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Detection of herpes viruses in Ghanaian patients with periodontitis

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Abstract

Aim: The complexity of periodontitis in both etiology and progression has raised many questions, necessitating enormous research in recent years. The aim of the present study was to detect the presence of herpes viruses in Ghanaian patients diagnosed with periodontitis.

Methods: Thirty-one patients were included in the study; 21 with periodontitis classified into localized chronic, generalized, and aggressive periodontitis, and 10 without the disease were used as controls. Subgingival samples were collected, followed by DNA extraction. Multiplex polymerase chain reaction was used to amplify viral DNA for the detection of herpes viruses. Data was analyzed using Stata 14.

Results: The mean age for patients with aggressive periodontitis was 32.2 years (standard deviation [SD]: 8.50), while those for localized chronic periodontitis and generalized chronic periodontitis were 40.6 years (SD: 7.83) and 46.3 years (SD: 12.12), respectively. Viruses were detected only among patients clinically diagnosed with aggressive periodontitis. Of the total number of aggressive periodontitis patients, herpes simplex virus 1 (HSV-1) and Epstein-Barr virus (HBV) were found in four (44%) and one (11%), respectively. The mean age for patients found to have HSV-1 or EBV was 29 years (SD: 6.93).

Conclusion: We found HSV-1 and EBV in the subgingival plaque samples of Ghanaian patients clinically diagnosed with aggressive periodontitis. While our finding requires further investigation, the role of HSV in periodontitis, if elucidated, could transform and inform the clinical management of the condition.

KEYWORDS

aggressive periodontitis, chronic periodontitis, Ghana, herpes viruses, periodontitis

1 | INTRODUCTION

Periodontitis is a complex, multifactorial disease that leads to the gradual destruction of the supporting structures of the teeth.[1] It is a chronic condition that is among the most prevalent microbial diseases worldwide and among the most common oral presentations in Ghana.[2,3]

The complexity of periodontitis in both etiology and progression has raised many questions, necessitating enormous research in recent years. Despite known and well-described etiopathological

factors and associations, such as the role of biofilm, the host's immune factors, specific indicated bacteria, and environmental factors, many have intimated the role of viruses in its pathogenesis.[4]

Herpes viruses are known to be the most common group of viruses found in humans, infecting 80%-90% of the global adult population, consisting of an eight-membered family known to cause human disease: Epstein-Barr virus (EBV); human cytomegalovirus (HCMV); herpes simplex virus (HSV) 1 and 2; varicella zoster virus; and human herpes virus 6, 7, and 8.[5] While periodontal disease itself has varying forms of clinical manifestations, ranging from gingivitis to chronic and aggressive periodontitis, HCMV, EBV and HSV have been detected in the subgingival samples of some patients.[6] The latent form of HCMV has also been seen in many chronic periodontitis sites, which some have suggested could account for the slow progression of the disease.[7] Some authors have attempted to quantify the herpes viruses in periodontal pockets to demonstrate the association with periodontal disease severity, suggesting that the prevalence and number of herpes viruses in periodontal pockets could vary according to type of periodontal disease.[8] The association of herpes viruses with bacteria has also been explored to improve existing understanding of the etiopathogenesis of periodontal disease.[9]

The global picture of periodontal disease in general is overwhelming, with the World Health Organization iterating its control and prevention as an essential non-communicable disease intervention.[10] Africa shares a greater part of this burden, as is demonstrated by the comparatively higher prevalence of the disease, and sub-Saharan Africa in particular.[11] Incidentally, sub-Saharan Africa also has the highest incidence of HSV infection.[12] However, while inquiries peak to establish the role of HSV in the etiopathogenesis of periodontitis, the region that has the highest independent incidents of both conditions has contributed the least to the evidence. The aim of the present preliminary study, the first of its kind in Ghana, was to detect the presence of herpes viruses in patients with periodontitis.

