Chapter

The Presence of HPV in Dental Calculus: It's Role in Pathogenesis of Oral and Cervical Cancer

Sunardhi Widyaputra, Natallia Pranata, Ignatius Setiawan and Jamas Ari Anggraini

Abstract

Human papillomavirus (HPV) infection accounts for approximately 5.2% of the worldwide human cancer burden. Molecular epidemiologic evidence clearly indicates that certain types of HPV are the principal cause of both cervical and oral cancers. Major oncoproteins E6 and E7 can inactivate p53 and pRB proteins because it happened genome instability and dysregulation host cell cycles. This virus is an epithelial tropism, vulnerable area mainly at the basal layer and epithelial stem cell, because it still has a high proliferation capacity, so it can support the replication of the virus. Virions bind initially to the glycosaminoglycan (GAG) chains of heparan sulphate proteoglycan (HSPG). More than 99% cervical cancer arise at the cervical transformation zone. In oral cavity, exposed areas of the basal layer will be very susceptible to HPV infection. The HPV presence in the oral area is considered as one of the etiologics of oral cancer in those who do not have bad habits such as smoking, betel chewing, or poor oral hygiene. Our study successfully identified HPV type 58 in dental calculus. Dental calculus, calcified oral plaque biofilm, has been shown to be an abundant, nearly ubiquitous, and long-term reservoir of the ancient oral microbiome, including bacteria, archaea, eukaryote, and viruses. During biomineral maturation process, several biological contents around the oral region should be trapped, including the exfoliated virus contained cells. Dental calculus is a promising source of HPV and carcinogens molecules in the oral cavity and could be used as a biomarker for early detection.

Keywords: HPV, biosource, dental calculus, oral cancer, cervical cancer, OSCC

1. Introduction

Human papillomavirus (HPV) is considered to be one of the oldest known viruses and also the most common sexually transmitted infection (STI). Annually, around 6 million people are diagnosed with the disease [1]. HPV-related diseases have been an important subject to study for many years and are becoming a major concern for public health at present [2, 3]. This virus is an epithelial tropism, a vulnerable area mainly at the basal layer and epithelial stem cells [3]. After infecting cells, HPV will change the cellular environment, avoiding the immune

response, so that the infection can persist [4]. This virus is very varied, there are about 228 genotypes that live in the human body [5]. If HPVs have 70% similarity in the DNA sequence, they are categorized as belonging to the same genus [3]. Alphapapillomavirus is a genus which mainly infects the mucosa both in the anogenital tract and in the oral cavity [3, 6]. HPV was confirmed to cause cervical cancer in early 1980s. It is estimated that around 70% of head and neck cancer cases are also caused by HPV infection of the genus alpha [6, 7]. Based on its role in carcinogenesis, HPV is divided into high risk (HR) and low risk (LR). LR-HPV such as HPV-6 and HPV-11 cause benign papilloma/condyloma, whereas HR-HPV such as HPV-16 and HPV-18 cause squamous intraepithelial lesions that can develop into squamous cell carcinoma [8].

A significant change in HPV endemic is indicated in the epidemiological data from the last decade. HPV is not only found in the genital area but also in the oral area [7, 9]. HPV infection in the oral cavity is frequently associated with sexual behavior. Oral sex is considered a risky sexual behavior that has the potential to transmit HPV from the anogenital to the oral cavity [10].

This chapter aims to describe the causality of HPV infection in the oral cavity and in the genital area, especially the causes of "endemic" triggered by changes in the behavior of the society. Knowledge of the history of HPV infection, risk factors, clinical manifestations, current prevention, and therapy strategies is indispensable prerequisite for health workers to improve the professionalism of dentists and other medical personnel involved in treating patients at risk of infection or patients with clinical risk manifestations of infection.

Since HPV infection is latent, to be able to study the pathogenesis of HPV-linked oral cancer, it is necessary to have a biosource that can detect the presence of the causative agent for a long time [7]. Dental calculus, as a biosource, can keep a variety of molecular information, including HPV DNA, for a long time [11]. Therefore, it is imperative to design sufficient prevention and management strategies to tackle HPV-related diseases, while promoting understanding and collaboration among health workers: the medical and dental communities, who may not yet familiarize themselves with this perspective.

2. Pathogenesis of HPV infections and cervical cancer

HPV is a small double-stranded circular DNA virus that commonly infects humans [12, 13]. HPV is almost entirely acquired from sexual exposure, when it enters the skin and mucous membranes of the mouth, anus, penis, and female reproductive tract [14]. Infections with different strains are linked to a variety of skin manifestations, ranging from common warts to malignancies [15]. HPV infection accounts for approximately 5.2% of human cancer burden worldwide, including the cancers of the anus, genital tract, and oropharynx [16].

