Herpes simplex virus type 1 in subgingival plaque and periodontal diseases. Meta-analysis of observational studies.

Herpes simplex virus type 1 in subgingival plaque and periodontal diseases. Meta-analysis of observational studies.

Paolo G. Arduino[1], Marco Cabras[1], Giovanni Lodi[2], Stefano Petti[3].

[1]Department of Surgical Science, Oral Medicine Section, CIR-Dental School, University of Turin, Turin, Italy

[2]Department of Biomedical, Surgical and Dental Sciences, University of Milan, Milan, Italy

[3]Department of Public Health and Infectious Disease, Sapienza University, Rome, Italy

Short title: HSV-1 in peridodontal disease: systematic review.

Computer system: mac OS 11.2.2.; Microsoft Word for Mac 16.16.27.

CORRESPONDING AUTHOR:

Prof. Paolo G. Arduino,

Department of Surgical Sciences, CIR-Dental School,

Via Nizza 230, 10126 – Turin, Italy;

Telephone: + 390116631522; Fax: + 39011618639

E-mail: paologiacomo.arduino@unito.it

Abstract

Background and objectives: This meta-analysis of observational studies (PROSPERO registration number CRD42021236054) sought to investigate strength and generalizability of the association of herpes simplex virus type 1 (HSV-1) in subgingival plaque with plaque-induced gingivitis and periodontitis, since the data from literature are contrasting.

Material and methods: Case-control and cross-sectional studies investigating HSV-1 in subgingival plaque/crevicular fluid and periodontal status, were searched through MEDLINE via PubMed, Scopus, Web of Science, Google Scholar. From each study the crude odds ratio (OR) with 95% confidence interval (95CI) was extracted and the pooled OR was assessed for any periodontitis, chronic and aggressive periodontitis, and gingivitis. The meta-analytic method was chosen on the basis of the level of heterogeneity. The generalizability of results, determined by the meta-analysis bias, was investigated through secondary analyses including sensitivity analyses for study quality, publication bias, and study inclusion, and subgroup analyses for quality of scientific journals that published the primary studies, world Region, subgingival plaque sampling method, study design.

Results: Twelve studies were included (738 cases, 551 controls). The pooled ORs were 4.4 (95CI, 1.9-10.2) for any periodontitis, 2.8 (95CI, 1.0-8.3) for chronic periodontitis, 11.8 (95CI, 5.4-25.8) for aggressive periodontitis, 4.8 (95CI, 2.1-11.0) for gingivitis. These estimates were statistically significant, excluding for chronic periodontitis that resulted marginally significant (p=0.05). Secondary analyses on any and aggressive periodontitis, and, partly, chronic periodontitis corroborated the results, while the material was insufficient for secondary analyses on gingivitis.

Conclusions: These results indicate that HSV-1 is associated with periodontitis, while data about gingivitis are inconclusive. HSV-1 investigation in subgingival plaque could help assess periodontitis risk and severity and, if causal association were confirmed, could contribute to its control.

Key words: HSV-1, plaque-induced gingivitis, periodontitis, systematic review, meta-analysis.

1. INTRODUCTION

Herpes simplex virus 1 (HSV-1) is probably the most common pathogen in the oro-facial region, infecting as many as three and half billion people worldwide.[1] Primary infection is generally acquired through direct contact with a lesion or with infected body fluids and asymptomatic individuals could also periodically shed infectious HSV-1 in saliva.[2] Primary herpetic gingivostomatitis usually arises in children and young adults.[3] Following an incubation period of up to 20 days, non-specific symptoms can arise, followed, 1 to 3 days later, by a typical mucocutaneous vesicular eruptions,[4] usually healing in two weeks without scarring.[5] The gingival features comprise diffuse, purple, boggy swelling of the free, and occasionally attached, gingivae, particularly anteriorly.[3] After primary infection, HSV-1 usually establish latent infection in peripheral trigeminal ganglia, and reactivation can occur causing cutaneous and mucocutaneous recurrent herpetic infection. Reactivation can be spontaneous or triggered by a number of factors.[6] Typically, recurrence gives rise to less severe clinical features than the primary infection; recurrent HSV-1 infection within the mouth is much less common than herpes labialis and thought to be rare in otherwise healthy patients.[7] Oral HSV-1 infection may also lead to several complications even in the immunocompetent host, such as herpetic esophagitis, more frequent than expected (prevalence of almost 2%) and usually undetected, herpetic pneumonia, occasionally observed in the immunocompetent host and more common in the immunocompromised host, erythema multiforme, that requires the concurrent role of lymphocyte abnormalities, and, possibly, periodontal diseases and apical periodontitis.[8]

HSV was first detected in the sulcular epithelium in 1973,[9] and since then the crevicular fluid was seen as an infection reservoir in absence of apparent oral lesions. Later on, HSV was isolated in patients with periodontitis, suggesting that the virus

could be associated to this disease.[10] Further studies found that patients with different periodontal diseases were more likely to yield HSV-1 in subgingival plaque and saliva than healthy individuals,[11] with differences between active and non-active sites of periodontal patients.[12,13] Chen et al[14] also hypothesized synergistic relationships between HSV-1 and periodontal pathogens, such as the exposure of periodontal tissues to bacterial infection promoted during the lytic phase of the viral infection, and the infection of periodontal pathogens that triggers the reactivation of the latent viral infection. Conversely, other studies failed to report such an association between HSV-1 and periodontal diseases.[15-18]

In order to reconcile these contrasting data, the association between HSV-1 infection and periodontitis was investigated through systematic reviews. While a qualitative review was inconclusive,[19] the results of two quantitative analyses were not reliable enough, as they were based on an insufficient number of primary studies; specifically, just two for chronic periodontitis,[20] and four for aggressive periodontitis.[21] With such a limited number of primary studies it was not possible to perform secondary analyses, pivotal to evaluate the robustness of the pooled estimates and, therefore, their generalizability.