2 | MATERIALS AND METHODS

2.1 | Study design and participants

The present study was carried out at the University of Ghana, School of Medicine and Dentistry Dental Clinic from March to April 2016. A case-control study design was employed using unmatched controls. Cases for the study were newly clinically-diagnosed patients with periodontitis attending the Dental Clinic, who consented to be part of the study. Controls were patients attending the same clinic, who were clinically found to have a healthy periodontium. Exclusion criteria included patients who had a history of smoking and chronic heavy alcohol consumption, and patients with comorbid conditions that did not allow for a longer stay at the clinic. Both cases and controls were consecutively selected, and patients who consented to participate in the study were screened using the basic periodontal examination (BPE). Those who had a score of 3 or 4 with the BPE were taken through a comprehensive periodontal examination for confirmation and clinical diagnosis. Confirmed cases were then recruited consecutively to participate in the study.

2.2 | Sampling and variables

Cases were further classified into gingivitis, localized chronic periodontitis, generalized chronic periodontitis, and aggressive periodontitis, derived from the 1999 International Workshop for a Classification of Periodontal Disease and Conditions.[13] Cases referred to patients with specific-site probing pocket depth of ≥3 mm and clinical attachment loss of ≥1 mm. Localized periodontitis was described as ≤30% of sites affected, and generalized periodontitis was described as >30% of sites affected. For all patients, the plaque index and gingival index were determined. Other independent variables obtained were age, sex, and educational level. Subgingival plaque or calculi samples were obtained from all patients by a single-stroke sterile curettage of the deepest pocket on probing for cases, and crevicular region of the lower and upper first molars in the controls. Samples were deposited into autoclaved 1.8 mL cryovials containing pre-prepared tris ethylenediaminetetraacetic acid buffer of pH 8.0. Samples were then labelled and transported into a freezer immediately and stored until they were ready to be analyzed.

2.3 | DNA extraction

Samples were weighed and crushed, and DNA extracted using the QIAamp DNA blood mini kit (Qiagen, Germantown, MD, USA) following the manufacturer's protocol. Briefly, buffer ATL and proteinase K were added and incubated for 2 hours with 15 minutes of pulse vortexing. Buffer AL was added and incubated at 70°C for 10 minutes; 96% ethanol was then added and the mixture transferred into the QIA amp spin column, centrifuged and washed with buffers AW1 and AW2, and the filtrate discarded. DNA was eluted with buffer AE into a new 2 mL Eppendorf tube, which was used for multiplex polymerase chain reaction (PCR).

2.4 | Multiplex polymerase chain reaction

A 25 μL PCR reaction was prepared containing 12.5 μL already prepared premix (Taq polymerase, dNTP, $MgCl_2$, and buffer), 2 μL (0.5 μL) forward and 2 μL (0.5 μL) reverse primers of the viruses, 6.5 μL molecular-grade water (Thermo Fisher Scientific, Waltham, MA), and 2 μL DNA template.

The primers used in the study (Thermo Fisher Scientific) were as follows: HSV-1: 5′-CGTACCTGCGGCTCGTGAAGT-3′ (forward) and 5′-AGCAGGGTGCTCGTGTATGGGC-3′ (reverse), HSV-2: 5′-TGGTATCGCATGGGAGACAAT-3′ (forward) and 5′-CTCCGTCCA GTCGTTTATCTTG-3′ (reverse), CMV: 5′-ACGTGTTACTGGCGGAG TCG-3′ (forward) and 5′-TTGAGTGTGGCCAGACTGAG-3′ (reverse), and EBV: 5′-AGCACTGGCCAGCTCATATC-3′ (forward) and 5′-TTGACGTCATGCCAAGGCAA-3′ (reverse).

Multiplex PCR conditions were as follows: Initial denaturation (95°C for 5 minutes), 45 cycles of denaturation (95°C for

30 seconds), annealing (54°C for 30 seconds), and extension (72°C for 30 seconds). Final extension was at 72°C for 5 minutes. Samples were held at 4°C and until the PCR product was detected by gel electrophoresis.

