

Relationship between the prevalence of oral human papillomavirus DNA and periodontal disease (Review)

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Abstract. Human papillomavirus (HPV) is a small DNA virus that infects the basal keratinocytes of squamous epithelium in the skin, and in the oral and genital mucosa. Smoking and sexual behavior have been recognized as significant risk factors for oral HPV infection. In the present review, the findings of recent studies of oral HPV infection in relation to periodontitis are discussed, as well as periodontopathic bacteria and periodontal herpes virus. Previous research suggests that HPV localizes to the inflammatory periodontal tissue. Inflammatory periodontal pockets may thus act as a reservoir for HPV. The interactions between HPV and periodontopathic bacteria remain unclear, but it is hypothesized that oral HPV infection may be related to a characteristic oral microbiome. Smoking is associated with HPV and periodontitis, as smoking induces destruction of periodontal tissue and suppresses the host defense, allowing HPV to infect periodontal tissue. Carcinogenic HPV and periodontitis may lead to the development of oral cavity cancer. However, oral HPV E6/E7 expression (transcriptionally active HPV) has not yet been fully investigated in patients with periodontitis. Collectively, the evidence suggests that oral HPV prevalence may be associated with periodontitis. The effect of clinical factors (age, sex, smoking, immunosuppressive condition and vaccination) on oral HPV DNA prevalence should be considered when clarifying the relationship between oral HPV and periodontitis. Additionally, the sampling method should be carefully chosen to directly detect HPV DNA in periodontal tissues.

Contents

1. Introduction
2. Association between oral HPV and periodontitis
3. Association between oral HPV and periodontopathic bacteria
4. Association between herpes virus and periodontitis
5. Association between oral HPV and herpes virus
6. Association of smoking with oral HPV infection and periodontitis
7. Conclusion

1. Introduction

Human papillomavirus (HPV) is a small DNA virus that infects the basal keratinocytes of the squamous epithelium through micro-wounds and abrasions in the skin and mucosa (1). To date, >150 HPV genotypes have been identified, of which ~40 infect the mucosa (mucosal HPV types) (1). Mucosal HPV types that infect the anogenital mucosa have also been shown to also infect the mucosa of the oral cavity (2). Smoking and sexual behavior have been recognized as predominant risk factors for oral HPV infection (3).

Several studies have been performed to investigate the association between HPV prevalence and periodontal disease (4-15). Periodontal diseases such as gingivitis and periodontitis are polymicrobial infectious diseases that affect epithelial tissue (the gingiva), tooth-supporting connective tissue (the periodontal ligament) and alveolar bone. Periodontal disease is a major cause of tooth loss, and is caused by interactions between periodontopathic bacteria, host immune responses and environmental factors (for example, smoking) (16). *Periodontopathic bacteria* such as *Treponema denticola*, *Tannerella forsythia* and *Porphyromonas gingivalis* serve vital roles in the pathogenesis of periodontal disease (17). Importantly, periodontal disease causes destruction of the crevicular epithelium (16), which may increase the opportunity for HPV to infect basal cells in the epithelium.

Oral virome analyses have revealed that several human viruses are stable members of the microbiota, and oral viruses display a sex-specific prevalence (18). Previous research has demonstrated that, together with periodontal disease-related bacteria, the herpes virus serves a significant role in the pathogenesis of periodontal disease (19). However, it remains to be

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Abbreviations: HPV, human papillomavirus; HCMV, human cytomegalovirus; EBV, Epstein-Barr virus

Key words: HPV, periodontitis, periodontopathic bacteria

elucidated whether oral HPV is implicated in periodontitis and the virulence of periodontopathic bacteria. In the present review, the findings of recent studies of oral HPV prevalence in relation to periodontitis are discussed, as well as periodontopathic bacteria and periodontal herpes virus.

