: acquisition of clinical data, interpretation of the data, drafting, revising the manuscript for intellectual content and final approval of the version to be published.

López-Arias, E; Ávila-Gómez, P; Pérez-Mato, M, Sobrino, T; Campos, F: biochemical analysis, interpretation of the data, drafting, revising the manuscript for intellectual content and final approval of the version to be published.

Leira, R; Blanco, J: design, conceptualization of the study, interpretation of the data revising the manuscript for intellectual content and final approval of the version to be published.

Table 1. Baseline characteristics of 94 patients with CM.

Variables	Whole sample	No PD (N=39)	Mild PD (N=14)	Moderate PD (N=22)	Severe PD (N=19)	P Value
Age (years)	47.1±10.5	45.1±11.1	47.0±10.5	49.1±11.0	48.3±8.8	0.600
Females, %	92 (97.9)	38 (97.4)	14 (100)	22 (100)	18 (94.7)	0.634
BMI (kg/m^2)	27.4 [24.1,31.2]	26.1 [23.4,30.4]	26.1 [21.8,29.9]	29.3 [24.6,33.4]	29.7 [25.5,31.2]	0.187
Hypertension, n (%)	12 (12.8)	3 (7.7)	1 (7.1)	4 (18.2)	4 (21.1)	0.384
Diabetes mellitus, n (%)	1 (1.1)	1 (2.6)	0 (0.0)	0 (0.0)	0 (0.0)	0.700
Hypercholesterolemia, n (%)	14 (14.9)	3 (7.7)	1 (7.1)	4 (18.2)	6 (31.6)	0.085
Bruxism, n (%)	11 (11.7)	5 (12.8)	1 (7.1)	2 (9.1)	3 (15.8)	0.854
Depression, n (%)	39 (41.5)	11 (28.2)	6 (42.9)	13 (40.9)	13 (68.4)	0.036
Current smokers, n (%)	14 (14.9)	4 (10.3)	1 (7.1)	3 (13.6)	6 (31.6)	0.137
Stress, n (%)	21 (22.3)	6 (15.4)	4 (28.6)	3 (13.6)	8 (42.1)	0.084
Asthma, n (%)	13 (13.8)	5 (12.8)	3 (21.4)	2 (9.1)	3 (15.8)	0.756
Fibromyalgia, n (%)	12 (12.8)	4 (10.3)	1 (7.1)	3 (13.6)	4 (21.1)	0.614
Obesity, n (%) [a]	32 (34.0)	11 (28.2)	3 (21.4)	10 (45.5)	8 (42.1)	0.333
Low education level, n (%)	47 (50.0)	17 (43.6)	5 (35.7)	13 (59.1)	12 (63.2)	0.281
PISA (mm^2)	550.9±404.0	390.2±332.8	473.1±276.2	533.1±304.8	958.9±457.3*	<0.0001
Time of CM evolution (months)	25.8±13.9	25.6±14.3	20.2±14.8	26.1±14.0	30.0±11.7	0.271
Intensity of headache	8.0 [7.0,10.0]	8.0 [7.0,9.0]	8.5 [8.0,10.0]	8.0 [6.7,10.0]	8.0 [8.0,10.0]	0.263
Number days with headache/month	18.8±5.7	18.7±5.3	19.1±8.0	17.6±5.3	20.2±4.9	0.551
Aura, n (%)	7 (7.4)	1 (2.6)	3 (21.4)	1 (4.5)	2 (10.5)	0.334
Allodynia, n (%)	58 (63.7)	22 (59.5)	8 (61.5)	15 (68.2)	13 (68.4)	0.876
Duration of migraine attack						0.273
< 12 h	7 (7.4)	2 (5.1)	3 (21.4)	0 (0.0)	2 (10.5)	
12-24 h	18 (19.1)	9 (23.1)	1 (7.1)	4 (18.2)	4 (21.1)	
>24 h	69 (73.4)	28 (71.8)	10 (71.4)	18 (81.8)	13 (68.4)	
Analgesic overuse, n (%)	20 (21.3)	6 (15.4)	3 (21.4)	4 (18.2)	7 (36.8)	0.298
Preventive treatment, n (%)						
Topiramate	28 (30.1)	11 (28.2)	2 (14.3)	5 (23.8)	10 (52.6)	0.082
β-blockers	38 (40.4)	14 (35.9)	9 (64.3)	8 (36.4)	7 (36.8)	0.273
Amitriptyline	37 (39.4)	18 (46.2)	6 (27.3)	7 (36.8)	18 (46.2)	0.527
Flunarizine	15 (16.0)	5 (12.8)	4 (28.6)	3 (13.6)	3 (15.8)	0.565
OnabotA	66 (70.2)	28 (71.8)	9 (64.3)	15 (68.2)	14 (73.7)	0.933

Migraine acute treatment, n (%)						
Triptans	68 (72.3)	26 (66.7)	9 (64.3)	17 (77.3)	16 (84.2)	0.442
NSAIDs	84 (89.4)	35 (89.7)	13 (92.9)	19 (86.4)	17 (89.5)	0.941
Opioids	17 (18.1)	5 (12.8)	2 (14.3)	2 (9.1)	8 (42.1)	0.024

PD: periodontitis; BMI: body mass index; CM: chronic migraine; PISA: periodontal inflamed surface area; OnabotA: onabotulinumtoxin A; NSAIDs: non-steroidal anti-inflammatory drugs.

