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Review Article

Protein-based salivary biomarkers for the diagnosis of periodontal diseases: Systematic review and meta-analysis

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الملخص

أهداف البحث: هدفت هذه المراجعة المنهجية والتحليل التلوي إلى تحديد نقاط التعبير التفاضلي للمؤشرات الحيوية القائمة على البروتين والتي يمكن اكتشافها في اللعاب لأمراض اللثة الرئيسية من خلال مراجعة منهجية وتحليل تلوي.

طريقة البحث: أجريت مراجعة الأدبيات حتى 31 يناير 2022. تم تقييم الجودة المنهجية وخطر التحيز باستخدام مقياس نيوكاسل أوتاوا لدراسات الحالات والشواهد. تم إجراء التحليل الإحصائي للنتائج باستخدام أداة "ميتا المفتوحة" (محلل). تم إجراء تحليل المسارات البيولوجية داخل علامات البروتين التفاضلية باستخدام أداة تحليل تكامل "ستيتش"، اقتصرت على التفاعلات مع مستويات ثقة عالية (0.7).

النتائج: من بين جميع المؤشرات الحيوية القائمة على البروتين التي تم اكتشافها، يمكن تحليل 12 مؤشرا تلويا: انترليوكن-1بيتا، والبروتينات الالتهابية الضامة-1ألفا، والألبومين، وعامل نخر الورم-ألفا، والتيلوبيبتيد المتقاطع من الكولاجين ألفا -1 ، والغلوبولين المناعي أ ، واللاكتوفيرين، بروتيناز فلزي شبكي-8، وانترليوكن-6، وانترليوكن-8، وانترليوكن-17 وبروستاقلاندين-2. كانت العلامات اللعابية ذات التطبيق الرئيسي هي انترليوكن-1بيتا، للتمييز بين مجموعة أمراض اللثة المزمنة والتهاب اللثة مع التعبير الكلي 73.5 بيكوجرام/ملي، والتيلوبيبتيد المتقاطع من الكولاجين ألفا -1 لأمراض اللثة المزمنة مقابل مرضى التحكم الصحي مع التعبير الكلي 0,091 نانوجرام/ملي، وبروستاقلاندين-2 لأمراض اللثة المزمنة مقابل مرضى التحكم الأصحاء الذين لديهم تعبير الكلي 36,3 بيكوجرام/ملي.

الاستنتاجات: المؤشرات الحيوية ذات أعلى تعبير تفاضلي وأكبر إمكانات للتطبيق السريري هي انترليوكن-1بيتا للتمييز بين التهاب اللثة والمرحلة المتقدمة من التهاب اللثة، التيلوبيبتيد المتقاطع من الكولاجين ألفا -1 والبروستاقلاندين-2 لالتهاب اللثة مقابل المرضى الأصحاء.

الكلمات المفتاحية: المؤشرات الحيوية؛ التهاب اللثة؛ أمراض اللثة؛ المرحلة المتقدمة من التهاب اللثة

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Abstract

Objective: This systematic review and meta-analysis was aimed at determining differentially expressed protein-based biomarkers detectable in the saliva for the diagnosis of major periodontal diseases.

Methods: A literature review was conducted through January 31, 2022. The methodological quality and risk of bias were assessed with the Newcastle–Ottawa scale for case-control studies. Heterogeneity among studies was analysed with the Q statistical test and the I^2 test. p-values lower than 0.10 and I^2 values higher than 50% indicated high heterogeneity among studies; therefore, the random-effects model was used. The analysis of biological pathways associated with the differentially expressed protein markers was performed with the STITCH integration analysis tool and was limited to interactions with high confidence levels (0.7).

Results: Of all protein-based biomarkers detected, 12 were suitable for meta-analysis: IL-1β, MIP-1α, albumin, TNF-α, ICTP, Ig-A, lactoferrin, MMP-8, IL-6, IL-8, IL-17 and PGE2. The salivary markers with high applicability were IL-1β for differentiating patients with chronic periodontal disease from patients with gingivitis with an OE = 73.5 pg/mL; ICTP for differentiating patients with chronic periodontal disease from healthy control patients with an OE = 0.091 ng/mL; and PGE2 for differentiating patients with chronic periodontal disease from healthy control patients with an OE = 36.3 pg/mL.

Conclusions: The biomarkers with the highest differential expression and the greatest potential for clinical applicability are IL-1β for differentiating periodontitis from gingivitis, and ICTP and PGE2 for differentiating periodontitis from healthy status.

Keywords: Biomarkers; Gingivitis; Periodontal diseases; Periodontitis

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Introduction

Gingivitis can be diagnosed according to specific signs of inflammation on the gums in response to the biofilm accumulated on the tooth surface over several days. Various factors, such as the systemic conditions of the patient or the intake of specific drugs, may favour development of this disease.[1] The pathology of gingivitis is characterised on the basis of clinical reversibility and the presence of a moderate to high bacterial load. In addition, gingivitis is understood as a precursor of periodontal disease and therefore must be controlled to prevent irreversible outcomes.[2]

Periodontal disease is a chronic inflammatory disease of the periodontium, which results in loss of the periodontal ligament and the destruction of the underlying alveolar bone.[3] The host's immune system defends against bacteraemia through an inflammatory response, which results in chronic inflammatory infection. Additionally, the immune response modulates the expression and virulence of biofilms, which interfere with multiple protective homeostatic mechanisms.[4]

Periodontal diseases affect as much as 50% of the world's population and rank sixth among the most prevalent pathologies worldwide.[5] Currently, the diagnosis of periodontitis is a precise, consistent and simple process enabling identification of disease stage, and the standardisation of specific treatments.[6] However, these clinical and radiological methods are useful only for confirming the presence and severity of this disease, whereas information on the biological behaviour at the stage of diagnosis is lacking. Moreover, clinical monitoring is a time-consuming activity in daily practice.[7]

Saliva is a known reservoir of large amounts of molecules and may potentially be influenced by bacterial dysbiosis and simultaneous effects of the host's immune system response. Many of these molecules are proteins that are involved in various molecular processes and can be quantified with diverse molecular techniques to identify diagnostic cut-offs for periodontal pathologies.[8]

This systematic review and meta-analysis was aimed at exploring the available literature to identify differentially expressed protein-based biomarkers that can be detected in saliva and used for diagnosis of major periodontal diseases.