2.1 Characteristics of HPV

HPV is a heterogeneous viral group of the papillomaviridae family that infects the basal layer of either the vertebrates mucosal epithelial or cutaneous, causes neoplasia, or persists without symptoms [17]. HPV contains a double-stranded circular non-enveloped DNA genome that codes for eight genes and a noncoding region that manages a replication of the viral and controls cellular and transcription of the viral [16, 18]. All protein-coding genes are located on the same DNA strand. The genes are divided into early (E) and late (L) genes, E1, E2, E4,E5, E6, E7, L1, and L2, with the late genes encoding the major and minor capsid proteins [18, 19]. The capsid is

the protein shell that surrounds the viral DNA. HPV can integrate into the host cell chromosomes and/or persist in episomal form [20].

One of important factor in HPV-related diseases is epigenetic regulation of viral gene expression [21, 22]. Another investigation is that the viral genome can be methylated de novo by host DNA methyltransferase (DNMT), implying an innate response to pathogens [23]. Thus methylation of the viral genome may be in part a mechanism by which the host attempts to suppress viral gene expression and thereby HPV pathogenicity [21].

HPV are characterized according to their tissue tropism and they are subdivided into five main genera (Alpha-, beta-, gamma-, nu- and mu-papillomaviruses) depending on the DNA sequences, HPV life cycle characteristics and disease associations [24]. Traditionally HPV is distinguished, based on the tropicalism of specific epithelium, on the skin type and mucosa: the first infects the skin of the hands and feet, the second prefers the mucosal surface of the upper gastrointestinal tract, the anogenital area, the urethra and conjunctive [25]. The HPV can be further subdivided according to the epidemiological classification as ones with low and high risk oncogenic potentials depending on the viruses' ability to promote the proliferation of infected cells and lead to malignant transformations [26]. HR-HPV is associated with an increased risk of developing cancer and is often referred to as a 'cancer related' or 'oncogenic' type [27]. This group has HPV genotypes such as 16–18–31-33-35, 39,45,51,52,53, 56,58,59, 66, 67,70,73,68, 82) [28]. HR-HPV is associated with potentially and obviously malignant lesions (e.g. anogenital cancer) [3]. LR-HPV has genotypes such as 2, 4, 27 (skin type) and mucosal types 6, 11, 13, 32, 42) [28]. LR-HPV is more commonly associated with non-malignant diseases (e.g. ordinary warts, condyloma, focal epithelial hyperplasia, squamous cell papilloma) [29].

2.2 Effect of HPV on the basal layer and epithelial stem cells

Papillomavirus infections are usually long-lived and persistent and the dividing basal cells must provide a continual reservoir of infected cells for the overlying virus producing tissue [30]. Thus, HPV need a robust mechanism to retain their episomal genomes within the nucleus of dividing epithelial cells [31]. In normal squamous epithelium of the cervix, the basal layer is the area of active cell division [14]. After division, the cells migrate up from the basal layer and no longer progress through the cell cycle and become terminally differentiated keratinocytes [32]. Since epithelial cells have stopped dividing at this stage, the number of virus copies per cell has increased considerably and the level of viral gene expression has also increased [14]. Most of the replication of the viral genome occurs after epithelial cells are shed from the basal layer [32]. The histopathological changes characteristic of typical low-grade HPV-induced lesions reflect active replication of the virus [14]. These include koilocytosis, multinucleation, and nuclear enlargement and are due to the assembly of the viral particles in the upper epithelial layers [30]. The epithelium is then shed, and infectious HPV virions are released, which can then infect a new host [31].

New insights have identified the capacity for HPV early region genes to dysregulate adult tissue stem cell self-renewal pathways ensuring that the expanded population preserve its stem cell characteristics beyond the stem cell niche. HPV-infected cells acquire additional transforming mutations that can give rise to intraepithelial neoplasia (IEN), from environmental factors such as sunlight or tobacco induced mutations in skin and oral cavity, respectively. With establishment of IEN, HPV viral replication is sacrificed with loss of the episome, and the tissue is predisposed to multiple cancer stem cell-driven carcinomas [33].

2.3 HPV and the potential for malignancy

Recent molecular and epidemiological studies showed HPV infection is now a well-established cause of cervical cancer and there is growing evidence of HPV being a relevant factor in other anogenital cancers (anus, vulva, vagina and penis) as well as head and neck cancers [34, 35]. Kian Ang's research (2010) suggested the role of HPV infection in the pathogenesis of oropharyngeal cancer; 63,8% of patients with oropharyngeal cancer (206 of 323) were HPV-positive [36].