2. Association between oral HPV and periodontitis

The PubMed search engine was used to search papers using the following key words: '(HPV OR human papillomavirus) AND (periodontitis OR periodontal disease)', yielding 118 papers published between June 1980 and October 2020. After reviewing the titles and abstracts for relevance, reviews and meta-analyses (n=25), case reports (n=23), *in vitro* or animal studies (n=8), articles that focused on cancer and pre-malignant lesions (n=20), hyperplastic lesions (n=2), periapical lesions (n=5) or odontogenic cysts (n=1), as well as other articles, including news (n=3), editorials and commentaries (n=3), letters to editors (n=1), congress (n=1), questionnaire surveys (n=1) or a demand study (n=1), were all excluded (Fig. 1). Additionally, clinical research papers with no data regarding the association between oral HPV infection and periodontitis (n=7), clinical research papers investigating HPV E6/E7 mRNA rather than HPV DNA (n=2), and articles not written in English (n=3) were excluded. This resulted in 12 original papers that investigated the association between oral HPV DNA prevalence and periodontal disease (4-15). Table I presents a summary of the studies included in the present literature review. Of the 12 papers that reported the association between oral HPV DNA and periodontal disease, periodontal tissue samples were used in 5 studies (4,6,7,9,11), oral swab samples were used in one study (8), oral rinse samples were used in 5 studies (10,12-15) and crevicular fluid samples were used in one study (5). A nucleic acid amplification assay (for example, PCR) was the most common detection method for HPV DNA and was employed in 11 of the 12 studies (5-15). Nucleic acid hybridization assays (such as southern blotting) were used for detection of HPV DNA (4,6).

To assess the prevalence of oral HPV DNA using periodontal tissue samples, HPV DNA was detected in adult periodontitis and rapidly progressive periodontitis using gingival papilla specimens and southern blotting (4). Carcinogenic HPV DNA was detected in 26% of the gingival tissues obtained from patients with periodontitis by PCR (6). Furthermore, HPV DNA was visualized in the nucleus of the junctional epithelium using *in situ* hybridization (6). These results indicate that local periodontal inflammation may provide an opportunity for HPV to infect epithelial basal cells. Additionally, a higher HPV DNA positive rate was found in gingivitis and/or periodontitis biopsy samples from kidney transplant patients compared with those from non-transplanted patients (11). Conversely, Horewicz *et al* (7) reported that HPV16 DNA was not detected by PCR in any paraffin embedded-gingival tissues (chronic periodontitis, gingivitis or healthy periodontium) of Brazilian patients with good general health. Furthermore, HPV16 DNA was not detected by PCR in the marginal periodontium of systemically healthy patients, excluding pregnant women, patients with uncontrolled systemic diseases and smokers (9). It is speculated that the prevalence of HPV DNA is low in normal periodontal tissues. Patients with general good health maintain an effective local immune system which may provide

a defense against HPV, and may explain the absence of HPV in periodontal tissues.

Oral swab samples from the external gingival epithelium and the internal gingival epithelium (the periodontal pocket epithelium) were used to detect HPV DNA by PCR in women with HPV-associated gynecological diseases, and 13.3% of external gingival epithelium samples and 16.7% of internal gingival epithelium were HPV DNA positive (8). However, there was no positive correlation between HPV DNA and the incidence and severity of periodontitis (8). The relationship between oral HPV DNA and oral health status (for example, plaque accumulation and bleeding in the gingival sulcus) was investigated using oral swab samples in individuals with no history of HPV vaccination (20). The detection rate for high risk type HPV was greater in the individuals with a high gingival bleeding index compared with those with a low index (20). A significant independent association was found between oral HPV DNA detection and plaque accumulation or gingival sulcus bleeding after adjustment for age and sex (20). Bleeding of the ulcerated epithelial surface of the periodontal pocket is considered a significant indicator of periodontal inflammation caused by periodontal pathogens (21). Thus, it is likely that oral HPV infection is significantly associated with periodontal inflammation.