Thus, the present meta-analysis sought to investigate the strength and the nature of the association between HSV-1 infection of periodontal tissues and periodontal diseases.

2. MATERIAL AND METHODS

This meta-analysis of observational studies was reported acknowledging the PRISMA 2020 guidelines.[22] The review was recorded under the PROSPERO registry (registration number CRD42021236054).

2.1 Review question

Since the primary objective of this review was to assess the strength of the association of HSV-1 detection in subgingival plaque/crevicular fluid with gingivitis/periodontitis in adult patients, the items of the PECOS question were the following,

P (population/patients) – Adult patients referring to oral healthcare settings;

E (exposure) – HSV-1 detected in subgingival plaque/crevicular fluid;

C (comparator) – HSV-1 undetected in subgingival plaque/crevicular fluid;

O (outcome) – Plaque-induced gingivitis and plaque-induced periodontal diseases;

S (study) – Observational studies.

The present review was directed to oral healthcare providers to help them decide whether to investigate the presence of HSV-1 in subgingival plaque/crevicular fluid of periodontal patients and to implement specific protocols aimed at controlling HSV-1 infection in periodontal tissues.[23]

2.2 Primary study eligibility. Inclusion and exclusion criteria

The eligibility criteria of primary studies were derived from the PECOS.

- Study design. Longitudinal studies investigating periodontitis development or progression in patients with/without HSV-1 infection in periodontal tissues would be highly desirable, as they could help assess whether the viral infection precedes, follows or exacerbates the periodontal diseases. However, it is anticipated here that similar studies were not detected. Therefore, this review necessarily included observational studies without longitudinal design, namely, cross-sectional and case-control studies performed in healthcare settings, excluding community settings. Case reports and series were not considered, because there were no comparators.

- Case and control patients. Otherwise healthy patients, aged 15 years or older, without underlying chronic disorders or syndromes, no concurrent oral or systemic infectious diseases, were considered. Pregnant women were excluded. Cases were patients diagnosed with plaque-induced gingivitis, chronic, and aggressive periodontitis. Although the diagnostic criteria for these conditions have been recently redefined,[24] less recent criteria were accepted on condition that they were clear and were provided in the text. Controls were patients without gingivitis, chronic and aggressive periodontitis, who were recruited in the same settings as cases. Both cases and controls must not be subjected to any periodontal treatment and/or topic or systemic antibiotic/antimicrobial regimens in the past three months.
- HSV-1 detection. Studies that investigated HSV-1 in subgingival plaque or crevicular fluid samples collected from gingival sulcus/periodontal pocket were considered. HSV-1 analysis was specific for HSV-1 DNA. Therefore, studies that investigated the presence of HSV-1 in saliva and periodontal tissues through gingival biopsy, or investigated HSV-1 antigens or antibodies, were not considered.

2.3 Search strategy

The literature search was performed without time and language restrictions and, in order to increase the likelihood to locate as many published articles as possible, used broad search terms. These terms were the same for all the bibliographic databases and referred solely to the exposure and the conditions under investigation. Specifically, they were, (1)“Herpes simplex” AND “period*”; (2)“HSV” AND “period*”; (3)“Herpes simplex” AND “gingivitis”; (4)“HSV” AND “gingivitis”. It was expected that the use of such generic terms necessarily provided many studies that were unrelated

to the present review. However, unrelated studies were removed during the following screening phase. Studies written in languages different from English were read using GOOGLE Translate.

The bibliographic databases were PubMed/MEDLINE, Scopus, and Web of Science, while the grey literature was investigated using GOOGLE Scholar (search limited to the first 500 most relevant items).

Study search was performed by one reviewer (MC) and was repeated twice at an interval of one month. Duplicates were removed and titles and abstracts of the remaining material were screened independently by three reviewers (PGA, MC, SP). Results were compared and any article considered by at least one reviewer was included in the set of articles to retrieve. This procedure was chosen in order not to miss potentially relevant studies. Full texts were then retrieved, and eligibility was assessed by two groups of reviewers independently (PGA-MC, GL-SP). An inclusion/exclusion form, based on the aforementioned eligibility criteria, was used. Results were compared and the final list of primary studies to include in the meta-analysis was assessed through open discussions.

2.4 Data extraction and exposure-disease association measure

The characteristics extracted from the primary studies were, publication year, country, number of patients with age and gender, periodontal diseases under investigation, subgingival plaque/crevicular fluid sampling method, proportion of HSV-1 positive cases and controls.

Viral load of HSV-1 positive subjects and lowest HSV-1 detection limit of the method used to assess HSV-1 were initially included in the list of the data to extract. However, it is anticipated here that this information was sporadically available. Therefore, since the quantitative analysis was impossible, the association between HSV-1 and

periodontal diseases was assessed using the odds ratio (OR), a measure for dichotomous exposures and outcomes. The numbers of HSV-1 positive cases, HSV-1 positive controls, HSV-1 negative cases, HSV-1 negative controls were extracted and 2x2 tables were made. In case of “0” values in one or more boxes, the value of “0.5” was added to all values in the four boxes. OR was preferred to prevalence ratio (PR) without a specific reason. The 95% confidence interval (95CI) of each OR was assessed. Although OR adjusted for covariates and interaction was desirable, primary studies did not assess it, therefore, the crude OR with 95CI for gingivitis/periodontal diseases relative to HSV-1 detection was extracted or calculated.

Data were extracted by the four authors during an online meeting.

2.5 Adjustment for study quality

Study quality was evaluated using the Newcastle-Ottawa Scale (NOS) for case-control studies.[25] For cross-sectional studies, a modified version of NOS was used.[26] NOS assessment forms were specifically designed for the present review (available upon specific request) and scores were evaluated independently by the four reviewers. Discrepancies were reconciled through open discussions.