HPV-induced carcinogenesis occurs as a multi-step process [16]. It begins by primary infection of the proliferating basal cells of the squamous epithelium [32]. If the infection is caused by a HR-HPV type, and there are presence of failure of the immune system to control and clear the infection plus the presence of some co-factors, after a period of time, HPV infection continues to accumulate sufficient genomic instability and leads to epithelial neoplastic transformation [18]. HPV is carcinogenic, partly because proteins E6 and E7 cause abnormal regulation of p53 and Rb, control of apoptosis and regulation of cell cycle [37]. It is believed that the circular genome is linearized and integrated as a late event in the infection process, destroying the region of the E1/E2 gene, destroying the E2 gene, releasing the suppression of the viral genome, leading to the overexpression of E6 viruses and E7 genes to maintain the malignant phenotype [38]. E5, E6 and E7 proteins are the most important for oncogenic transformation [39]. In the early stages of carcinogenesis the E5 protein plays a role and appears to increase cellular EGFR signaling, leading to up-regulation of viral gene expression and cell proliferation [37]. Generally, the high-risk HPV E6 protein activates many cellular proteins, including the cellular ubiquitin ligase E6AP, which targets the degradation of the TP53 protein, leading to loss of TP53-mediated processes, including apoptosis mediated by TP53 and the cell cycle checkpoints, DNA damage response and chromosome stability [19, 40]. Low risk E6 will not degrade TP53 [19]. The E7 protein promotes the proliferation of HPV-infected cells by degrading the RB1 protein, releasing E2F transcription factors, and boosting the expression of S-phase cell cycle genes and their proteins (including CDKN2A and its protein p16INK4a) [41]. This protein can be used as a surrogate marker for HPV expression [42]. The expression of E6 and E7 genes not only eliminates the two most important cellular tumor suppressor pathways, namely RB1 and TP53, but also affects the expression of a variety of tumor suppressor genes, DNA damage response genes and oncogenes, resulting in carcinogenic transformation [41].

3. HPV in the oral cavity

In oral cavity, exposed areas of the basal layer will be very susceptible to HPV infection. The presence of HPV in the oral cavity is thought to be the etiologic of oral cancer in those who do not have bad habits such as smoking, betel chewing or poor oral hygiene.

3.1 Characteristics of oral microbiome

Microbiome is the community of microbial residents in our body. It is the ecological community of symbiotic, commensal, and pathogenic microorganisms [43]. The human microbiome defines either the microorganisms (bacteria, archaea, lower and higher eukaryotes, and viruses) found in and on the human body or their collective genomes. The microbiota takes part in regulating the immune response, affecting the appetite, and therefore changing the food intake pattern,

participating in vitamin biosynthesis, and protecting human beings by producing antimicrobial substances [44].

The gut microbiome has been most extensively studied, however, microorganisms actually inhabit all the barrier surfaces of the human body including the skin, the oral cavity, the nasopharynx, the esophagus and stomach, and also the vagina, the urinary tract, the lungs and others. The composition of the microbiome varies according to the anatomic site. Different individuals have different compositions of microbiome [45]. The most common reproducible microbiome archetypes, or community-state types (CSTs) found in cervical intraepithelial neoplasia (CIN) patients are CSTs characterized by Lactobacillus depletion, anaerobic bacteria predominance, and Lactobacillus iners dominance. These CSTs are significantly associated with preinvasive diseases, increased disease severity, and disease invasiveness [46]. *L. crispatus*, L. iners, and ureaplasma parvum are associated with the pro-inflammatory inflammasome molecules IL-1α and IL-18, concomitantly with the antagonist IL-1ra generating a balance between anti-inflammatory and pro-inflammatory responses. This equilibrium is imbalanced by the presence of pathogens, which diverts it toward the inflammation [47].

Oral microbiome is defined as the collective genome of microorganisms that live in the oral cavity. After the gut, the oral cavity is the second largest microbial community in humans [43]. The oral cavity of healthy individuals contains hundreds of different bacterial, viral, and fungal species. Many of these can join to form biofilms which are resistant to mechanical stress or antibiotic treatment. Most are also commensal species, however, they can become pathogenic when triggered by changes in the environment or in the oral cavity, including changes in the quality of an individual's personal hygiene.

Those microorganisms can have very dynamic behavior, adapting to a wide range of environments and interactions with other microbial species in biofilms. The formation of biofilms may occur on many kinds of surfaces in the oral cavity. The epithelial cells, saliva-coated enamel, dental surfaces, primary colonizing bacteria, and orthodontics together provide suitable environments for the establishment of mixed-species biofilms [48]. Most organisms can only survive in the oropharynx when they stick to either the soft tissues or the hard surfaces. Otherwise, they may be removed by swallowing and chewing movements, nose blowing force, tongue movements and oral hygiene implements, the wash-out effect of the saliva, nasal and crevicular fluid outflow, and the active motion of the cilia of the nasal and sinus walls [49].