Materials and Methods

Protocols and inclusion criteria

A literature review was conducted through January 31, 2022. The review was organised according to the PRISMA guidelines[9] for systematic reviews. The PICO elements were as follows: P: patients with gingivitis (GV), chronic periodontal disease (CPD) or aggressive periodontal disease (APD); I: salivary protein study; C: healthy control patients (HCG); O: differences in salivary protein expression between CPD and GV, compared with HCG.

The inclusion criteria were as follows: 1) human case-control studies in English; 2) studies including quantified salivary protein-based markers; and 3) studies using ELISA and/or equivalent analytic methods with quantitative expression determination (bromocresol green method, Immunoturbidimetry and LUMINEX).

The exclusion criteria were as follows: 1) studies performing differential expression proteomic analysis through mass spectrometry or LC-MS/MS; 2) in vitro or animal studies; 3) studies quantifying protein expression in units of measurement not transformable to ng/dl; 4) studies in which the marker was expressed below a minimum cut-off point; 5) studies with unclear or poorly defined control groups; and 6) studies with a risk of bias rating of less than 7 on the Newcastle–Ottawa Scale.[10]

Sources

The electronic research was conducted in MEDLINE through PubMed; EMBASE through OVID; Web of Science; Scopus; Cochrane Library; Clinical Trials; the five regional bibliographic databases of the WHO; and databases from the Conference Citation Index.[11] The terms used in this process were as follows: gingivitis OR chronic periodontitis OR periodontal disease OR aggressive periodontal disease AND salivary protein OR biomarker OR salivary biomarker OR protein expression.

Study selection and data extraction process

The research was performed by five observers (EAA, MGOA, SBBL, JBC and MPS), and CMCP acted as an independent observer in the event of any discrepancies. First, the title and abstract of the retrieved articles were read. Subsequently, the full text of all eligible studies was examined, and a decision was made regarding study inclusion. The data were extracted with a standardised, pilot-tested form.

The form included the following components: title; authors; publication year; study group; sample size; biomarker; quantitative expression; analysis method; and diagnostic criteria for the pathology under study.

Diagnostic criteria

The GV definition was established according to Armitage's 1999 classification defining GV as gingival disease induced by dental plaque without any other contributing factor.[12] The diagnosis of CPD varied from radiographic to arbitrary criteria based on probing depth thresholds (PPD) or clinical attachment loss (CAL) for a determined number of affected sites, or PPD or CAL averages for the full mouth. Because of modifications that have occurred over the years, broad criteria were established as follows: CPD Group: Two or more sites with PPD $\geq$4 mm and CAL $\geq$2 mm. APD group: any PPD $\geq$6 mm with associated CAL >2 mm. GV group: $\geq$20% bleeding on probing, with <3% of sites with PPD $\geq$4 mm and no sites with CAL $\geq$2 mm. HCG group: no systemic diseases, no immunosuppressive medication, <20% of sites bleeding on probing, <3% of sites with a probing depth $\geq$4 mm and no sites with clinical insertion loss $\geq$2 mm.[12]

Risk of bias assessment, data synthesis and analysis

The methodological quality and risk of bias were assessed with the Newcastle–Ottawa scale for case-control studies.[10] Two researchers (MPS and EAA) performed this analysis independently, and in the event of any discrepancies, a third party (XMM) served as a mediator. Studies with a score greater than 7 were included.

Qualitative analysis

The expression of each protein biomarker in saliva was analysed, in conjunction with the author and year of publication. The expression estimates are shown as integer values with standard deviation for each biomarker and subgroup. The weighted mean difference between groups was obtained for each model, thus yielding the overall expression (OE) and 95% confidence interval (CI 95%).

Quantitative analysis

A meta-analysis was performed when at least two articles provided data on the same biomarker and units of measurement. Our analysis strategy was to include all expression studies regardless of their threshold values. The statistical analysis of the results was performed with the OpenMeta tool (Analyst).

Functional networks and pathway mapping

The analysis of biological pathways associated with the differentially expressed protein markers was performed with the STITCH integration analysis tool and was limited to interactions with high confidence levels (0.7)[13 (p4)].

Assessment of heterogeneity

First, we evaluated the threshold effect of expression graphically by examining the forest plot; second, we statistically evaluated the threshold effect with the Q test to determine the I^2 index. A p-value >0.1 and an index $I^2 < 50\%$ indicated low heterogeneity among studies. In contrast, a p-value of <0.1 and an index $I^2 > 50\%$ indicated the presence of considerable heterogeneity. Study specific estimates were combined with random effects models.

Results

A total of 81 studies were included in the qualitative assessment, of which 26 were suitable for quantitative analysis[14–39] (Figure 1). Of all the detected protein-based biomarkers (Supplementary 1), 12 were suitable for meta-analysis (Table 1).