In order to account for study quality, “the most radical approach is to directly incorporate information on study quality as weighting factors in the analysis”.[27] Therefore, study weights were multiplied by quality related weights, thus increasing the weights of high-quality primary studies relatively to low-quality studies. Since the highest NOS scores for case-control studies and cross-sectional studies were different, quality scores were homogenized assessing the relative quality weights with formula,

[(study NOS score) / (highest possible NOS score for that type of study)]

Quality related weights ranged between 0 and 1. Adjustment for study quality was considered a form of sensitivity analysis.

2.6 Meta-analysis method

The choice of the meta-analysis method was important, as the fixed-effects model assumed that there was only one true association between HSV-1 in subgingival plaque and periodontal diseases, while the random-effects model implied that there were several true associations between exposure and outcome due to different characteristics of the primary studies. In this case, the overall variance observed in the analysis was due to within-study variance, plus between-study variance, the latter known as heterogeneity.[28] The presence of significant heterogeneity was tested with the Cochran's Q, a χ^2 test, and was quantified with the I^2 which denoted how much of the total variance was due to heterogeneity.[29] As a rule of thumb, the random effects model was preferred to the fixed-effects model for I^2>50%, suggesting that between-study variance overwhelmed within-study variance. The DerSimonian and Lair method was used to estimate the between-study variance, and it was preferred to other methods because it is consistent with the Cochran's Q, and provides between-study variance higher than zero for statistically significant heterogeneity. However, meta-analyses with a small number of primary studies, may produce artificially low Q and I^2 values, that hamper the assessment of between-study variance even in presence of heterogeneity.[30]

Meta-analyses were made for gingivitis, any periodontitis, chronic periodontitis, and aggressive periodontitis.

2.7 Adjustment for publication bias

Another important problem of meta-analyses of observational studies was that the results provided by the available primary studies could systematically differ from the

results provided by unavailable or unpublished studies. This problem would be due to lack of publication of small studies (publication bias), their publication in low quality journals difficult to detect (selection bias/language bias), and the lack of reporting non-significant results regarding the focused question (selective nonreporting bias). These forms of bias together are generally known as publication bias.[31]

Publication bias was investigated assessing funnel plot symmetry, both visually and through formal tests. The choice of the axes of the funnel plot was pivotal, as different variables in the axes provide different funnel shapes and could artificially suggest asymmetry when there is not and vice versa. In order to investigate funnel plot symmetry, the natural logarithm of OR in the x axis and its standard error (inverse order) in the y axis were the most appropriate variables in the present case.[32]

The test of Peters and colleagues, a weighted linear regression with the logarithm of the OR as dependent variable, the inverse of the total sample size as independent variable, and the inverse of the variance as weight, was chosen as formal test, because the widely used tests of Egger and colleagues and Begg and Mazumdar are not recommended for application to OR.[33]

In presence of significant funnel plot asymmetry, possibly, but not necessarily, denoting the presence of publication bias,[34] the trim and fill method was used to adjust the set of primary studies and the pooled OR estimate was reassessed. The number of potentially missing studies was assessed using the R_0 estimator. Adjustment for publication bias was considered a sort of sensitivity analysis.[35]

2.8 Risk of bias and robustness of the pooled estimates

As already explained, the problem of heterogeneity in meta-analyses of observational studies is often due to the different characteristics of the primary studies.[34] In order to

investigate the risk of bias of the pooled OR estimates and their generalizability, a set of sensitivity and subgroup analyses was performed.

Sensitivity analyses to study quality and publication bias were previously described. In addition, sensitivity analysis to study inclusion was performed, excluding each study in turn and investigating whether the 95CI of the pooled OR estimate overlapped the 95CI of the estimate performed with all primary studies included.

The aim of subgroup analysis was to detect and deal with potential sources of heterogeneity. Therefore, four hypotheses were made. First, that studies published in high-quality scientific journals provided more consistent data. Therefore, subgroup analysis according to the quality of the scientific journals where the studies were published, was performed. Primary studies were split into those published in scientific journals with Impact Factor higher than zero (IF>0) and those with no IF at all (IF=0). In the second subgroup analysis studies were split according to the world Region where they have been performed. In the third subgroup analysis, studies were split according to subgingival plaque sampling method. In the fourth subgroup analysis studies were split according to their design, namely, case-control and cross-sectional. Only subgroups with at least three primary studies were considered, since performing meta-analyses using one or two studies is meaningless.

2.9 Statistical evaluation

Statistical analyses were performed using MedCalc Version 14 (MedCalc Software Ltd), and StatView 5.0.1 (SAS Institute Inc). Statistically significance was set at $p<0.05$.

3. RESULTS

The literature search was performed between 10 February and 13 March 2021 and provided almost 7,000 titles. As expected, the majority of them were unrelated to the present review, with 53 titles remaining for screening (Figure 1). Forty-one studies were excluded; the reasons for their exclusion are reported in Table S1. The remaining twelve studies were considered for the present meta-analysis.[15,16,18,36-44] They were published in a relatively narrow period, between 2004 and 2020; one of them was performed in Ghana,[36] one in Turkey,[43] three in India,[37,40,44] three in Latin America,[38,39,41] and four in Europe.[15,16,18,42] The characteristics of primary studies are displayed in Table 1. Globally, 1,289 patients were involved, cases were 738, and controls 551. Two studies investigated gingivitis,[39,44] nine chronic periodontitis,[15,16,36,38-42,44] seven aggressive periodontitis,[18,36-39,42,43] with any periodontitis investigated in all the twelve studies.

Study quality (Table 2) of case-control studies was averagely good, with NOS scores ranging between 5 and 9. NOS score of the two cross-sectional studies was 5. The quality related weight, incorporated in the quality-adjusted meta-analysis was ranging between 0.556 and 1.000.

The crude ORs for periodontal diseases extracted from the primary studies were varying (Table S2, Figure 2), ranging between 0.1[41] and 106.3[43] for any periodontitis, 0.1[41] and 18.5[44] for chronic periodontitis, 1.0[18] and 106.3[43] for aggressive periodontitis, and 4.6[39] and 5.2[44] for gingivitis.