The oral cavity has three different sites, including mucosal surfaces, hard tissues, and exocrine gland tissue, all of which present unique characteristics for microbiota composition. The tongue, the gingiva, the buccal mucosa, and the palate are mucosal surfaces, while the teeth are hard tissues in the oral cavity [44]. Based on their anatomical location there are different oral mucosal surfaces. The oral mucosa surfaces, in general, can be divided into masticatory and non-masticatory mucosa. The attached gingiva around the teeth, the hard palate, and the upper surface of the tongue are the masticatory mucosa, which also known as keratinized stratified squamous epithelium. The taste buds of the lingual papillae are found on the upper surface of the tongue. The rest of the oral cavity including buccal and labial sites, as well as at the floor of the mouth are non-masticatory mucosa or stratified squamous nonkeratinized epithelium. Teeth are hard structures, which are in contact closely with mucosa in the oral cavity. There is no structure in the human body like the condition of the oral cavity. In the oral cavity there is also the gingival sulcus, which is located between the teeth and the mucosal gingiva, is an important anatomical site for the formation of dental plaque biofilm [44].

Saliva also has an important role in oral health. Saliva is excreted by the major and minor salivary glands. The main salivary gland openings are located at the floor of the mouth, the caruncles sublingual, while those in the buccal mucosa are called the Stensen's duct. About 1–2 L/day of saliva is naturally produced and swallowed. Saliva is fundamentally composed of water, electrolytes, mucus, antibacterial material, and enzymes that help to process food and kill bacteria. Saliva has very essential function in maintain oral health. The prevalence of oral diseases, such as dental caries, gingivitis, and periodontitis, increments fundamentally without saliva [44].

The total volume of oral microbial is around 1011 microbes/mL. The primary type of microbial found in the oral cavity is Streptococcus. The others are Leptotrichia, Porphyromonas, Veillonella, Prevotella, Haemophilus, Propionibacterium, Staphylococcus, and Treponema [44]. There is a symbiotic relationship among the microorganisms in the oral cavity to gain mutual benefits. The commensal populations are harmless and maintain a check on the pathogenic species by not allowing them to adhere to the mucosa. The bacteria become pathogenic only after they breach the barrier of the commensals, causing infection and disease [43].

Oral microbiome profiles from both healthy controls and HPV-negative oral cavity cancer (OCC) and oropharyngeal cancer (OPC) patients suggested that the presence of HPV affected the composition of the oral microbiome [46]. HPV-positive OCC and OPC patients both showed an abundance of Gemella and Leuconostoc, while Haemophilus correlated with HPV infection. The 16S rRNA sequencing on saliva and oral rinse samples of OCC and OPC patients showed a decrease in richness and diversity when compared to control patients. This decrease in diversity was opposite the case of cervical patients and indicated that a few dominating, pathogenic bacteria might have influence on HPV persistence and carcinogenesis in the oral environment [46]. Interestingly, Lactobacillus spp. were found to be significantly associated with the saliva samples from HPV-positive OPC patients [3, 50]. In a follow-up study, species-level context was provided for the Lactobacillus spp. using high-resolution 16S rRNA analysis. A subset of OPC patient samples were enriched with commensal species from the vaginal flora, including L. gasseri/johnsonii and *L. vaginalis*. This was not observed in control groups nor in the saliva from the Human Microbiome Project [51]. This suggested that these normally commensal vaginal species could have been transferred to the oral flora during oral sex, which, if validated, would have interesting implications in the role of vaginal-associated Lactobacillus in oral HPV disease.

3.2 Correlation between oral microbiome, chronic inflammation, and HPV infection

As part of the digestive tract, the oral cavity has diverse microorganisms and oral microbiota is a complex microbial community. The oral microbiota plays an important role in human health, and dysbiosis of oral microbiota can induce many kinds of local and systemic diseases [50]. In this dysbiosis of oral microbiota, the host's immune system will be stimulated by the inflammatory process. If this condition persists, the inflammation will become chronic [52]. Chronic inflammation occurs the most frequently in tooth supporting tissues [53]. The average prevalence of periodontitis in the general population is 30% [54].

Periodontitis is an advanced gingival disease induced by dysbiosis of bacterial and it can eventually result in tooth missing. It begins as gingival bleeding in response to inflammation, after that bacterial biofilm accumulates around the tooth cervical surfaces [44, 49]. The damage continues to spread to the periodontal tissue. There is a migration of the junctional epithelium toward the apical,

the gum groove becomes more than 3 mm deep, which is called the periodontal pocket. In the connective tissues, there is an increase in angiogenesis, chronic inflammatory infiltrates, fibrosis, loss of connective tissues, clinical attachment loss (CAL) and resorption of the alveolar bones/alveolar bone loss (ABL) [55].

Risk factors for oral and pharyngeal cancers are age, tobacco use, frequent use of alcohol, and exposure to sunlight. A higher incidence of cancer development is also found in individuals with chronic inflammatory conditions. An increased risk of developing oral squamous cell carcinoma (OSCC) associated with periodontitis suggests a possible role of inflammation caused by the microbiome with oral cancer. Periodontitis is a typical example of an infectious disease causing chronic inflammation in the oral cavity [56]. Recent evidence proved the role of microbiome-derived signals in the pathogenesis of several chronic inflammatory diseases. Periodontal diseases have been associated with the risk for precancerous lesions, tumors, and oral neoplasms. The third National Health and Nutrition Examination Survey (NHANES III) discovered that periodontitis was significantly related to HPV status in patients with oropharyngeal cancer [49].