2.5 | Detection of amplified products

The detection of amplified products was done by electrophoresis on 2% agarose gel containing 1 × TAE (tris acetate ethylenediaminetetraacetic acid) stained with ethidium bromide; 10 μL of each amplified product was mixed with 3 μL orange dye (Thermo Fisher Scientific) and was loaded onto the gel, one product in each lane, along with the 100 bp DNA ladder for locating the accurate amplicon size of positive test samples. Electrophoresis was performed at 100 V for 1 hour to confirm the amplification of amplicons. The gel was visualized under a UV transilluminator. The amplicon size of known samples of HSV-1, HSV-2, HCMV, and EBV were detected as 271, 231, 368, and 326 bp, respectively.

2.6 | Data entry, analysis, and quality control

Sociodemographic data are described in Tables 1 and 2. Frequency graphs were also used to show the distribution of the different herpes viruses among periodontitis patients (Figures 1 and 2). Associations between the presence of herpes viruses and the different presentations of periodontitis were done by cross-tabulating the presence of herpes viruses against different categorical variables from the background characteristics collected.

2.7 | Ethical considerations

Ethical approval for the study was granted by the Ethical and Protocol Review Committee for the School of Biomedical and Allied Health Sciences of the University of Ghana. Individual written consent was obtained from all patients included in the study.

3 | RESULTS

Thirty-one participants in total participated in present the study (19 males and 12 females, representing 61.3% and 28.7%, respectively). The age of the participants ranged from 19 to 72 years, with median and mean ages of 33 and 34.58 (standard deviation [SD]: 11.62), respectively.

The mean age for patients with aggressive periodontitis was 32.2 years (SD: 8.50), whereas those for localized chronic periodontitis and generalized chronic periodontitis were 40.6 years (SD: 7.83) and 46.3 years (SD: 12.12), respectively. All of the participants had at least primary education, with most participants having tertiary education (Figure 1). Most of the participants (N = 17) were visiting the dentist for the very first time (Figure 2).

Only two viruses (HSV-1 and EBV) were detected among the participants out of the viruses tested (HSV-1, HSV-2, HCMV, EBV), with their distribution shown in Table 1. These were found only among patients clinically diagnosed with aggressive periodontitis. Of the total number of aggressive periodontitis patients, HSV-1 and EBV were found in four (44%) and one (11%), respectively. The mean age for patients found to have HSV-1 or EBV was 29 years (SD: 6.93). However, the detection of herpes virus in patients with periodontitis was not found to be statistically significant (Fisher's exact test, $P = 0.147$).

While all cases with detected viruses were found to be males, there was no statistical evidence of the association of sex, educational level, or income with either the type of periodontitis observed or the presence of subgingival virus.

4 | DISCUSSION

Based on the hypothesis that the presence of herpes viruses would be significantly higher among patients with periodontitis, our study showed that its presence was high in aggressive periodontitis (55%), although this was not statistically significant (Fisher's exact test, $P = 0.147$). This seems to have been the conclusion for several other studies,[6,14] which found that herpes viruses target host cells by attaching onto the cell surface through glycoproteins present in the viral envelope, stimulating local cytokine production through macrophages and other inflammatory cells. This phenomenon coincides with the state of elevated localized inflammatory burden due to increased gingival crevicular fluid cytokine levels in aggressive periodontitis, which leads to the impairment of local periodontal immune defense.[6] This association has also been suggested to be modified by

TABLE 1 Distribution of detected viruses in periodontal diseases

Diagnosis	Proportion (%)	CI	Presence of HSV-1 (proportion %)	CI	Presence of EBV (proportion %)	CI
Clinically-healthy periodontium (control)	32.3	0.17-0.51	0.0	–	0.0	–
Localized chronic periodontitis	16.1	0.07-0.35	0.0	–	0.0	–
Generalized chronic periodontitis	22.6	0.11-0.42	0.0	–	0.0	–
Aggressive periodontitis	29.0	0.15-0.48	12.9 (4)	0.05-0.31	3.2 (1)	0.004-0.215

CI, confidence interval; EBV, Epstein-Barr virus; HSV-1, herpes simplex virus 1.

