Oral human papilloma virus infection: an overview of clinical-laboratory diagnosis and treatment

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Abstract. – OBJECTIVE: The aim of this review is to describe the "hot points" of current clinical governance for oral HPV comprising the use of new diagnostic molecular procedures, namely, Pyrosequencing and Next Generation Sequencing.

MATERIALS AND METHODS: The data on oral HPV was collected through two levels of research. First for all, we used the canonical medical search engines, PubMed, and Medline, followed by the study of current commercial tools for HPV diagnosis, particularly within commercial companies involved in the molecular procedures for HPV detecting and genotyping.

RESULTS: Different medical procedures are now described and used throughout the world in HPV diagnosis and treatment. However, the laboratory methods are often validated and used for genital infections, and, in these cases, data are missing in the literature as regards the clinical approach for oral lesions.

CONCLUSIONS: Dental care units are often the front line for a clinical evaluation of a possible HPV lesion in the oral cavity, which means that correct clinical governance could avoid a viral neoplastic progression of this disease with great advantages for the patient. In this case, the problem is due to the difficulty in lesion recognition but also and more especially the absence of correct laboratory diagnosis and subsequent treatment in the clinical course.

Key Words:
Human papilloma virus, Oral pathology, Oral infections, Laboratory tests, Clinical features.

Introduction

Human Papillomavirus (HPV) is a DNA virus belonging to the *Papillomaviridae* family that infects different animals, from birds and reptiles to mammals[1]. In humans, this virus is involved in the pathogenesis of different skin and mucosa lesions[2,3] and is responsible for the main sexually transmitted infection (STI) with 79 million affected people and 14 million new infections assessed every year in the USA, CDC report 2014. The prevalence of oral HPV infection varies among different studies, countries, and patient habits. For example in Hispanic men attending a clinic for sexually transmitted infections (STI), the prevalence was assessed at 20.0%[4], while in Scotland a study of male and female patients from different primary care and dental practice centres showed a prevalence of 5.5% for HPV infection[5]. HPV infection of the oral mucosa is mainly associated with oral sex or high-risk sexual behaviour, even though other means of transmission are possible, such as contamination from infected medical instruments or vertically from mother to child and also by autoinoculation[6]. Patient habits play a significant role in promoting the infection and in the subsequent neoplastic development in the infected tissues. Indeed, many studies have described how smoking and alcohol abuse have a significative synergic action with HPV, since they seem to modify tissue permeability to the virus and have

a negative influence on host immune response. It therefore appears that the host's alimentary habits play a role in virus progression in the oral cavity[7,8]. As far as epigenetic factors are concerned, recent publications have reported that the complex resident bacteria community, now known as oral microbiota, may have a possible role in HPV infection development and progression[9]. The purpose of this review is to provide a critical written account of the current state of knowledge of oral HPV infection, focalizing on clinical and laboratory approaches.

Structure of HPV Genome

HPV is a small non-enveloped dsDNA virus with a virion size of about ± 55 nm in diameter. The genome organization of the papilloma virus reflects the typical structure of alpha papillomaviruses[10]. It results as being a double-stranded DNA genome of about 8000, 7854 base pair long for type 18, GenBank accession No. KU298886. The viral gene expression pattern leads (i.e., in undifferentiated keratinocytes) six non-structural viral regulatory proteins and two structural viral capsid proteins (L1 and L2) (Figure 1).

E6 and E7 proteins represent viral oncogenes that induce host cell immortalization and transformations able to inactivate cell tumor suppressor proteins[11,12]. HPV viral strains can cause vari-

ous types of lesions in the oral mucosa, described as: (i) benign, (ii) premalignant, and (II) malignant lesions[13-15]. They are associated with the expression of proteins such as E1, E2, involved in viral replication[16] and E6, E7 which target the tumor suppressor proteins p53 and pRb[12]. These oncogenes interfere with the cell-cycle regulatory pathways inducing malignant cell proliferation[17]. In general, the L1 region (capsidic protein) is used in the genotyping methods for HPV[17-22]. More than 200 genotypes can be differentiated through the nucleotide sequence of the HPV L1 region. These are mainly divided into either low or high risk categories, according to their oncogenic potential[23].

Benign lesions are commonly related to low risk genotypes 2, 4, 6, 11, 13, 32:

- HPV-2 and HPV-4 are not sexually transmitted and related to oral verruca vulgaris[24];
- HPV-6 and HPV-11 cause the most frequent oral lesions, squamous papilloma, and *condiloma accuminata*[25,26];
- HPV-13 and HPV-32 are related to focal epithelial hyperplasia, known as "Heck disease"[27].