According to the Newcastle–Ottawa Scale, 2 (6.7%) were determined to be of low quality, 20 (66.7%) were determined to be of moderate quality, and 8 (26.6%) were determined to be of high quality (Supplementary 2).

The biomarkers included were IL-1β, MIP-1α, albumin, TNF-α, ICTP, Ig-A, lactoferrin, MMP-8, IL-6, IL-8, IL-17 and PGE2. Forest plots were drawn for the different subgroups, CPD vs. GV (Figure 2), CPD vs. HCG (Figure 3) APD vs. HCG (Figure 4) and GV vs. HCG (Figure 5). The most studied biomarker was IL-1β. All markers were positive and overexpressed in the CPD group, in contrast to the GV and HCG groups, except for albumin (CPD vs. HCG; OE = −16.8 mg/mL; CI 95%: −50.3 to 16.6 mg/mL, $p = 0.324$; $I^2 = 98.02\%$, $p < 0.001$) and Ig-A (CPD vs. HCG; OE = −47.9 mg/mL; CI 95%: −223.9 to 127.9 mg/mL, $p = 0.593$; $I^2 = 95.59\%$, $p < 0.001$). MMP-8 was the most overexpressed marker in the CPD vs. HCG group, although it had a high degree of heterogeneity (OE = 319.4 ng/mL; CI 95%: 206.1–432.8 ng/mL, $p < 0.001$; $I^2 = 92.20\%$, $p < 0.001$).

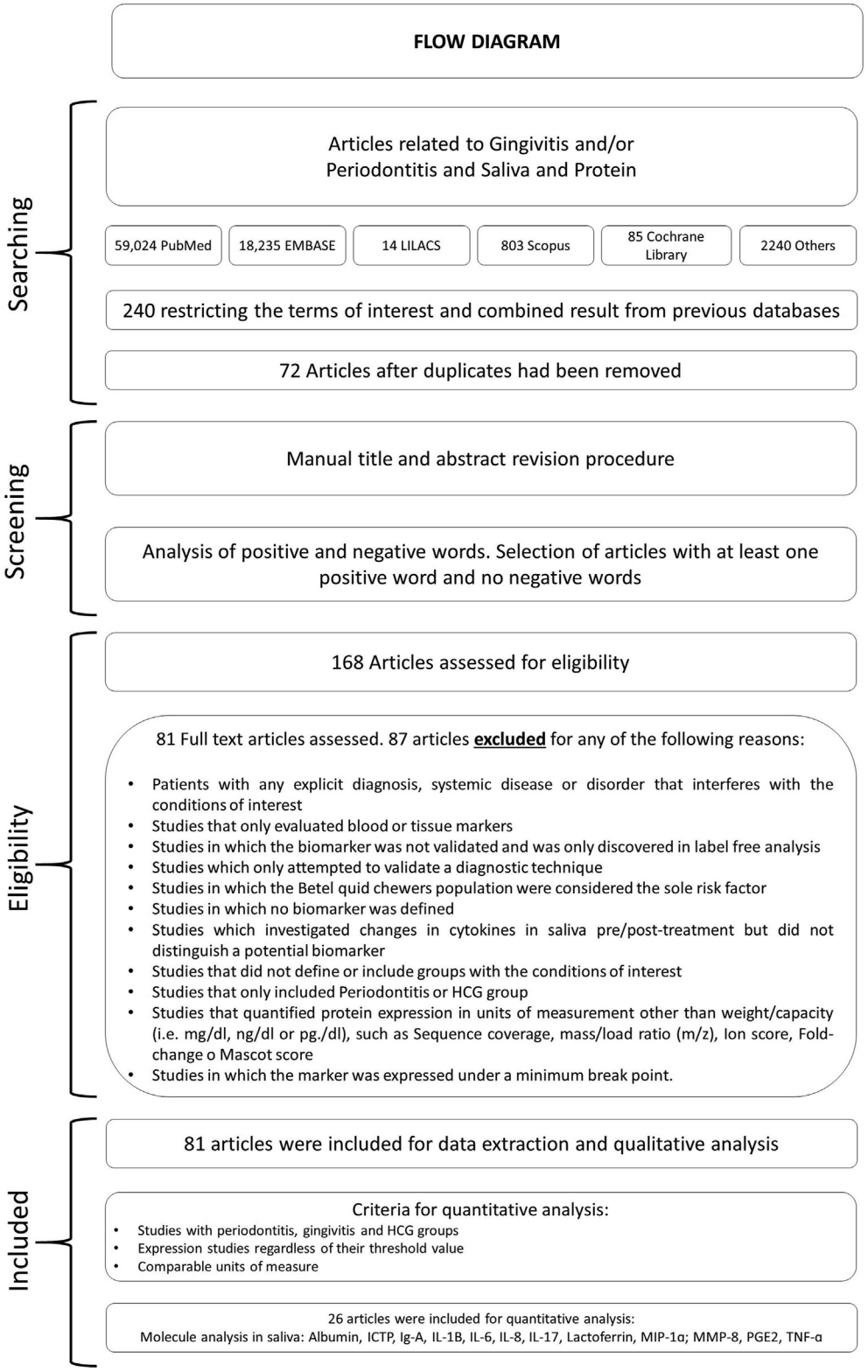

Figure 1: Flowchart of the systematic review.

Table 1: Complete description of the overall expression values (OE) of the different salivary biomarkers according to the subgroup under study. CPD (chronic periodontal disease), GV (gingivitis), APD (aggressive periodontitis), HCG (healthy control), CI (confidence interval).