Oral rinse samples were employed in 5 previous studies to investigate oral HPV prevalence (10,12-15). HPV DNA detection and genotyping were performed using oral rinse samples in 740 Hispanic adults (13). The prevalence of oral HPV DNA was significantly higher amongst individuals with severe periodontitis (11.3%) than those with mild or moderate periodontitis (5.3%) or no periodontitis (2.6%) (13). The same group also reported a significant association between oral HPV infection and the severity of periodontitis in Hispanic participants of the San Juan Overweight Adults Longitudinal Study between 2011 and 2013 (14). Sun *et al* (12) investigated the relationship between oral HPV16 DNA and periodontal health status (including presence of bleeding on probing, dental calculus and periodontal pockets) by PCR, using oral rinse samples in patients at a dental school clinic. The HPV16 DNA detection rate was higher in individuals without periodontal disease (5.3%) than in those with periodontal disease (3.4%); however, no significant association was found between the oral HPV16 DNA detection rate and periodontal health status (12). Wiener *et al* (10) performed a study based on the National Health and Nutrition Examination Survey data and revealed that 58.7% of HPV positive participants had periodontitis, whereas 38.7% of HPV negative participants had periodontitis (10). A significant association was found between the presence of oral HPV DNA and periodontitis (10). However, no independent association was found between oral HPV DNA and periodontitis after adjustment for clinical factors such as sex, ethnicity, education, age, income to poverty ratio, smoking, alcohol use and number of lifetime sexual partners (10). Additionally, McDaniel *et al* (15) performed a study based on the National Health and Nutrition Examination Survey data between 2011 and 2012 as well as 2013 and 2014, and reported that the median predicted oral HPV prevalence rates were higher in individuals with periodontitis than in those without periodontitis amongst non-HPV-vaccinated individuals (15).

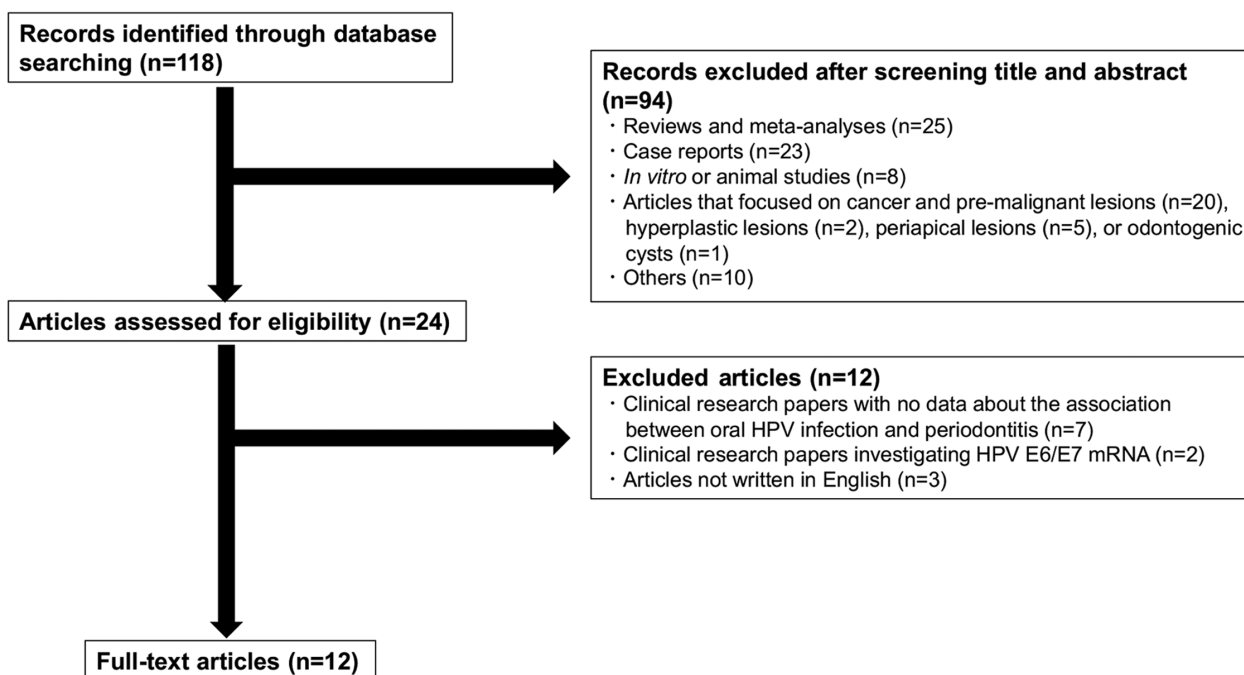


Figure 1. Flow chart of the search strategy employed in this review. HPV, human papillomavirus.