Well-known periodontal pathogens, such as Tannerella forsythia, Porphyromonas gingivalis (P. gingivalis), and Treponema denticola, are not usually detected in the oral cavities of healthy human beings [44]. Oral bacteria could affect the outcome of viral infection. This is evident in the case of P. gingivalis, which upregulates expression of CCR5 [48]. P. gingivalis also causes the expression of the B7-H1 and B7-DC receptors in primary OSCC, which are upregulated in a variety of cancers and contribute to chronic inflammation [57].

Chronic infection with P. gingivalis and Fusobacterium nucleatum has been recently demonstrated to promote tongue tumors in a murine model through direct interaction with oral epithelial cells, leading to upregulation of the IL-6-STAT3 pathway in a TLR2-dependent manner [56]. P. gingivalis was also shown to cause gingival epithelial cells (GECs) to migrate in a manner which depends on the overexpression of Zeb1, an activator of the epithelial-mesenchymal transition (EMT). Moreover, P. gingivalis increases proliferation and promotes invasion and migration in an in vitro model of persistent infection. Furthermore, P. gingivalis infection hinders the activity of glycogen synthase kinase 3 (GSK3b), an important EMT regulator, in primary oral epithelial cells. In addition, other EMT-associated transcription factors, as well as mesenchymal intermediates, such as vimentin, MMP-2, MMP-7, and MMP-9, increase and are associated with higher levels of cell migration [58].

Expression of pro-inflammatory cytokines in periodontal disease such as IL-1 and TNF-a has been related to microbial triggered carcinogenesis [59]. In a study comparing the microbiome of gingival squamous cell carcinoma (GSCC) with periodontitis microbiome, members of the genera Fusobacterium, Peptostreptococcus, and Prevotella were found more abundant in cancerous, periodontal tissues. In contrast, saliva or soft mucosa concealed more periodontal health-related bacteria [60].

What shall we do to minimize diseases caused by the oral microbiota? The most obvious recommendation is to improve oral hygiene; however, people with sufficient oral hygiene can still develop chronic infections due to the composition of resident microbiota and changes in the host's immune response.

3.3 Transmission of oral HPV infection

Most HPV transmission is thought to occur as a result of microscopic mucosal erosion during sexual activity [61, 62]. HPV can cause latent infection in basal cells after mucosal epithelial surface erosion by low HPV DNA copy; transmission of infection can occur only when the number of the viruses is sufficient [63].

HPV can also cause subclinical infection that is active but asymptomatic; or clinical infection leading to benign, potentially malignant or malignant lesions [39, 63]. Most HPV infections are cleared by the immune system; the individual is not aware he or she has had the infection and does not develop visible lesions or cancer [64, 65].

Unlike many viruses, HPV requires the infected cells to divide and differentiate. The epidermis is composed of multiple keratinocyte layers, and is the component that papillomaviruses target [3]. HPV infection starts when the viruses enter epithelial basal cells which are referred to as the target cells of the virus [32]. HPV binds epithelial cell heparin sulfate proteoglycans and cell specific receptors to gain entry by both clathrin-dependent and -independent endocytosis [66]. Infection leads to the establishment of the HPV circular double-stranded genome as a stable episome within some cells of the basal layer [67]. In the case of alpha-HPV, the viral genome can integrate into the host genome, whereas for beta-HPV, the viral genome remains episomal [68].

After entering the host cell, HPV infection can manifest in two clinical circumstances: 1) Subclinical or invisible infection, i.e. the tacit presence of the viral genome to the inoculation site without clinical and/or histological and/or cytologic changes in the cervical mucosa; 2) clinical infections, expression of proliferation of infected keratinocytes and associated with clinical and histological lesions of the cervical mucosa [69–71]. These lesions are usually benign when the infection is sustained by LR-HPV [72]. Otherwise HR-HPV infection, especially when settled for more than 18–24 months and it is accompanied by the integration of viral DNA into eukaryotic DNA in basal cells, may be associated with malignant and potentially malignant development [73]. This latter form of infection is recognized as the cause of CSCC. Clinically, HPV infects basal cells of the skin's epithelium and mucous membranes [73]. Because HPV can affect the site of epithelial cells, infections are found in the oral mucosa, esophagus, larynx, trachea, conjunctiva as well as the genitals and rectum [74]. This phenomenon explains the increased frequency of HPV-related OSCC [29].