The results of the Q and I^2 tests showed significantly high heterogeneity among studies on any periodontitis and chronic periodontitis (Table 3). Therefore, the preferred meta-analytic model was the random-effects model. Heterogeneity accounted for only 24% of the total variance among aggressive periodontitis studies. However, since there were few available studies, the values of Q and I^2 could be

artificially low and no preference between the fixed- and the random-effects models could be made. As for gingivitis studies, the inverse-variance weighting method was the only possible meta-analytic model. The pooled OR estimates (Table 3, Figure 2) were 4.4 (95CI, 1.9-10.2) for any periodontitis, 2.8 (95CI, 1.0-8.3) for chronic periodontitis, 11.8 (95CI, 5.4-25.8 –fixed-effects model) for aggressive periodontitis, and 4.8 (95CI, 2.1-11.0) for gingivitis. These estimates were statistically significant, excluding for chronic periodontitis that resulted marginally significant (p=0.05).

The formal tests for funnel plot asymmetry (Table S3) revealed significant asymmetry for any and chronic periodontitis studies, while the test was non-significant for aggressive periodontitis studies. Consistently with this test, the trim and fill method detected one and three missing studies for any and chronic periodontitis, respectively, and no study for aggressive periodontitis. The inclusion of missing studies changed the shapes of the funnel plots improving their symmetry (Figure 3). The funnel plot for gingivitis with just two studies was meaningless and was displayed only for completeness.

The ORs for any periodontitis and chronic periodontitis adjusted for study quality and publication bias did not change from the unadjusted OR appreciably, although the adjusted point estimates resulted higher than the unadjusted estimates (Table 4). The study-quality adjusted ORs for aggressive periodontitis and gingivitis were similar to the unadjusted ORs.

The exclusion of each primary study in turn did not lead to important changes in the pooled OR estimates for any periodontitis, aggressive periodontitis, and gingivitis (Table S4). However, there were five studies on chronic periodontitis[38-40,42,44] whose exclusion caused a drop in the pooled OR estimate to a statistically non-significant level.

The subgroup analysis was incomplete due to the small number of available studies in some subgroups (Table S5). However, for any and chronic periodontitis, the studies published in scientific journals with Impact Factor higher than zero provided lower pooled point OR estimates, than studies published in journals without Impact Factor, while the reverse was found for aggressive periodontitis. As for the Regions, the highest pooled point OR estimates were provided by Indian studies, followed by Latin American studies, and European studies. The use of curette as sampling method provided higher pooled point OR estimates than the use of paper points. For any periodontitis and chronic periodontitis, the pooled estimates provided by the case-control studies were statistically significant, while those provided by the cross-sectional studies were not. In the case of any periodontitis the difference between subgroups was statistically significant. In general, however, the majority of differences between subgroups were statistically not significant.

4. DISCUSSION

4.1 Generalizability of the pooled association estimates between HSV-1 and periodontal diseases

The assessment of external validity (i.e., generalizability) of the pooled estimates is probably the most important problem of meta-analyses of observational studies, that suffer from the lack of an independently developed methodology, with the majority of recommendations and checklists often transferred from guidelines for meta-analyses of RCTs.[45] Even precise and apparently highly significant results of meta-analyses of observational studies, misleadingly suggesting high generalizability, could be spurious if insufficient attention is given to heterogeneity. Heterogeneity of observational studies must be expected, due to the different conditions of the primary studies, and

non-significant heterogeneity test results are frequently due to the low power of these tests when the number of primary studies is small.[34] In the present analysis, the frequent use of the random-effects model was therefore imposed by these considerations and the significant heterogeneity detected with formal tests. This model implies, as expected, that there was not a unique association between HSV-1 and periodontal diseases, but several types of association that depend on the form and severity of the periodontal disease in the samples under investigation, on patients' general characteristics, such as age, gender, ethnicity, on the methods used to assess exposure and outcome, etc.[46] Thus, the question here was to assess whether the set of primary studies used for the meta-analysis was large and various enough to capture all these different HSV-1/periodontal disease associations, and, consequently, whether the pooled estimates were reliable averages of these different exposure/outcome associations. Sensitivity and subgroup analyses were finalized at providing an answer to these questions.

As for any periodontitis, the primary study set allowed an adequate exploration of heterogeneity. Indeed, the pooled ORs assessed in the sensitivity analyses regarding study quality, publication bias (Table 4) and study inclusion (Table S4), as well as the subgroup analyses (Table S5), were very similar to the pooled unadjusted OR estimate (Table 3), thus suggesting that the odds of any periodontitis in HSV-1 positive patients was between two and ten times higher than in HSV-1 negative patients, with high external validity.

A different situation was reported for chronic periodontitis. The unadjusted pooled OR estimate (Table 3) was marginally significant, and while the sensiyivity analyses for publication bias and study quality (Table 5), as well as the subgroup analysis according to study design (Table S5) corroborated the HSV-1/chronic periodontitis

association, the sensitivity analysis to study inclusion showed that there were five out of nine primary studies whose exclusion produced a drop in the strength of the association to non-significant levels (Table S4). In addition, such an association was statistically non-significant in the subgroup of studies published in high-quality journals (Table S5). This inconsistency between secondary analyses, therefore, suggests that the generalizability of the reported association between HSV-1 in subgingival plaque/crevicular fluid and chronic periodontitis must be cautious, although it is highly likely.

As for aggressive periodontitis, although there were not enough studies to perform highly reliable secondary analyses, the unadjusted estimate (Table 3), the sensitivity analyses for study quality (Table 4), and to study inclusion (Table S4), and the subgroup analyses, when feasible (Table S5), provided consistent pooled ORs, thus suggesting that the odds of aggressive periodontitis in HSV-1 positive patients was between four and thirty times higher than in HSV-1 negative patients, with good external validity.