Gingival crevicular fluid contains not only serum and blood cells, but also periodontal epithelial cells and subgingival plaque (22). Therefore, gingival crevicular fluid can be used to investigate the localization of HPV in periodontal pockets. Parra and Slots (5) investigated the prevalence of HPV DNA by PCR using gingival crevicular fluid in patients with advanced periodontitis or gingivitis. HPV DNA was detected in 16.7% of patients with advanced periodontitis, but was not detected in patients with gingivitis (5). In our previous study, the presence of HPV16 DNA in gingival crevicular fluid collected by inserting paper points into periodontal pockets was investigated in middle-aged and older Japanese individuals (23). Of the 89 participants, four women (4.5%) were HPV16 DNA-positive, but no men exhibited HPV16 DNA positivity (23). Postmenopausal women were more likely to be infected with HPV in the cervix because of sex-hormone-related immunosuppression (24). Female sex hormones, such as progesterone, enhances the regulatory response to HPV16 virus-like particles in peripheral blood mononuclear cells (25). Therefore, postmenopausal women may be more susceptible to oral HPV infection as well as cervical HPV infection. It is thus considered that factors specific to women, such as reduced levels of sex hormones, may increase the risk of HPV infection in periodontal pockets.

The oral HPV detection rate may be affected by differences in sample detection methods. Oral rinse samples contain a mix of saliva, bacteria, epithelial cells and blood cells derived from various sites in the oral cavity. Furthermore, contamination of the pharynx by gargling may have affected the positive rate of HPV DNA in oral rinse samples because tonsillar tissue acts as a reservoir for microorganisms due to its specific anatomical and histological structure (26). It is thus considered that sampling methods, such as gingival tissue biopsies and crevicular fluid samples may be more appropriate to determine the presence of periodontal disease-related HPV.

Collectively, the evidence suggests that oral HPV infection may be associated with periodontitis. A recent systematic review and meta-analysis revealed a positive relationship between oral HPV infection and periodontitis, although the certainty of the evidence is low (27). It is important to consider the effect of clinical factors contributing to oral HPV infection (age, sex, smoking, immunosuppressive condition and vaccination) on HPV DNA prevalence to clarify the presence of periodontitis-related HPV. Additionally, sampling methods should be carefully chosen to directly detect HPV DNA in periodontal tissues. Further studies are required to demonstrate the presence of HPV in periodontal tissues and clarify the biological role of HPV in periodontitis.

3. Association between oral HPV and periodontopathic bacteria

Our previous study revealed that increased HPV16 E6 viral copy numbers were associated with an increased number of oral bacteria in hospital patients, which suggests that poor oral hygiene may be related to oral HPV infection and viral replication (28). Additionally, the HPV16 DNA positivity of gingival crevicular fluid was significantly associated with the prevalence of *Treponema denticola* and *Fusobacterium nucleatum* (23). It is thus considered that oral HPV prevalence is related to the presence of periodontal bacteria. Analysis of the microbiome shows a strong association between the diversity of the vaginal microbiota and HPV infection (29). Vaginal HPV infection and its persistent infection are characterized by a reduced abundance of vaginal *Lactobacillus* spp. (29). A significant relationship between carcinogenic HPV infection and the *Prevotella* genus was found in the vagina of HIV-negative participants (30). These observations suggest that changes in the microbiome may potentially facilitate vaginal HPV infection. The composition of the oral microbiome has been shown

Table I. Summary of studies included in the present literature review.