Biomarker	Subgroup	OE	IC 95% (p)	Heterogeneity (I^2, p-value)
IL-1B	CPD/GV	73.598	70.660−76.536 ($p < 0.001$)	($I^2 = 0\%$, $p = 0.961$)
pg/mL	CPD/HCG	93.801	52.393−135.208 ($p < 0.001$)	($I^2 = 99.30\%$, $p < 0.001$)
	CPD-AGR/HCG	16.628	−1.265 to 34.521 ($p = 0.069$)	($I^2 = 0\%$, $p = 0.775$)
	GV/HCG	62.421	−41.856 to 166.698 ($p = 0.241$)	($I^2 = 90.38\%$, $p = 0.001$)
MIP-1α	CPD/GV	10.131	−1.491 to 21.754 ($p = 0.088$)	($I^2 = 99.79\%$, $p < 0.001$)
pg/mL	CPD/HCG	20.077	6.200−33.953 ($p = 0.005$)	($I^2 = 99.89\%$, $p < 0.001$)
	GV/HCG	9.954	7.700−12.208 ($p < 0.001$)	($I^2 = 95.48\%$, $p < 0.001$)
Albumin mg/mL	CPD/HCG	−16.867	−50.366 to 16.632 ($p = 0.324$)	($I^2 = 98.02\%$, $p < 0.001$)
TNF-α pg/mL	CPD/HCG	1.236	0.373−2.099 ($p = 0.005$)	($I^2 = 93.57\%$, $p < 0.001$)
ICTP ng/mL	CPD/HCG	0.091	0.019−0.164 ($p = 0.013$)	($I^2 = 16.22\%$, $p = 0.275$)
Ig-A mg/mL	CPD/HCG	25.755	−84.664 to 136.174 ($p = 0.593$)	($I^2 = 88.31\%$, $p < 0.001$)
Lactoferrin mg/mL	CPD/HCG	10.449	−7.580 to 28.477 ($p = 0.256$)	($I^2 = 95.91\%$, $p < 0.001$)
MMP-8 ng/mL	CPD/HCG	319.489	206.139−432.838 ($p < 0.001$)	($I^2 = 92.20\%$, $p < 0.001$)
IL-6 pg/mL	CPD/HCG	49.166	18.380−79.952 ($p = 0.002$)	($I^2 = 99.93\%$, $p < 0.001$)
IL-8 pg/mL	CPD/HCG	43.563	−330.540 to 417.667 ($p = 0.819$)	($I^2 = 71.59\%$, $p = 0.061$)
IL-17 pg/mL	CPD/HCG	1.843	−3.086 to 6.772 ($p = 0.464$)	($I^2 = 98.82\%$, $p < 0.001$)
PGE2 pg/mL	CPD/HCG	36.305	18.350−54.260 ($p < 0.001$)	($I^2 = 0\%$, $p = 0.850$)

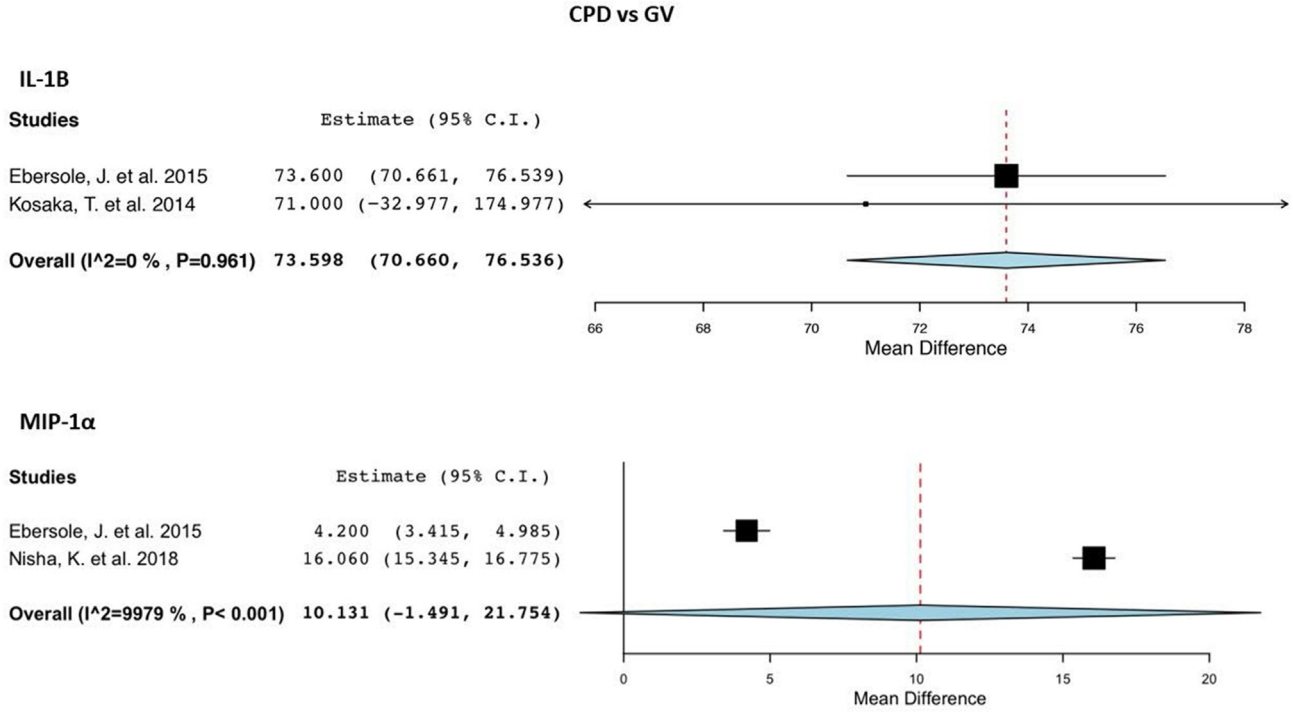

Figure 2: Forest plots of the chronic periodontitis (CPD) subgroups vs. the gingivitis (GV) group, and the IL-1β and MIP-1α biomarkers.