Although the two available studies on gingivitis consistently provided similar levels of exposure/outcome associations and the pooled OR estimate was statistically significant (Table 3), the unfeasibility of secondary analyses could not help to corroborate this result. Thus, although the association between HSV-1 in subgingival plaque/crevicular fluid was strong, that is, with high internal validity, its external validity was uncertain suggesting that more studies are needed.

4.2. Evidence of the association between periodontal diseases and herpesviruses

Systematic reviews consistently suggest that periodontal diseases are associated to two members of the Herpesviridae family, namely, Cytomegalovirus (CMV) and

Epstein-Barr virus (EBV). Indeed, regarding CMV, Botero and colleagues reported a pooled OR estimate for any periodontitis of 5.3 (95CI, 3.1-9.0)[47], Li and colleagues an OR of 3.6 (95CI, 2.1-6.1) for aggressive periodontitis,[21] Zhu and colleagues an OR of 3.6 (95CI, 1.4-9.2) for chronic periodontitis.[20] These estimates are not statistically significantly different. In addition, using a different methodology, Slots estimated that the median prevalence of CMV was 49% in aggressive periodontitis sites, 40% in chronic periodontitis sites, and only 3% in healthy periodontal sites.[11] As for EBV, Gao and Wang reported pooled OR estimates of 6.2 (95CI, 3.1-12.3) for any periodontitis, 6.6 (95CI, 3.0-14.3) for chronic periodontitis, and 8.4 (95CI, 2.1-33.1) for aggressive periodontitis,[48] Zhu and colleagues an OR of 5.7 (95CI, 2.5-13.0) for chronic periodontitis,[20] Li and colleagues an OR of 6.1 (95CI, 2.1-17.5) for aggressive periodontitis.[21] Once again, these estimates are not significantly dissimilar, and were corroborated by Slots who reported median EBV prevalence estimates of 45% in aggressive periodontitis sites, 32% in chronic periodontitis sites, and 7% in healthy periodontal sites.[11] These data on CMV and EBV are also corroborated by qualitative systematic reviews.[19,49]

The situation regarding the association between HSV-1 and periodontitis was more controversial, since the data provided by the two available meta-analyses were inadequate to perform reliable secondary analyses to investigate the generalizability of the pooled estimates.[20,21] In addition, Alzahrani reported conflicting results, namely, three studies with higher HSV-1 prevalence in aggressive periodontitis patients than in healthy controls, and two studies with comparable prevalence in the two groups.[19] Conversely, the median prevalence of HSV-1 reported by Slots was 63% in aggressive periodontitis sites, 45% in chronic periodontitis sites, and 12% in healthy periodontal

sites.[11] The present meta-analysis, reporting a reliable association between HSV-1 and periodontitis, reconciled such disagreement.

However, although the HSV-1/periodontitis association was strong, it is important to remember that it was not based on cohort studies and, therefore, temporality, one of the seven Hill's causation criteria, could not be satisfied.[50] Nevertheless, other criteria were satisfied, including biological plausibility,[14] although it remains to be determined whether HSV-1 infection precedes (or exacerbates) periodontitis, or vice versa, or even whether both events are possible. Indeed, Chen and colleagues postulated a two-way synergism between herpesviruses and periodontal bacteria.[14]

Despite a large body of compelling research data, definitive proof is still lacking that periodontal herpesviruses actually play a causal role in periodontitis development and do not occur merely as an epiphenomenon to the periodontal disease process.[11] The classical Koch's postulates to identify causative agents are applicable to diseases with a monocausal aetiology, but not to diseases that involve numerous cooperating pathogens, a high asymptomatic carrier state of pathogens, and crucial tissue-destructive immune responses.[11]

4.3 Clinical implications

Periodontitis is a complex disease that is among the most prevalent microbial diseases and chronic inflammatory diseases worldwide.[51] Although the process of periodontitis is considered to involve a multifactorial interaction between microbial, host, and environmental modulating factors,[52] microbial agents are of key importance in its development.[20,53]

Studies have reported that sites with the presence of herpesviruses increase level of other microbiota, and mainly affect different periodontopathic bacteria.[53] Presence of

both bacteria and human herpesviruses with immune responses by the host may lead to the destruction of periodontium.

The close relationship between herpesviruses and periodontitis justifies treatments that decrease the periodontal herpesvirus load and/or the destructive immune reactions of herpesvirus infections.[11] Systemic antiviral therapy may be indicated for severe periodontitis, which can harbour high herpesvirus counts within the gingival tissue and is inaccessible by topical treatment.[11,54]

The hypothesis of herpesvirus infections of the periodontium may help in a new level of understanding of the significance of preventing and controlling periodontal diseases also for medical purposes. Increased research into periodontal virology is encouraged given the outstanding preventive and curative opportunities it may offer.[54]

The main purpose of studies on periodontal herpesviruses is ultimately to prevent or cure periodontitis by controlling the viruses.[11] Further progress in delineating the periodontopathogenicity of herpesviruses depends on the implementation of cohort studies that help investigate the sequence of the events, using validated molecular detection methods to perform quantitative and multiple herpesvirus assessments in the periodontal tissues, that provide information on virus qualitative and quantitative changes, and sufficiently large samples of well-defined periodontitis and control patients.

As reported by a very recent systematic review about CMV and periodontitis, a limitation of our works is that studies that used virome sequencing were not included mainly because they are missing.[47] Forthcoming surveys using virome sequencing analysis, and more consistent sampling methods, could yield extra information in order to improve the understanding of the relations and connections between HSV-1 and periodontal diseases.

4.4 Conclusion

In conclusion, the results from this meta-analysis suggest strong association of HSV-1 with periodontitis, particularly aggressive periodontitis, while data about gingivitis are not conclusive.

REFERENCES

1. James C, Harfouche M, Welton NJ, et al. Herpes simplex virus: global infection prevalence and incidence estimates, 2016. *Bull World Health Organ* 2020;98:315-329.

2. Corey L, Spear PG. Infections with herpes simplex viruses. *N Engl J Med* 1986;314:749-757.

3. Arduino PG, Porter SR. Herpes Simplex Virus Type 1 infection: overview on relevant clinico-pathological features. *J Oral Pathol Med* 2008;37:107-121.