Author, year	Country	Subjects	Sample	Detection method	HPV DNA positive rate, % (positive n/total n)	Refs.
Madinier <i>et al.</i> , 1992	France	6 patients with adult periodontitis and 2 patients with rapidly progressive periodontitis	Gingival tissues	Southern blot	Percentages of HPV positivity were 16.7% (1/6) in patients with adult periodontitis and 50% (1/2) in patients with rapidly progressive periodontitis	(4)
Parra and Slots, 1996	USA	30 patients with advanced periodontitis	Creviceular fluid samples	PCR	16.7% (5/30)	(5)
Hormia <i>et al.</i> , 2005	Finland	38 individuals with clinically diagnosed periodontal disease	Gingival tissues	PCR, southern blot	25.8% (8/31)	(6)
Horewicz <i>et al.</i> , 2010	Brazil	56 systemically healthy adults with chronic periodontitis	Paraffin blocks of gingival tissues	PCR for HPV16 DNA detection	0% (0/56)	(7)
Fuster-Rossello <i>et al.</i> , 2014	Argentina	11 women with HPV-associated gynecological diseases and periodontitis	Oral swab samples	PCR	Unknown	(8)
Jacob <i>et al.</i> , 2014	India	67 systemically healthy participants with periodontitis	Gingival tissues	PCR for HPV16 DNA detection	0% (0/67)	(9)
Wiener <i>et al.</i> , 2015	USA	Participants with periodontal disease from the National Health and Nutrition Examination Survey data from between 2009 and 2012	Oral rinse samples	PCR	10.5% (309/2945)	(10)
Baez <i>et al.</i> , 2016	Brazil	74 kidney transplanted or non-transplanted patients with gingivitis and/or periodontitis	Gingivitis and/or periodontitis tissues	PCR	41.9% (31/74)	(11)
Sun <i>et al.</i> , 2017	Australia	89 participants with periodontitis	Oral rinse samples	PCR for HPV16 DNA detection	3.4% (3/89)	(12)
Ortiz <i>et al.</i> , 2018	Puerto Rico	Participants of the San Juan Overweight Adults Longitudinal Study between 2014 and 2016	Oral rinse samples	PCR	Percentages of HPV positivity were 5.3% in patients with mild/moderate periodontitis and 11.3% in patients with severe periodontitis	(13)
Ortiz <i>et al.</i> , 2018	Puerto Rico	Participants of the San Juan Overweight Adults Longitudinal Study between 2011 and 2013	Oral rinse samples	PCR	Percentages of HPV positivity were 4.4% (13/297) in patients with none/mild periodontitis, 4.1% (12/290) in those with moderate periodontitis, and 11.5% (17/148) in those with severe periodontitis	(14)
McDaniel <i>et al.</i> , 2020	USA	Participants of the National Health and Nutrition Examination Surveys from between 2011 and 2012 as well as 2013 and 2014	Oral rinse samples	PCR	Not determined	(15)

HPV, human papillomavirus.

to reflect differences in the periodontal health condition (31). *Prevotella*- and *Veillonella*-dominant oral microbiomes were associated with the active phase of periodontitis amongst Japanese individuals (31). Conversely, *Neisseria*-, *Haemophilus*-, *Aggregatibacter*- and *Porphyromonas*-abundant oral microbiomes reflected healthy periodontal tissue (31). The relationship between the oral microbiome and oral HPV infection has not been fully elucidated, limited by the small number of studies and small sample sizes in these studies (32,33). The association between the composition of the bacterial microbiota and HPV DNA in the oral cavity was investigated in 39 Finnish women (32). Unclassified *Bifidobacteriaceae* and *Finnegoldia* genera were significantly dominant, but the *Haemophilus* genus was less numerous in HPV positive cases than in HPV negative cases (32). *Capnocytophaga ochracea* was more abundant in HPV16 DNA positive periodontal granulation tissue than in HPV negative tissues in Indians (33). It has been reported that the oral viral community is significantly characterized according to the sex of the host (34), which indicates that human sex hormones may affect the composition of oral viromes. Furthermore, it is hypothesized that aging may be a significant factor affecting the composition of oral viromes as a result of declining immune function. Therefore, it is necessary to consider the effect of sex and age when evaluating the association between oral HPV and periodontopathic bacteria. Further analysis of microbial communities may provide greater insight into the relationship between HPV and specific oral bacteria.

4. Association between herpes virus and periodontitis

It is clear that the herpes virus is notably associated with periodontitis. Herpes viruses such as herpes simplex virus (HSV), human cytomegalovirus (HCMV) and Epstein-Barr virus (EBV) were detected with a wide range of positive percentages in gingivitis and chronic periodontitis (35-39). In two previous independent meta-analyses, it was suggested that oral EBV and HCMV are significantly associated with periodontitis (35,37). According to a review by Slots (38), the median prevalence of HSV, HCMV and EBV was 45, 40 and 32% in chronic periodontitis and 12, 3 and 7% in healthy periodontal tissue, respectively (38). Co-infection of herpes virus and periodontal disease-related bacteria induces the risk of periodontitis (40,41). Herpes virus-induced local proinflammatory cytokines in the presence of immunosuppression may contribute to periodontitis (38). Proinflammatory cytokines released by HCMV-infected gingival fibroblasts serve an important role in attracting cytotoxic T-cells and natural killer cells (41). Attachment of *Actinobacillus actinomycetemcomitans* to periodontal epithelial cells can be enhanced by HCMV (42). These results highlight the important role of herpes virus in periodontitis.