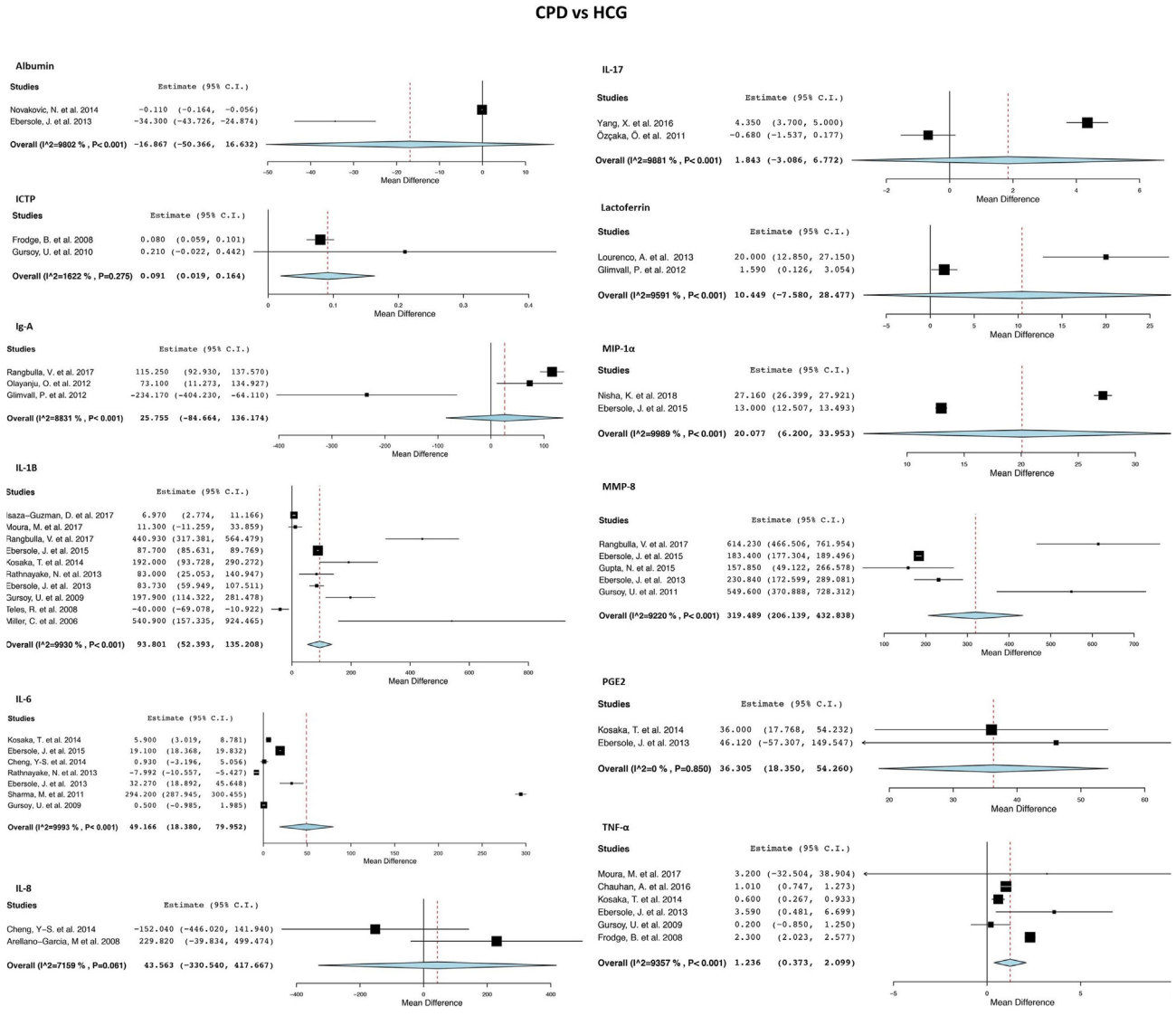

Figure 3: Forest plots of the chronic periodontitis (CPD) subgroups vs. the healthy control (HCG) group and the IL-1β, MIP-1α, albumin, TNF-α, ICTP, Ig-A, lactoferrin, MMP-8, IL-6, IL-8, IL-17 and PGE2 biomarkers.