4.Huber MA. Herpes simplex type-1 virus infection. *Quintessence Int* 2003;34:453-467.

5. Amir J, Harel L, Smetana Z, Varsano I. The natural history of primary herpes simplex type 1 gingivostomatitis in children. *Pediatr Dermatol* 1999;16:259-263.

6. Siegel MA. Diagnosis and management of recurrent herpes simplex infections. *J Am Dent Assoc* 2002;133:1245-1249.

7. Holbrook WP, Gudmundsson GT, Ragnarsson KT. Herpetic gingivostomatitis in otherwise healthy adolescents and young adults. *Acta Odontol Scand* 2001;59:113-115.

8. Petti S, Lodi G. The controversial natural history of oral herpes simplex virus type 1 infection. *Oral Dis* 2019;25:1850-1865.

9. Zakay-Rones Z, Ehrlich J, Hochman N, Levy R. The sulcular epithelium as a reservoir for herpes simplex virus in man. *J Periodontol* 1973;44:779-781.

10. Ogawa Y, Fujita H, Matsuoka H, et al. [Isolation of herpes simplex virus from patients with periodontal disease]. *Nihon Shishubyo Gakkai Kaishi* 1982;24:594-600.

11. Slots J. Periodontal herpesviruses: prevalence, pathogenicity, systemic risk. *Periodontol 2000* 2015;69:28-45.

12. Passariello C, Gigola P, Testarelli L, Puttini M, Schippa S, Petti S. Evaluation of microbiota associated with Herpesviruses in active sites of generalized aggressive periodontitis. *Ann Stomatol* 2017;8:59-70.

13. Ling LJ, Ho CC, Wu CY, Chen YT, Hung SL. Association between human herpesviruses and the severity of periodontitis. *J Periodontol* 2004;75:1479-1485.

14. Chen C, Feng P, Slots J. Herpesvirus-bacteria synergistic interaction in periodontitis. *Periodontol 2000* 2020;82:42-64.

15. Petrović SM, Zelić, K, Milašin J, Popović B, Pucar A, Zelić, O. Detection of herpes simplex virus type 1 in gingival crevicular fluid of gingival sulcus/periodontal pocket using polymerase chain reaction. *Srpski Arhiv za Celokupno Lekarstvo* 2014;142:296-300.

16. Puletic M, Popovic B, Jankovic S, Brajovic G. Detection rates of periodontal bacteria and herpesviruses in different forms of periodontal disease. *Microbiol Immunol* 2020;64:815-824.

17. Singhal R, Jain A, Rastogi P. Prevalence of herpesviruses in periodontal disease of the North Indian population: A pilot study. *J Indian Soc Periodontol* 2020;24:163-166.

18. Stein JM, Said Yekta S, Kleines M, et al. Failure to detect an association between aggressive periodontitis and the prevalence of herpesviruses. *J Clin Periodontol* 2013;40:1-7.

19. Alzahrani, AA. Association between human herpes virus and aggressive periodontitis: A systematic review. *Saudi J Dent Res* 2017;8:97-104.

20. Zhu C, Li F, Wong MC, Feng XP, Lu HX, Xu W. Association between Herpesviruses and Chronic Periodontitis: A Meta-Analysis Based on Case-Control Studies. *PLoS One* 2015;10:e0144319.

21. Li F, Zhu C, Deng FY, Wong MCM, Lu HX, Feng XP. Herpesviruses in etiopathogenesis of aggressive periodontitis: A meta-analysis based on case-control studies. *PLoS One* 2017;12:e0186373.

22. Page MJ, McKenzie JE, Bossuyt PM, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ* 2021;372:n71.

23. Counsell C. Formulating questions and locating primary studies for inclusion in systematic reviews. *Ann Intern Med* 1997;127:380-387.

24. Caton JG, Armitage G, Berglundh T, et al. A new classification scheme for periodontal and peri-implant diseases and conditions - Introduction and key changes from the 1999 classification. *J Periodontol* 2018;89 Suppl 1:S1-S8.

25. Wells GA, Shea B, O'Connell D, et al. Newcastle-Ottawa Scale (NOS) for Assessing the Quality of Nonrandomized Studies in Meta-Analyses. The Ottawa Hospital Research Institute: Ottawa, ON, Canada, 2013.

26. Corrêa-Faria P, Petti S. Are overweight/obese children at risk of traumatic dental injuries? A meta-analysis of observational studies. *Dent Traumatol* 2015; 31:274-282.

27. Jüni P, Altman DG, Egger M. Systematic reviews in health care: Assessing the quality of controlled clinical trials. *BMJ* 2001;323:42-46.

28. Higgins JP. Commentary: Heterogeneity in meta-analysis should be expected and appropriately quantified. *Int J Epidemiol* 2008;37:1158-1160.

29. Higgins JP, Thompson SG. Quantifying heterogeneity in a meta-analysis. *Stat Med* 2002;21:1539-1558.

30. Borenstein M, Hedges LV, Higgins JP, Rothstein HR. A basic introduction to fixed-effect and random-effects models for meta-analysis. *Res Synth Methods* 2010;1:97-111.

31. Page MJ, Sterne JAC, Higgins JPT, Egger M. Investigating and dealing with publication bias and other reporting biases in meta-analyses of health research: A review. *Res Synth Methods* 2021;12:248-259.

32. Sterne JA, Egger M. Funnel plots for detecting bias in meta-analysis: guidelines on choice of axis. *J Clin Epidemiol* 2001;54:1046-1055.

33. Peters JL, Sutton AJ, Jones DR, Abrams KR, Rushton L. Comparison of two methods to detect publication bias in meta-analysis. *JAMA* 2006;295:676-680.

34. Egger M, Schneider M, Davey Smith G. Spurious precision? Meta-analysis of observational studies. *BMJ* 1998;316:140-144.