The genotypes related to malignant and premalignant lesions in the oral cavity are mainly HPV-16 and HPV-18. They are involved in the pathogen-
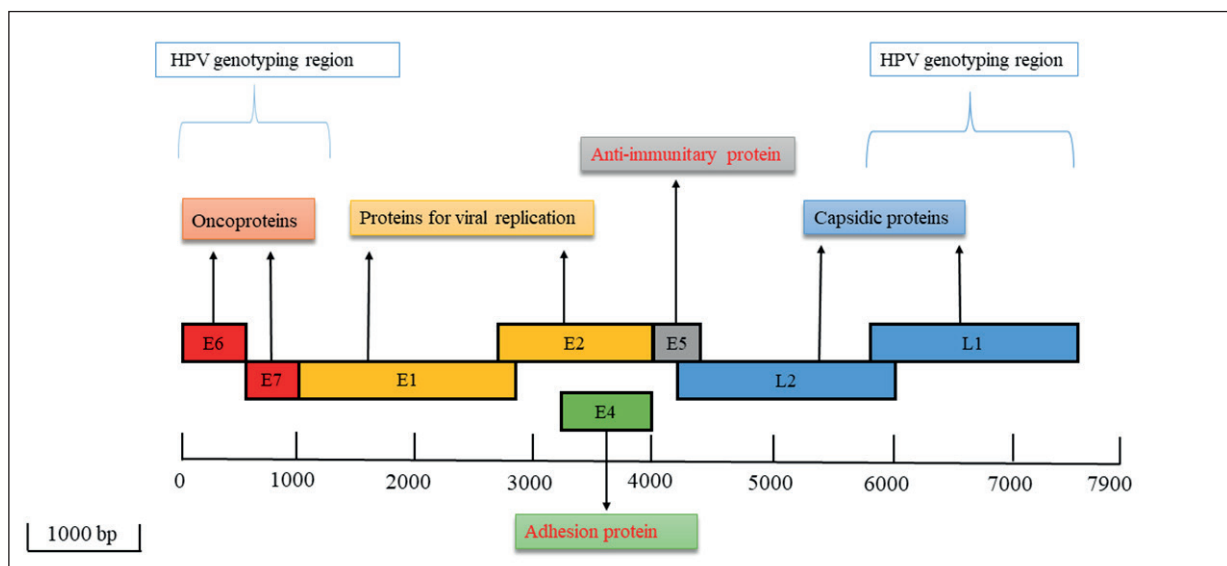
Figure 1. Schematic representation of the Human Papillomavirus genome. E6 and E7 are the oncogenic proteins that bind to p53 and pRb respectively; E1 and E2 involved in viral replication. More specifically, E1 is a helicase while E2 is a transactivator; E4 is expressed in later phases and are involved in the viral maturation and proliferation; E5 stops the apoptosis; L1 and L2 codify for capsid protein.

esis of more than 40.8% of premalignant lesions, such as oral erythroplakia, oral lichen planus, erythroleukoplakia, homogeneous leukoplakia, and nodular and verrucous leukoplakia[26,28]. HPV was found in 11% of malignant oral cancers (Oral squamous cell carcinoma/OSCC and Oral Verrucous Carcinoma/OVC) and in 47% of oropharingeal cancers[29,30]. HPV lesions are mainly found in sites exposed to microtraumas, such as the vermilion border, hard palate, labial mucosa, and labial commissures[31] (Figure 2), whilst the sites for tumors associated to HPV infection are localized in the oropharynx, most notably, at the base of the tongue and in the lingual/palatine tonsils[32].

The presence of an evident lesion may induce a diagnostic suspicion of the presence of HPV, but in the case of subclinical infection or in normal mucosa, no further investigations are carried out. At the moment, the diagnosis is based on bio-molecular methods but as far as the oral cavity is concerned, very different diagnostic tests are currently used to determine the presence of HPV in the mouth, and no studies have explored how oral HPV can effectively be prevented.

Discussion

Laboratory Diagnosis for HPV Oral Infection

Several methods for diagnoses are described in the literature and they include several sample and different sampling procedures: biopsies, mucosal scraping and mouthwashes, but biopsies being the most common procedures used for diagnostic purposes.