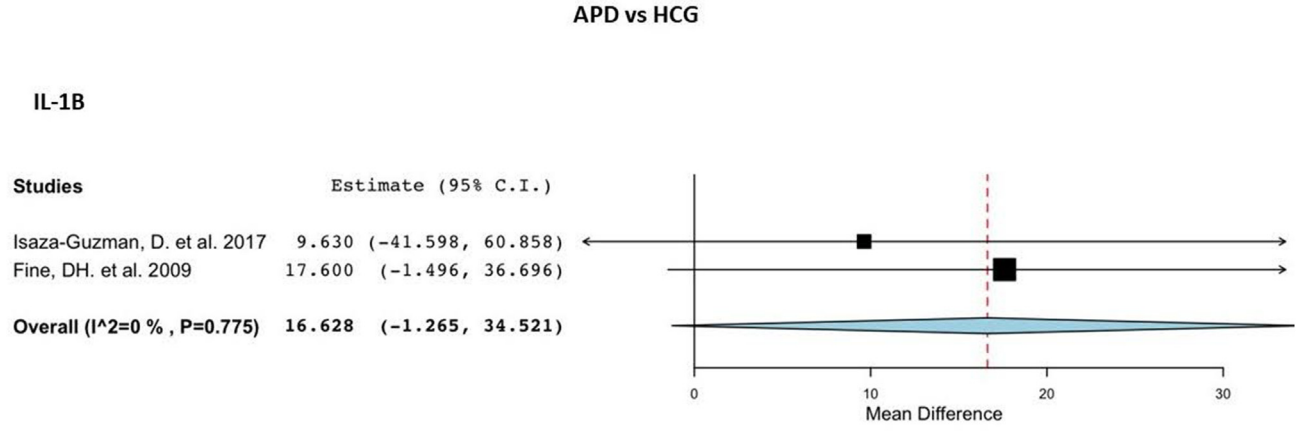

Figure 4: Forest plot of the aggressive periodontal disease (APD) subgroup vs. the healthy control (HCG) group, and the IL-1β biomarker.

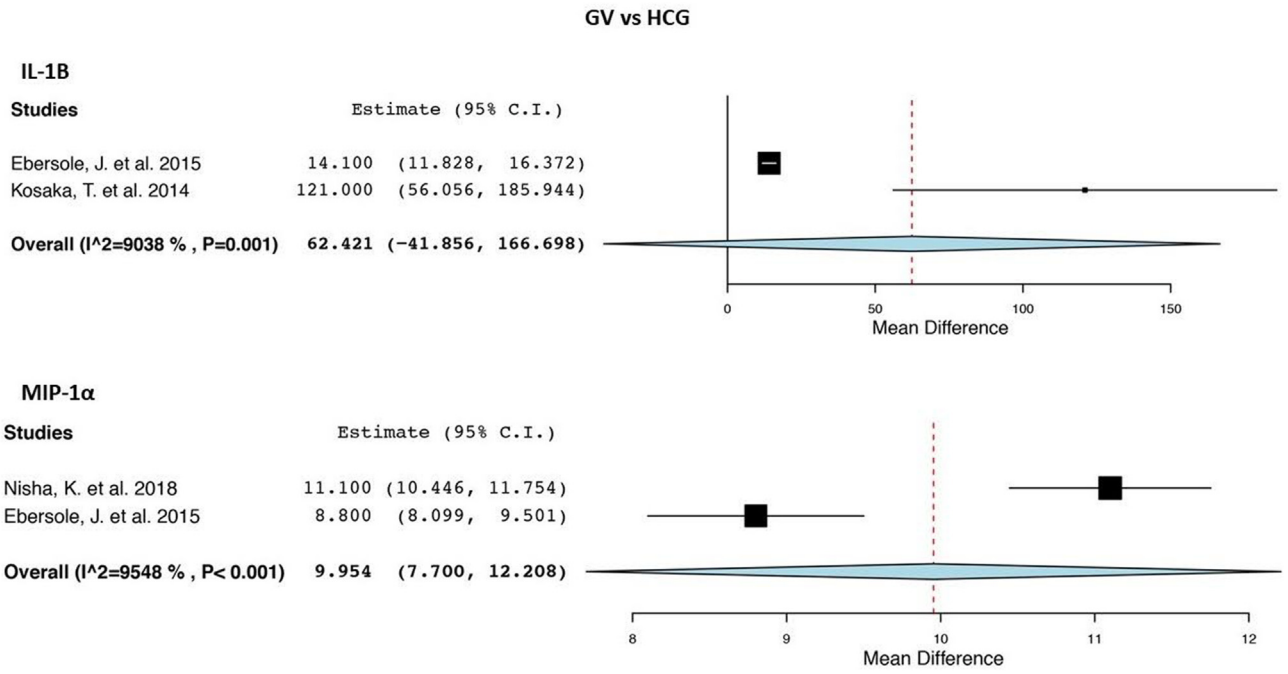

Figure 5: Forest plots of the gingivitis (GV) subgroup vs. the healthy control (HCG) group, and the IL-1β and MIP-1α biomarkers.

On the basis of the significance between groups and the low degree of heterogeneity of the results, the salivary markers found to have strong applicability were IL-1β for differentiating the I and GV groups with an OE = 73.5 pg/mL (CI 95%: 70.6−76.5 pg/mL, $p < 0.001$; $I^2 = 0\%$, $p = 0.961$); ICTP for differentiating CPD from HCG with an OE = 0.091 ng/mL (CI 95%: 0.019−0.164 ng/mL, $p = 0.013$; $I^2 = 16.22\%$, $p = 0.275$); and PGE2 for differentiating CPD from HCG with an OE = 36.3 pg/mL (CI 95%: 18.3−54.2 pg/mL, $p < 0.001$; $I^2 = 0\%$, $p = 0.850$). However, the number of studies included in the meta-analysis was highly low for these markers (Supplementary 3).

We conducted a functional interaction study for these three markers and observed a strong network between IL-1β and PGE2, whereas ICTP remained outside this node (Supplementary 4).

Discussion

This study was aimed at determining differentially expressed salivary proteins for diagnosis of the main periodontal diseases, CPD and GV. Despite analysing 81 studies, we were able to perform statistical comparisons of protein expression through meta-analysis on 26 studies on 12 previously described biomarkers.