35. Duval S, Tweedie R. A Nonparametric “Trim and Fill” Method of Accounting for Publication Bias in Meta-Analysis. *J Am Stat Assoc* 2000;95:89-98.

36. Blankson PK, Blankson HNA, Obeng-Nkrumah N, et al. Detection of herpes viruses in Ghanaian patients with periodontitis. *J Investig Clin Dent* 2019;10:e12386.

37. Dani S, Dwarakanath CD, Alampalli R, Bhat K, Savitha AN, Gundannavar G. Role of herpes simplex-1, Epstein barr and human cytomegalo viruses in aggressive periodontitis. *Int J Dent Res* 2013;1:19-24.

38. Hernández HH, Fernandes AS, Escalona LA, Correnti M. Herpes Simplex Virus 1, Cytomegalovirus and Epstein Barr Virus Detection in Patients with Chronic and Aggressive Periodontitis. *Open Access LibraryJournal* 2016;3:e2557.

39. Imbronito AV, Okuda OS, Maria de Freitas N, Moreira Lotufo RF, Nunes FD. Detection of herpesviruses and periodontal pathogens in subgingival plaque of patients with chronic periodontitis, generalized aggressive periodontitis, or gingivitis. *J Periodontol* 2008;79:2313-2321.

40. Kazi MM, Bharadwaj R, Bhat K, Happy D. Association of Herpes Viruses with Mild, Moderate and Severe Chronic Periodontitis*. J Clin Diagn Res* 2015;9:DC05-8.

41. Pallos D, Ruivo GF, Ferrari-Junior SH, et al. Periodontal disease and detection of human herpesviruses in saliva and gingival crevicular fluid of chronic kidney disease patients. *J Periodontol* 2020;9:1139-1147.

42. Santangelo R, D'Ercole S, Graffeo R, et al. Bacterial and viral DNA in periodontal disease: a study using multiplex PCR. *New Microbiol* 2004;27:133-137.

43. Saygun I, Sahin S, Ozdemir A, Kurtiş B, Yapar M, Kubar A, Ozcan G. Detection of human viruses in patients with chronic periodontitis and the relationship between viruses and clinical parameters. *J Periodontol* 2002;73:1437-1443.

44. Shah R, Mehta DS. Prevalence of herpesviruses in gingivitis and chronic periodontitis: relationship to clinical parameters and effect of treatment. *J Indian Soc Periodontol* 2016;20:279-285.

45. Mueller M, D'Addario M, Egger M, et al. Methods to systematically review and meta-analyse observational studies: a systematic scoping review of recommendations. *BMC Med Res Methodol* 2018;18:44.

46. Sutton A, Abrams KR, Jones DR, Sheldon TA, Song F. Meta-analysis of Epidemiological and Other Observational Studies. In, Sutton A, Abrams KR, Jones DR, Sheldon TA, Song F, eds. Methods for meta-analysis in medical research. Chichester, UK: Wiley; 2000, pp. 239-258.

47. Botero JE, Rodríguez-Medina C, Jaramillo-Echeverry A, Contreras A. Association between human cytomegalovirus and periodontitis: A systematic review and meta-analysis. *J Periodontal Res* 2020;55:551-558.

48. Gao Z, Lv J, Wang M. Epstein-Barr virus is associated with periodontal diseases: A meta-analysis based on 21 case-control studies. *Medicine* 2017;96:e5980.

49. Rodrigues PM, Teixeira AL, Kustner EC, Medeiros R. Are herpes virus associated to aggressive periodontitis? A review of literature. *J Oral Maxillofac Pathol* 2015;19:348-355.

50. Hill AB. The Environment and Disease: Association or Causation? *Proc R Soc Med* 1965;58:295-300.

51. Pihlstrom BL, Michalowicz B S, Johnson NW. Periodontal diseases. *The Lancet* 2005;366:1809–1820.

52. Thomasini RL, Bonon SH, Durante P, Costa SC. Correlation of cytomegalovirus and human herpesvirus 7 with CD3+ and CD3+ CD4+ cells in chronic periodontitis patients. *J Periodontal Res* 2012;47:114-120.

53. Saygun I, Kubar A, Ozdemir A, Yapar M, Slots J. Herpesviral-bacterial interrelationships in aggressive periodontitis. *J Periodontal Res* 2004;39:207-212.

54. Slots J, Slots H. Periodontal herpesvirus morbidity and treatment. *Periodontol 2000* 2019;79:210-220.

TABLE 1 Characteristics of the primary studies

First Author, year	Country	Study type*	Subjects	Disease[#]	Sampling method	Proportion HSV-1 positives
Blankson, 2019	Ghana	CC	31 subjects age range, 19-72 (mean 34.6) 61.3% males	CP, AP	curette	CP, 0/12 AP, 4/9 controls, 0/10
Dani, 2013	India	CC	30 subjects age range, 18-30 56.7% males	AP	curette	AP, 7/15 controls, 2/15
Hernandez, 2016	Venezuela	CC	33 subjects age range, 28-61 (AP, 14-39) 30.3% males	CP, AP	paper points	CP, 3/11 AP, 3/11 controls, 1/11
Imbronito, 2008	Brazil	CC	120 subjects mean ages, 27 (AP), 42 (CP), 25 (G), 28 (controls) 39.2% males	G, CP, AP	paper points	G, 16/30 CP, 12/30 AP, 26/30 controls, 6/30
Kazi, 2017	India	CC	600 subjects mean age, 43 male:female, 1:1.2	CP	curette	CP, 140/300 controls, 21/300
Pallos, 2019	Brazil	CS	23 subjects adults 48% males	CP	paper strips	CP, 0/21 controls, 0/1
Petrovic, 2014	Serbia	CC	66 subjects age range, 18-76 36.4% males	CP	paper points	CP, 13/35 controls, 11/31
Puletic, 2020	Serbia	CS	57 subjects mean ages, 42 (CP), 30 (controls) 49.1% males	CP	paper points	CP, 1/27 controls, 3/30
Santangelo, 2004	Italy	CC	66 subjects mean ages, CP, 55; AP, 29; controls, 40 53.4% males	CP, AP	paper points	CP, 3/22 AP, 3/22 controls, 0/22
Saygun, 2004	Turkey	CC	34 subjects age range, 17-31 50.0% males	AP	curette	AP, 14/18 controls, 0/16
Shah, 2016	India	CC	100 subjects age range, 18-60 53.0% males	G, CP	paper strips	G, 31/40 CP, 37/40 controls, 8/20
Stein, 2013	Germany	CC	130 subjects age range, 23-70 43.1% males	AP	paper points	AP, 1/65 controls, 1/65

*CC, case-control study; CS, cross-sectional study.