[a]BMI $\geq$ 30 kg/m^2.

[*]P <0.0001 compared to no PD.

Table 2. Biochemical parameters of 94 patients with CM.

Variables	Whole sample	No PD (N=39)	Mild PD (N=14)	Moderate PD (N=22)	Severe PD (N=19)	P Value
PTX3 (pg/mL)	1063.5±1551.0	516.6±1193.8	769.4±1220.9	1000.9±1551.5	2475.3±1646.8*	<0.0001
sTWEAK (pg/mL)	537.0±138.1	485.7±112.2	521.7±114.6	520.8±140.5	672.4±118.2*	<0.0001
hs-CRP (mg/dL)	0.3±0.5	0.2±0.3	0.3±0.6	0.3±0.4	0.4±0.9	0.501
C3 (mg/dL)	116.0 [103.7,133.5]	118.0 [100.0,128.0]	127.5 [108.7,136.7]	117.0 [100.5,135.2]	112.0 [103.0,142.9]	0.790
C4 (mg/dL)	25.6±8.1	25.2±9.0	22.9±5.8	26.4±7.6	28.1±7.7	0.227

PD: periodontitis; PTX3: pentraxin 3; sTWEAK: soluble tumor necrosis factor-like weak inducer of apoptosis; hs-CRP: high sensitivity C-reactive protein; C3: complement component 3; C4: complement component 4.

*P <0.0001 compared to no PD.

Table 3. Associations of PD with increased levels of inflammatory markers in CM patients.

Dependent variable	Severity of PD	β (95% CI)	P Value
PTX3 (pg/mL)			
	No PD [a]		
	Mild PD	113.5 (-700.2-927.2)	0.784
	Moderate PD	484.3 (-216.7-1185.3)	0.176
	Severe PD	1997.6 (1219.2-2776.0)	<0.0001
sTWEAK (pg/mL)			
	No PD [a]		
	Mild PD	33.1 (-38.8-105.2)	0.367
	Moderate PD	30.1 (-31.8-92.2)	0.340
	Severe PD	187.1 (118.2-256.0)	<0.0001
hs-CRP (mg/dL)			
	No PD [a]		
	Mild PD	0.1 (-0.2-0.4)	0.480
	Moderate PD	0.1 (-0.1-0.4)	0.460
	Severe PD	0.1 (-0.1-0.5)	0.263
C3 (mg/dL)			
	No PD [a]		
	Mild PD	5.8 (-6.6-18.2)	0.361
	Moderate PD	-3.6 (-14.4-7.0)	0.501
	Severe PD	-1.9 (-13.8-9.9)	0.746
C4 (mg/dL)			
	No PD [a]		
	Mild PD	-2.8 (-7.4-1.7)	0.231
	Moderate PD	0.2 (-3.6-4.2)	0.892
	Severe PD	1.9 (-2.4-6.3)	0.392

[a] Reference category.

PD: periodontitis; PTX3: pentraxin 3; sTWEAK: soluble tumor necrosis factor-like weak inducer of apoptosis; hs-CRP: high sensitivity C-reactive protein; C3: complement component 3; C4: complement component 4.

Models are adjusted for age, sex, obesity, depression, low education level and tobacco consumption.

Table 4. Associations of relevant clinical periodontal parameters with increased levels of inflammatory markers in CM patients.

Dependent variable	Clinical periodontal parameters	β (95% CI)	P Value
PTX3 (pg/mL)			
	PPD	799.0 (136.0-1462.0)	0.019
	CAL	485.2 (15.9-954.3)	0.043
sTWEAK (pg/mL)			
	PPD	82.1 (23.4-140.9)	0.007
	CAL	66.0 (25.3-106.6)	0.002
hs-CRP (mg/dL)			
	PPD	0.1 (-0.1-0.3)	0.431
	CAL	0.1 (-0.0-0.2)	0.195
C3 (mg/dL)			
	PPD	-1.1 (-10.4-8.2)	0.810
	CAL	-0.0 (-6.6-6.4)	0.982
C4 (mg/dL)			
	PPD	-0.0 (-3.5-3.3)	0.963
	CAL	0.1 (-2.2-2.6)	0.877

PTX3: pentraxin 3; sTWEAK: soluble tumor necrosis factor-like weak inducer of apoptosis; hs-CRP: high sensitivity C-reactive protein; C3: complement component 3; C4: complement component 4; PPD: probing pocket depth; CAL: clinical attachment level. Models are adjusted for age, sex, obesity, depression, low education level and tobacco consumption.

FIGURE LEGEND

Figure. Correlation between PISA (mm^2) and: **A)** PTX3 serum levels (pg/mL); **B)** sTWEAK serum levels (pg/mL).