5. Association between oral HPV and herpes virus

Concurrent infection of the herpes virus and HPV was found in individuals with advanced periodontitis (5). The prevalence of both HPV DNA and EBV DNA in gingivitis and periodontitis tissue biopsies was 25% in kidney transplant patients, but 0% in non-transplanted patients (11). Kidney transplant

patients receiving immunosuppressive therapy exhibited high HPV and EBV DNA positivity, which indicates that immunosuppressive conditions may elicit susceptibility to concurrent viral infection in the periodontium.

Several *in vitro* studies have been performed to investigate the biological relationship between HPV and the herpes virus (43-45). HSV facilitated integration and amplification of the HPV genome in HPV18 DNA-transfected cervical cancer cells (43). Co-expression of EBV latent membrane protein-1 and HPV16 E6 induced malignant transformation in primary mouse embryonic fibroblasts through NF- κ B signaling (44). HPV can increase EBV genome stability and lytic reactivation of EBV in oral keratinocytes (45), which indicates that HPV promotes the pathogenicity of oral EBV. Collectively, these results suggest that HPV and the herpes virus induce adverse oncogenic events.

6. Association of smoking with oral HPV infection and periodontitis

Smoking causes the destruction of periodontal tissue through microcirculatory dysfunction and impairment of host immune systems (46). Therefore, smoking is a significant risk factor for periodontal disease (47). Notably, smoking is thought to cause dysbiosis of the periodontal microbiome (48), which suggests that the smoking-induced imbalance in the microbiome is detrimental to periodontal health. Several studies have demonstrated that smoking is a major risk factor for oral HPV infection (49-54). Our previous meta-analysis showed the association between oral HPV infection and smoking (3). Current smoking was a significant risk factor for oral HPV infection (3). Furthermore, smoking increased the duration of high-risk HPV infection in the oral cavity (55). These results suggest that smoking is a major risk factor for both oral HPV infection and periodontitis. It is thus speculated that HPV tends to infect the smoking-deteriorated periodontal tissue due to the suppression of the host defense by smoking.

Chronic inflammation in periodontitis contributes to the development of several types of cancer caused by carcinogens, such as ROS, produced by activated inflammatory cells in response to periodontal pathogens (56-59). Periodontopathic bacteria are reported to be a risk factor for oral squamous cell carcinomas (60,61). Furthermore, a history of periodontitis is importantly associated with the HPV status of patients with oral cavity cancer (62,63). Therefore, it is hypothesized that periodontitis can increase the possibility of adverse oncogenic events independently or cooperatively with oncogenic HPV.

HPV and smoking are thought to interact in carcinogenesis in the following manner. Smoking upregulates the number of HPV genome copies and promotes integration of viral genomes into the host genome in HPV-infected cells (64). Next, the HPV oncoproteins E6 and E7 inhibit p53 function, which results in accumulation of chromosomal instability and loss of cell cycle control (64). Finally, HPV-induced immortalization and tobacco smoke-associated DNA damage cause carcinogenesis (64). Therefore, persistent carcinogenic HPV infections induced through smoking may contribute to the development of HPV-related oral cavity cancer.

7. Conclusion

HPV localizes to inflammatory periodontal tissue and is thought to infect basal keratinocytes in the ulcerated gingival sulcus epithelium. Inflammatory periodontal pockets serve a significant role as a reservoir for HPV. Although the interactions between HPV and periodontopathic bacteria remain unclear, oral HPV infection may be associated with a characteristic oral microbiome. Smoking induces destruction of periodontal tissue, and HPV then tends to infect periodontal tissue due to the smoking-induced suppression of the host defense. Carcinogenic HPV and periodontitis are likely to contribute to the development of oral cavity cancers. However, to date, oral HPV E6/E7 expression (transcriptionally active HPV) has not been fully investigated in individuals with periodontitis. Collectively, the available literature suggests that oral HPV may be associated with periodontitis. To clarify the association between oral HPV and periodontitis, the effects of clinical factors contributing to oral HPV DNA prevalence should be considered. Additionally, methods of sampling that can directly detect HPV DNA in inflammatory periodontal tissues should be further investigated.

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Authors' contributions

HS contributed to the conception of the study and wrote the manuscript. MS and KO aided in writing the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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