Oral HPV infection can be acquired by oral-genital contact, by mouth-to-mouth contact, or possibly by autoinoculation and in infants by mother-to-child transmission [35]. The natural history of HPV infection in the oral cavity and oropharynx is not entirely clear although there are some characteristics similar to those described for the cervix of the uterus [75]. Histological similarities between the service vaginal and oropharyngeal regions, both coated with squamous epithelium or slightly keratin, and the capacity of the virus to perpetuate human oral keratinocytes in vitro, make it possible to transfer the concept of HPV induction oncogenicity occurring in gynecology to the oral cavity [76]. Although the way HPV is transmitted in the oral cavity is still not fully known, epidemiological data shows that detection of HPV (i.e. HPV 16) in chipped cells in the mouth increases the risk more than 14 times that of oropharyngeal cancer (tonsils and base of the tongue) and 3.8 times the risk of oral cancer [77, 78]. Syrjänen et al.'s findings suggest that the oral mucosa is a reservoir of infection, the virus can easily pass through the oral cavity and sometimes remain at riskier sites, such as tonsil kriptus similar to cervical squamosa cell connections [18, 64, 79]. The target of viral infection can also be at sites where basal keratinocytes do not differentiate [80]. The results of the meta-analysis Kreimer et al. detected confirmed the presence of HPV in the oral mucosa and showed only 4.5% (95% CI: 3.9–5.1) of the 4070 positive subjects for HPV and 3.5% (95% CI: 3.0–4.1) of the 4441 subjects had HPV carcinogenic mucosa and concluded the oral mucosa was a reservoir of infection [81]. Dayakar, Shipilova and Gupta's research shows more precisely that the reservoir is located in the gingiva pocket [82].

The oral cavity is a significant reservoir for HPV infection that may not be entirely independent of the cervical reservoir [35]. Because the high discordance of infections may reflect differences in the risk factors for or natural history of infection at the two sites, it may not be entirely appropriate to extrapolate the vast literature on cervical HPV natural history to oral HPV infection [35, 83].

3.4 HPV and oral potentially malignant disorders (OPMDs)

A large number of oral cancers are preceded by visible clinical changes that occur in the oral mucosa in the form of chronic white or red patches [84]. Some lesions and this condition carry malignant potential and are listed as premalignant [85]. WHO (2005) recommends that the term lesions and oral pre-malignant conditions be replaced with the term OPMDs. Based on these recommendations, oral leukoplakia (OL), oral erythroplakia (OE), oral proliferative verrucous leukoplakia (PVL), oral submucosal fibrosis, oral lichen planus (OLP) and actinic cheilitis have been classified as OPMD [86, 87].

A subgroup of HPVs, The HR-HPVs, can cause precancerous lesions [19]. Recent investigations of significant HPV detection rates are recorded in several OPMDs. Studies have reported the prevalence rate of HPV's relationship with OPMD ranges from 0–85% [88]. The most common OPMDs are OL, PVL, OE, and OLP [86]. OL, the most common disorder among OPMD and therefore the most studied in the literature, current evidence suggests that OL shows an increased risk of HPV infection with respect to clinically healthy mucosa, with a prevalence of about 20%, without significant differences in clinical presentation [89]. OE is a rare OPMD characterized by a large neoplastic risk [90]. Due to its very low frequency, references to viral infections are very rare in the literature. The latest data published by Syrjänen et al. reported that of the 11 OE tested for HPV, 54.5% were found to be HPV positive 16 [90]. OLP is also associated with viral infections, with the frequency of infections ranging from 27 to 65% [90]. Some authors hypothesize the influence of erosive OLP in increasing the risk of HPV infection, although this hypothesis has not been confirmed by subsequent research [91]. In the review Syrjänen et al. prevalence of HPV infection in OLP was 5.12%, with genotype 16 most commonly involved [29].

In the context of maligna's transformation from OPMD, the potential role of HPV promoters is still debated. Szarka et al. reported an increase in HPV prevalence in OPMD with increased malignant potential: 32.8%, 40.9% and 47.7% in OLP, OL and OSCC [92].

4. Dental calculus: novel promising biosource for HPV-induced oral cancer study

The oral cavity is a place where various microorganisms live. On the surface of teeth, supra or subgingival, the biofilm of these microorganisms with the additional contribution of saliva and gingival crevicular fluids (GCF) can calcify into dental calculus [93]. This process starts with the formation of plaque. A thin layer (film) of salivary protein will adhere to the surface of the tooth. It is then called the acquired enamel pellicle (AEP). AEP is the main barrier between the enamel and bacteria and food acids. The next layer is a colony of microorganisms with a bacterial density of more than 200 million bacterial cells per milligram. Plaque is bound by a matrix of bacterial extracellular polymeric substances (EPSs), in which desquamated cells, oral microorganisms, food debris, microscopic particles, and biomolecules such as DNA, RNA and protein can be trapped [93–95]. Calcium phosphate ions from saliva and GCF can also affect this process [49, 96].

Calcium phosphate is the most dominant mineral in dental calculus. The calcification process occurs periodically, beginning from the layer closest to the teeth, so each layer has a different morphology and stoichiometric composition. Hydroxyapatite (HAP) is the layer that sticks to the surface of teeth. The next layers, from inside to the outside, are layers of whitlockite (TCP-b), octocalcium phosphate (OCP), and brushite (B) [93]. Irregular tooth surfaces, pits and fissures are also predisposing factors for the accumulation of dental calculus [93, 94].