IL-1β, a cytokine produced by macrophages, is a key mediator of the inflammatory response and is stimulated in the presence of bacterial components associated with the osseous reabsorption process in periodontal diseases. Furthermore, clinical studies have indicated that high levels of IL-1β are associated with gingival inflammation, the severity of periodontitis and periodontal disease progression.[40] In the present study, the IL-1β levels were significantly higher in the CPD group with respect to the GV and HCG groups. The APD and GV groups presented lower values of OE than the CPD group, and no significant differences were detected with respect to the HCG group (Table 1). IL-1β was the marker assessed in the most studies in the meta-analysis (10 for the CPD−HCGcomparison,[17,18,20,23,28–30,34,36,37,39] 2 for the CPD−GV comparison,[29,36] 2 for the CPD/AGR−HCG comparison[18,19] and 2 for the GV−HCG comparison[29,36]).

Research conducted by Miller in a case study from the USA reported the highest average expression of IL-1β among all studies included in the meta-analysis, with a level of 540,900 pg/mL (CI 95%: 157,335−924,565) in the CPD and HCG groups.[39] Rangbulla[23] reported an IL-1β expression of 440.9 pg/mL (CI 95%: 317.3−564.4). In contrast, the studies including populations from Medellín, Colombia[18]; Belo Horizonte, Brazil[28]; and Boston, USA[37] showed an IL-1β expression lower than the average in the meta-analysis (Figure 3). The differences in detection for the studies conducted by Miller[39] and Rangbulla[23] compared with the study conducted by Teles,[37] which had the highest and lowest IL-1β expression in the meta-analysis, respectively, might have been due to differences in disease levels/severity, the collection and storage methods, processing and centrifugation, or salivary analysis, as well as potential cytokine degradation. The patients included in the study conducted by Miller[39] presented relatively more severe periodontal disease. This explanation might also apply to the findings of Rangbulla,[23] who selected patients with a profile similar to those included in the study conducted by Miller.[39]

MIP-1α, a cytokine secreted by a wide variety of cells, contributes to the pathogenesis of a wide range of inflammatory diseases through processes including cell recruitment, stem cell inhibition and immune response maintenance.[35] MIP-1α is associated with the formation of multinuclear cells, thus indicating its potential role in bone destruction associated with periodontal diseases.[41] In the present work, we observed differences in MIP-1α expression between CPD−HCG and GV−HCG. However, no significant

differences were observed among pathological conditions. Nonetheless, all comparisons showed high heterogeneity, with I^2 values of approximately 95%. The variability of MIP-1α expression observed among studies may be associated with the sample sizes, given that in the study by Nisha, the same number of individuals was tested in each group,[35] whereas in the study by Ebersole, the CPD group had more than twice the number of participants as the GV group.[17] The variability might also be associated with the detection methods, given that Nisha used ELISA,[35] and Ebersole used the Luminex 100IS system.[17]

Albumin is the most abundant protein in human blood, and its main role is regulating the osmotic pressure in the blood plasma; it exhibits an esterase-like activity with broad substrate specificity.[42] Novakovic[16] has reported significantly higher levels of albumin in healthy patients, and suggested that non-enzymatic antioxidants may be a promising indicator of periodontal health. In the present study, we observed no significant differences in albumin expression in the CPD−HCG comparison. Furthermore, high heterogeneity was observed ($I^2 > 95\%$). The wide heterogeneity in the results may be explained by use of different analytic methods.

TNF-α, a cytokine secreted predominantly by macrophages, can induce cell death in certain tumour cell lines, stimulate cell proliferation and induce cell differentiation; it also plays a key role in the pathogenesis of periodontal disease.[42] In the present work, we observed significant differences in the CPD−HCG comparison, with high heterogeneity values ($I^2 = 93.57\%$). Ding[43] has reported contradictory results in the association between periodontitis and TNF-α. However, each of the studies included in the meta-analysis observed that the TNF-α expression was higher in CPD than in HCG.

Pyridinoline cross-linked carboxyterminal telopeptide of type-I collagen (ICTP) is a type 1 bone collagen molecule that is released into the circulation after bone resorption and the degradation of the collagen matrix by protease and bacterial collagen. This marker indicates the progression of periodontal collapse, including alveolar bone loss.[44] Al-Shammari[45] has observed high levels of ICTP in gingival crevicular fluid in periodontitis. In the present work, we observed significant differences in ICTP expression in the CPD−HCG comparison, with reasonably low heterogeneity values ($I^2 = 16.22\%$). Among the selected studies, Frodge[21] did not observe any significant differences in ICPT expression or any correlation between ICTP expression and smoking/non-smoking status. In contrast, in the study conducted by Gursoy,[22] a significant difference was observed only between the CPD and HCG when the participants were non-smokers. These differences might have been due to differences in harmful habits or the geographical origin of the study populations, given that Frodge studied an American population,[21] whereas Gursoy studied a Finnish population.[22]

Ig-A is predominant in saliva and it is derived from plasma cells of the salivary glands.[46] Rangulla has highlighted that the decreased Ig-A levels following treatment suggest an association between this biomarker and periodontal disease.[23] In the present work, we observed no significant differences in Ig-A expression in the CPD−HCG comparison. However, significant differences were observed between CPD and HCG in the studies by Rangbulla[23] and Olayanju,[24] whereas Glimvall[25] did not report any differences. These differences might have been because smokers accounted for almost 50% of Glimvall's CPD group, whereas the samples assessed in the other two studies contained only non-smokers. Therefore, given that Ig-A is an inflammatory marker, the vasoconstrictive effects of tobacco have been suggested to affect Ig-A expression.