TABLE 2 Gingival index and plaque score per disease type

Diagnosis	Gingival index	SD	Plaque index	SD
Clinically-healthy periodontium (control)	0.088	0.080	0.304	0.220
Localized chronic periodontitis	0.624	0.293	1.039	0.338
Generalized chronic periodontitis	0.943	0.976	0.959	0.572
Aggressive periodontitis	0.341	0.294	0.602	0.356

SD, standard deviation.

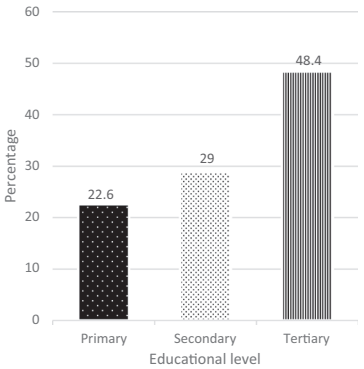

FIGURE 1 Educational background of participants

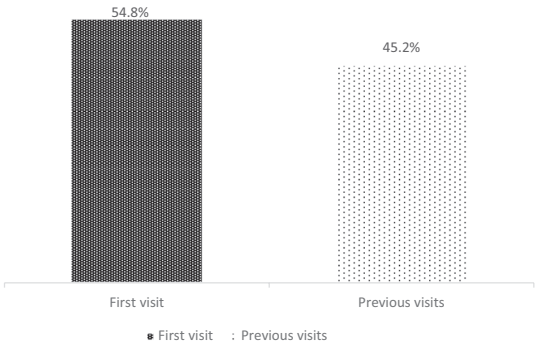

FIGURE 2 First dental visit of patients

several environmental factors, such as comorbidities, oral hygiene, stress, smoking, and alcohol.[15] However, in the present study, we excluded patients with a history of smoking or chronic alcohol consumption, as well as those with comorbid conditions, thus reducing the chances of interaction. Our finding of EBV in 11% of patients clinically diagnosed with aggressive periodontitis compared favorably to those from other studies, in which ranges from 7.5% to 72.2% were reported. Our finding of 0% for both HSV-2 and HCMV was also supported.[6]

While the prevalence of periodontitis in Ghana, and aggressive periodontitis in particular is not known, global prevalence studies suggest that Africa has the highest burden of aggressive periodontitis.[16] The average age of patients with aggressive periodontitis found in the present study was lower than that of chronic periodontitis (32.2 and 46.3 years, respectively). This finding also corroborates current thinking that aggressive periodontitis, which is associated with rapid attachment loss and bone destruction, is associated with comparatively younger patients, although some authors suggest that it usually affects patients <30 years.[17]

In the present study, we showed that the level of plaque accumulation and gingival inflammation might not be clinically comparable to the level of damage in aggressive periodontitis, evidenced by lower plaque and gingival indices in comparison to chronic periodontitis (Table 2). This finding is supported by Vendana et al., who also found a risk factor comparison between chronic periodontitis and aggressive periodontitis.[18]

Periodontitis in general is influenced by several socioeconomic factors,[19] of which it could be assumed that educational level might play an important role in health-seeking behaviors. There was, however, no evidence of the presence of periodontitis in less-educated individuals. Most of the participants were also visiting the dental clinic for the first time (Figure 2), a phenomenon which has been suggested to be an indicator of a high level of unmet need for oral health care in Ghana.[3] Although periodontal disease generally has a worldwide prevalence, its higher occurrence in Africa might require a greater effort in its control.[16] Not only is there the need to improve on systemic efforts to control the condition but also to expand on current clinical management regimes.

The small sample size is a limitation of the present study, thereby potentially influencing interpretation of the findings. Likewise, the use unmatched controls in our patient selection ignores possible interacting characteristics, such as age, sex, and ethnicity.[1]

5 | CONCLUSION

In the present study, we found HSV-1 and EBV in the subgingival plaque samples of Ghanaian patients clinically diagnosed with aggressive periodontitis. While our finding requires further investigation, the role of HSV in periodontitis, if elucidated, could transform and inform clinical management of the condition.

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