Dental calculus can be found in all human populations, in the past and at present, especially in groups of people with poor oral hygiene. Clinically it can be observed easily, and it accumulates around the neck of the teeth, causing chronic inflammation of the periodontal tissue. This condition causes the formation of periodontal pockets. This hallmark periodontitis is an ideal reservoir for HPV [82, 97].

The target cells of HPV are cells in the basal layer because they have a high proliferation capacity, so they can support the replication of the virus [70, 98]. In the periodontal pocket, the basal layer is exposed to the outside environment [97]. The junctional epithelium in this area has a high proliferative capacity [70, 82, 97]. Virions bind initially to the glycosaminoglycan (GAG) chains of the heparan sulphate proteoglycan (HSPG) of epithelial cells [4]. This protein is expressed more in the healing process in the periodontal pocket [97, 99].

GCF is a very specific oral cavity fluid that represents periodontal health [99]. In several studies, this fluid has been used as a biological source of detection for the presence of HPV [97, 100]. HPV DNA is detected in advanced cases of periodontitis, but not detected in patients with gingivitis [101, 102]. There is a tendency that it is detected more often in women [100]. Women's specific factors, such as decreased levels of sex hormones, may increase the risk of HPV infection in the periodontal pocket [103].

HPV has several characteristics including selecting basal cells as its target, being latent, and its virions being released into the external environment together with desquamated cells [104]. Desquamated cells from the entire mouth will be carried in saliva, some of which will be precipitated into dental calculus. Saliva only shows the state of the moment [105]. Meanwhile, the part that precipitates into dental calculus will last if it is has not been cleaned, so the dental calculus is able to store data longer and to be the evidence of the presence of HPV in the past. This is consistent with the latent nature of the HPV infection.

The involvement of HPV in cancer will greatly influence the treatment plan and prognosis, so detection of its involvement is very important [4, 106]. Various studies have been carried out to develop the examination designs. The method of examination, the molecular targets, and the biological sources used were considered in those studies [11, 106, 107].

Various methods have been developed, ranging from observation of tissue morphology to visualization of molecular markers. In microscopic observation, pathognomonic koilocytotic cells have been observed [108]. The HPV-infected cells show perinuclear halo, enlarged cell nucleus, increased ratio of nucleus and cytoplasm, dysplasia, and minimal keratinization [106, 108]. Observation of these morphological changes really depends on the operator's carefulness [106]. Various stains are used, from the most conventional – hematoxylin & eosin, Papanicolaou and immunohistochemical staining (IHC) [106, 109]. The protein used in IHC can HPV origin, for example E6 and E7 or p16 from host [110]. IHC staining method is simple but the results are less consistent [106]. HPV infection is latent, it takes a long time for the tissue to show pathognomonic signs [8].

The initial step in early detection of HPV-induced cancer is to confirm the presence of the virus [111]. HPV cannot be cultured in vitro [112]. Molecular analysis is

developed to detect HPV even before tissue changes can be observed. The molecular targets include DNA, RNA, HPV proteins, and host antibodies [106]. The detection methods that can be used are nucleic acid-hybridization assays, signal amplification assays and nucleic-acid amplification [113]. Detection kits in various brands have been widely circulating in the market [112].

The biological source of samples also determines the detection of HPV. If the cancer is clinically visible, it is easier to determine the biological source. However, for early detection, the collection of the sample must be non-invasive, and the life cycle of HPV must be considered. This virus is epithelial tropism, infecting mainly cells in the basal layer. It is latent, and when it is mature, virions will exit the cell [4, 104].

Routine cytology examination for early detection of cervical cancer has become a health program in various countries [114, 115]. The oral cavity is very different from the cervix. The oral mucosa is very broad, with various anatomical landmarks more varied than those of the cervix. Several studies have used oral swabs, oral rinse, and saliva of non-oral cancer individuals [110, 116, 117]. These various biological sources represent only the condition of the oral cavity then, while HPV is latent [8]. No biological source has been acknowledged as the standard source in routine examinations of the oral cavity.

Dental calculus is formed from calcified plaque which can accumulate sub gingivally or supragingival [118]. During the maturation process, the dental calculus can trap organic material, for example cells with integrated viral genomes, DNA, RNA, proteins, molecules, and other biological data [93, 95]. This makes dental calculus a potential biological source for molecular examination of latent pathogens [95]. To our knowledge, for the first time, our study was able to detect the presence of latent HPV in the dental calculus of the periodontal pockets of patients with OSCC accompanied by chronic periodontitis. These results strongly suggest that dental calculus is a promising biological source for the detection of HPV in the oral cavity and can be used as a biomarker for early detection as shown in **Figure 1** [11].