Lactoferrin, a metalloprotein with antimicrobial, anti-inflammatory and anticancer characteristics, can be found in tears, saliva, sweat, colostrum and milk. It is also found in polymorphonuclear leukocyte granules,[25] Glimvall has correlated the salivary lactoferrin concentration with the progression of periodontal disease.[25] In the present study, we observed no significant differences in lactoferrin expression in the CPD−HCG comparison. However, the study conducted by Lourenço[34] has indicated significantly higher lactoferrin expression in the CPD group than the HCG group, and similar results have been observed by Glimvall.[25] Both studies used the ELISA method to detect lactoferrin, although the study performed by Lourenço[34] in a Brazilian population observed expression seven times greater than those found by Glimvall[25] in a study in a Swiss cohort.

Extracellular matrix metalloproteinase-8 (MMP-8) is involved in extracellular matrix breakdown, and in various physiological and pathological processes. Multiple active forms of the enzyme exist, which act in the degradation of type I−III collagens.[42] Sorsa[47] has observed high MMP-8 levels in gingival crevicular fluid in patients with periodontal disease, which were correlated with periodontitis levels. Gursoy[22] has concluded that gingival inflammation can affect secretion of both the MMP-8 and the matrix metalloproteinase inhibitor (TIMP-1), thus modulating saliva concentrations. In the present work, we observed significant differences in the CPD−HCG comparison, with high heterogeneity ($I^2 = 92.20\%$). Within each study, significantly higher expression of MMP-8 was observed in the CPD group. The study by Rangbulla has indicated that the MMP-8 expression in CPD patients significantly decreased after periodontal treatment.[23] Johnson[48] has noted several limitations in the detection of high levels of MMP-8, including the sample size and the cross-sectional nature of the study.

Interleukin-6 (IL-6) is a cytokine that acts on inflammation and B-cell maturation, and it is predominantly produced in areas with high and chronic inflammation, where serum is secreted.[42] We observed significant differences in the CPD−HCG comparison, with high heterogeneity ($I^2 = 99.93\%$). All but two included studies[15,17,29,32,36] showed significantly higher IL-6 expression in CPD.[20,30]

Interleukin-8 (IL-8), a chemoattractant cytokine produced by a variety of tissue and blood cells, attracts and activates neutrophils, which migrate to regions of inflammation and release granulate enzymes,[49] Finoti has observed higher levels of protein in the gingival tissues of patients with chronic periodontal disease in the presence of high expression of the IL-8 gene.[49] In contrast, we found no significant differences in the CPD−HCG comparison, with high heterogeneity ($I^2 = 71.59\%$). Cheng[32] has not observed significant differences in the presence of IL-8 in saliva between the CP and control groups. Likewise, Arellano-Garcia

has not observed any differences in IL-8 expression in the saliva between the CPD and HCG groups.[33] Teles and Cheng[32,37] underscored existing doubts regarding the use of salivary cytokines as biomarkers for periodontal disease. Teles[37] has also highlighted the need for standardisation of disease level in these types of studies. Furthermore, Cheng[32] has noted the importance of using the total number of salivary proteins in unstimulated saliva as a point of reference for standardisation in salivary protein biomarker research.

Interleukin 17 (IL-17), a pro-inflammatory cytokine produced by T lymphocytes in tissues with periodontal disease, is involved in the modulation of T helper 1 (Th1) lymphocytes and the enhancement of inflammatory reactions via gingival fibroblast-derived mediators. It is also involved in osteoclastic bone resorption.[50] In the present systematic review and meta-analysis, we found no significant differences ($p = 0.464$) in IL-17 expression between patients with periodontitis and healthy patients, and extreme heterogeneity in values was observed ($I^2 = 98.82\%$). Özçaka[27] has observed a significant difference in salivary IL-17 concentration between the COMPOUND and HCG groups ($p = 0.009$). Likewise, Yang[14] has reported a higher IL-17 concentration in CPD than HCG, and observed a significant decrease in concentration after treatment of periodontal disease.

Prostaglandin E2 (PGE2) is a product of cyclooxygenase in arachidonic acid metabolism whose functional effects include fever, pain, vasodilation and bone resorption. Cyclooxygenase-2 expression is elevated in inflamed gingival tissues, and this enzyme is responsible for PGE2 production, thus stimulating proinflammatory reactions.[51] In the present systematic review and meta-analysis, we observed significant differences ($p < 0.001$) in PGE2 expression in the CPD−HCG comparison, and there was but no heterogeneity in the data included in the meta-analysis ($I^2 = 0\%$). Kosaka[29] has observed a significant and proportional correlation between PGE2 concentration and the Community Periodontal Index.

Conclusion

The most studied protein based salivary biomarker was IL-1β. The biomarkers with the highest differential expression and the greatest potential for clinical applicability are IL-1β for differentiating periodontitis from gingivitis; ICTP for differentiating patients with periodontitis from healthy patients; and PGE2 for differentiating patients with periodontitis from healthy patients. The number of studies must be increased in the future to support clinical translation of the findings.

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Conflict of interest

The authors have no conflict of interest to declare.

Ethical approval

Not applicable.

Authors contributions

EA: Conceptualization, Investigation, Writing - Original Draft, Visualization. MGOA: Methodology, Investigation, Writing - Original Draft, Visualization. CMC: Methodology, Validation, Investigation, Visualization, Supervision. Xabier Marichalar-Mendia: Formal analysis, Investigation, Visualization. SBB: Investigation, Writing - Review & Editing, Visualization. JB: Investigation, Writing - Review & Editing, Visualization. MP: Conceptualization, Methodology, Formal analysis, Investigation, Writing - Review & Editing, Visualization, Supervision, Project administration. All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jtumed.2022.12.004.

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