[#]G, gingivitis; CP, chronic periodontitis; AP, aggressive periodontitis.

TABLE 2 Quality of primary studies according to the Newcastle-Ottawa scales adapted to the present study, and quality related weights used to adjust the meta-analysis for study quality. Formula to assess weights, [(study NOS score)/(highest possible NOS score for that type of study)]; range, 0.000-1.000.

Study	Item 1	Item 2	Item 3	Item 4	Item 5	Item 6	Item 7	Item 8	Item 9	NOS score	Quality weight
Case-control studies*											
Blankson, 2018	1	1	1	1	0	0	1	1	1	7	0.778
Dani, 2013	1	0	0	1	1	1	1	1	1	7	0.778
Hernandez, 2016	1	0	1	1	1	1	1	1	1	8	0.889
Imbronito, 2008	1	0	1	1	1	1	1	1	1	8	0.778
Kazi, 2017	1	0	1	1	0	0	1	1	1	6	0.667
Petrović, 2014	1	0	0	1	0	0	1	1	1	5	0.556
Santangelo, 2004	0	0	1	0	0	1	1	1	1	5	0.556
Saygun, 2004	1	1	1	1	1	1	1	1	1	9	1.000
Shah, 2016	1	0	1	1	1	1	1	1	1	8	0.889
Stein, 2013	1	1	1	1	1	1	1	1	1	9	1.000
Cross-sectional studies#											
Pallos, 2019	0	1	1	1	1	1	0	0	-	5	0.625
Puletic, 2020	0	1	1	1	1	1	0	0	-	5	0.625

*Items case-control studies, 1-case definition; 2-representativeness of cases; 3-control selection; 4-control definition; 5-comparability, age; 6-coparability, gender distribution; 7-exposure assessment; 8-same exposure assessment method; 9-similar non-response rate.

#Items cross-sectional studies, 1-sample representativeness; 2-sample selection procedure; 3-eligibility criteria; 4-case and control definition; 5-exposure ascertainment; 6-response rate; 7-adjustment for confounders; 8-study bias.

TABLE 3 Pooled unadjusted odds ratio (OR) estimates of the association between HSV-1 detection in subgingival plaque/crevicular fluid and periodontal diseases; all the pooled OR estimates were statistically significant with $p<0.05$, excluding the random-effects model for chronic periodontitis ($p=0.05$)

Disease	Method	Pooled OR	95CI*	Cochran's Q (df)	p-value for Q	I^2 value
Any periodontitis	Fixed effects	6.8	4.8-9.6	35.25 (11)	0.0002	68.8%
	Random effects	4.4	1.9-10.2			
Chronic periodontitis	Fixed effects	6.0	4.2 -8.6	33.22 (8)	0.0001	75.9%
	Random effects	2.8	1.0-8.3			
Aggressive periodontitis	Fixed effects	11.8	5.4-25.8	7.90 (6)	0.24	24.1%
	Random effects	10.3	3.8-28.1			
Gingivitis	Fixed effects	4.8	2.1-11.0	0.02 (1)	0.88	0.0%

*95% confidence interval; df, degrees of freedom, corresponding to the total number of studies included in the analysis minus one.

TABLE 4 Pooled OR estimates of the association between HSV-1 detection in crevicular fluid/subgingival plaque and periodontal diseases adjusted for publication bias and study quality (all the pooled OR estimates were statistically significant with $p<0.05$)

Adjustment	Method	Pooled OR	95CI*	Cochran's Q (df)	p-value for Q	I^2 value
			Any periodontitis			
Publication bias	Fixed effects	6.5	4.6-9.2	36.11 (12)	0.0003	66.8%
	Random effects	4.5	2.0-10.1			
Study quality	Fixed effects	7.1	4.6-11.0	23.71 (11)	0.01	53.6%
	Random effects	5.1	2.1-12.5			
			Chronic periodontitis			
Publication bias	Fixed effects	6.8	4.7-9.9	45.61 (11)	0.000003	75.9%
	Random effects	5.6	1.9-15.9			
Study quality	Fixed effects	6.2	3.9-9.8	21.3 (8)	0.006	62.4%
	Random effects	3.3	1.0-10.6			
			Aggressive periodontitis			
Study quality	Fixed effects	10.9	4.3-27.5	7.32 (6)	0.29	18.1%
	Random effects	10.2	3.4-30.1			
			Gingivitis			
Study quality	Fixed effects	4.9	2.0-11.9	0.02 (1)	0.88	0.0%

*95% confidence interval; df, degrees of freedom, corresponding to the total number of studies included in the analysis minus one.

FIGURE 1 Flowchart of study selection (PRISMA flow diagram 2020)

FIGURE 2 Forest plots of the unadjusted association between HSV-1 detection in subgingival plaque/crevicular fluid and periodontal diseases

FIGURE 3 Funnel plots with the logarithms of the OR (lnOR) in the x axis and their Standard Error in the y axis (inverted), unadjusted and, when statistically significant asymmetry was found, adjusted for publication bias. Blue dots are the primary studies, red dots are the missing studies identified with the trim and fill method included in the primary study set to make the funnel plot symmetric. Adjustment was unnecessary for aggressive periodontitis and unfeasible, due to the limited number of studies, for gingivitis