DNA isolates from the dental calculus of OSCC patients were amplified with the universal primer MY09 /11. In visualization, 29% of the samples had a clear

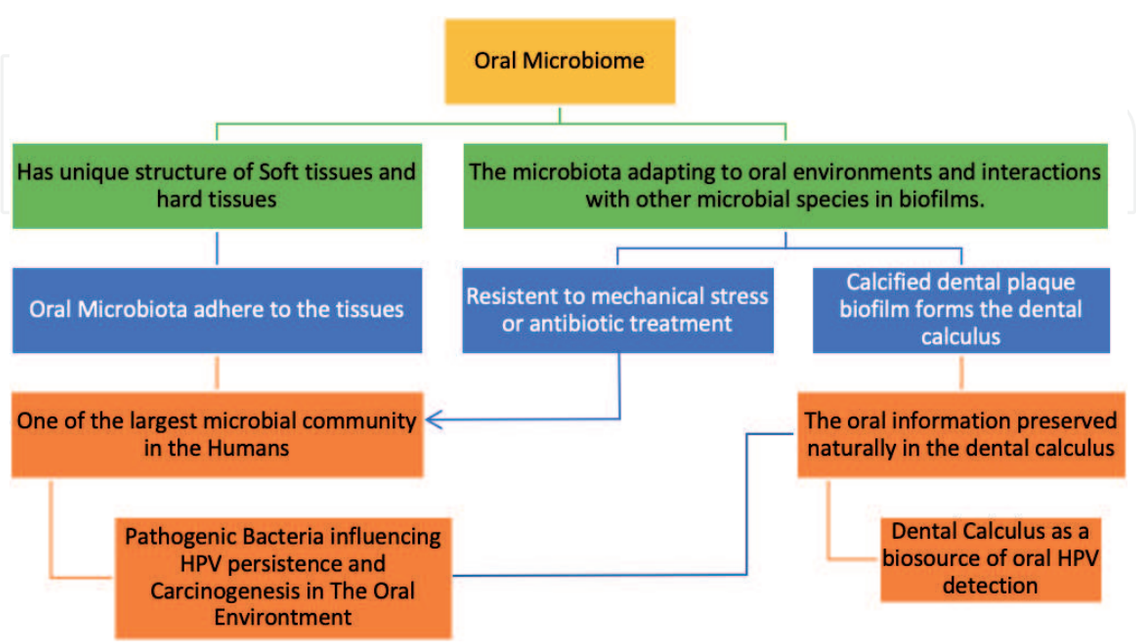

Figure 1.
Dental calculus as a potential biosource of HPV detection. This diagram shows the potential reasons why dental calculus will have an important role in the future of oral cancer study.

single band, at 450 bp. The Sanger method was performed to determine the DNA sequence, the sequence was compared with the data on GenBank using NCBI BLAST online on the website https://blast.ncbi.nlm.nih.gov [119]. HPV 58 was identified in 75% of the samples, while the rest was identified as unclassified HPV. Type 58 is included as high risk HPV and is the most common genotype found in cervical cancer after HPV 16 and 18 in East Asia and in Thailand [120, 121].

It is predicted that in 2035 there will be an increase in the global incidence of malignancies of the lips, oral cavity, and pharynx by about 62% [122]. It is also predicted that 95% of this malignancy is OSCC [123]. There have been only few studies about HPV genotype in the oral cavity, [124, 125]. so further studies need to be done to make sure this prediction will not come true. Interestingly, this study showed that the remaining positive samples were identified as unclassified papillomaviridae. Further research on unclassified HPV is still ongoing. This finding suggests the possibility of the presence of other papillomaviridae viruses that have not been identified.

The goal of any developing technology for HPV detection in clinical samples is to approach the gold standard for sensitivity and specificity while maximizing efficiency, simplicity, reproducibility, and transferability to the routine diagnostic laboratory. Our research is currently developing dental calculus as the standard of biological source for the detection of HPV in the oral cavity.

5. Conclusion

The oral cavity contains hundreds of different microorganisms that can associate to form biofilms. Biofilms are resistant to mechanical stress or antibiotic treatment. Oral cavity also has unique structure that cannot be found anywhere else in the human body. These conditions led oral cavity to become one of the largest microbial community in the humans. The oral plaque biofilm are calcified to dental calculus. During biomineral maturation process, several biological contents around the oral region should be trapped in dental calculus, including the exfoliated virus contained cells. Hence, dental calculus is a promising biosource of HPV and carcinogens molecules detection in the oral cavity.

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Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this chapter.

Author details

Sunardhi Widyaputra[1*], Natallia Pranata[2], Ignatius Setiawan[3] and Jamas Ari Anggraini[1]

1 Department of Oral Biology, Faculty of Dentistry, Universitas Padjadjaran, Bandung, Indonesia

2 Department of Oral Biology, Faculty of Dentistry, Maranatha Christian University, Bandung, Indonesia

3 Department of Dental Public Health, Faculty of Dentistry, Maranatha Christian University, Bandung, Indonesia

*Address all correspondence to: sunardhi.widyaputra@fkg.unpad.ac.id

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