Nowadays, both medical and dental organizations feel that the best way to screen for HPV-related oral and oropharingeal cancer is through a visual and tactile exam and, therefore, screening for oral HPV is not recommended. Only after the discovery of evident lesions, the dentist or doctor performs a biopsy to see whether the lesions are cancerous, after which the biopsy samples are also probably tested for HPV[33,34]. The laboratory diagnosis appears crucial for a correct clinical governance of HPV infection in the oral cavity. In fact, for example, the long-term clinical pathway of a HPV initial lesion could be influenced by the viral genotype identified[35]. However, at least two different steps are fundamental in the laboratory pathway: (I) oral sample transport and maintenance (II) analytical laboratory procedures.

Pre-analytical Procedures (Sample Storage-Transport)

Many clinicians often neglect this point but if it is not correctly performed, it can influence the destiny of the entire laboratory diagnosis with a possible false negative result. Following different protocols described in the literature for cervical-vaginal samples, different possibilities exist for the correct recovery of DNA, depending on the presence or absence of a DNA storage solution. The first situation requires the rapid freezing of the sample (fresh tissue or swab) during its
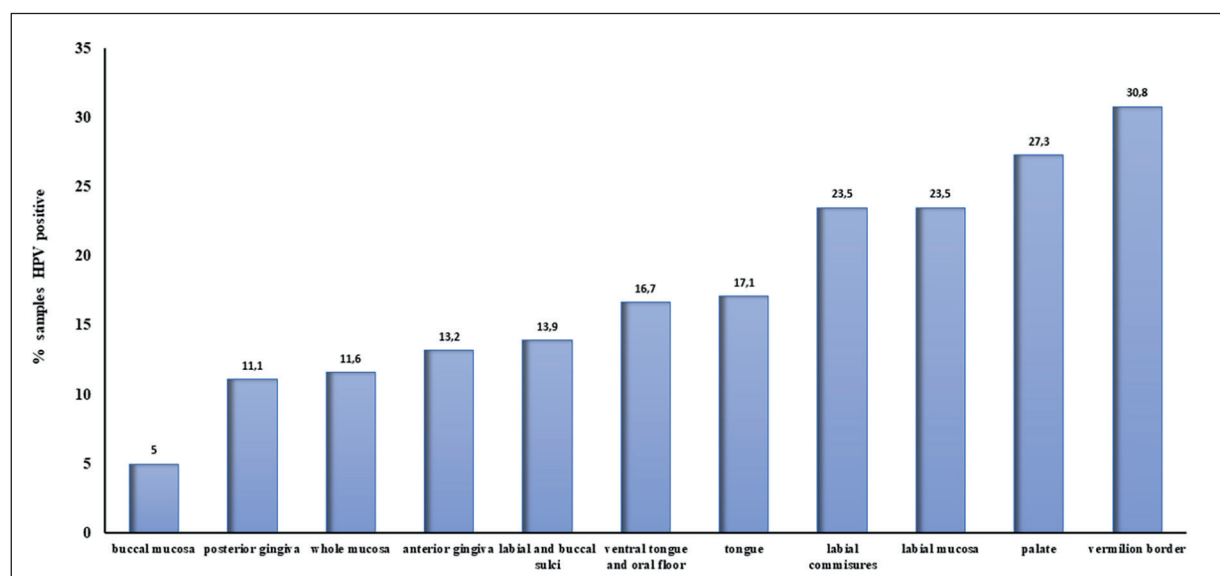

Figure 2. Percentage of lesions related to HPV in different oral sites[31].

storage, as well as during its transportation to the laboratory (max -20°C temperature, e.g., by dry ice). This is necessary to prevent any changes in pH variation and mucosal bacterial growth, with subsequent HPV-DNA degradation.

In the second option, the sample can be immersed in a tube containing a storage DNA solution and then the manufacturer's instructions are followed as far as storage temperature and transport are concerned, for example, many of these require room temperature or -20°C. The sample can be stored for several months[36-40].

Analytical Methods

Molecular tests are mandatory and new diagnostic tests are now starting to be used[41,42][25]. For example, in the US, a non-invasive salivary HPV test is now commercially available and, since the same types of HPV that infect the genital areas can also infect the mouth and throat, the tests designed for genital HPV infection have started to be also validated for oral lesions[43,44]. The majority of these tests are designed as Real-Time protocols, a few as Hybrid-capture non-PCR methods[45], while others use microarray or sequencing systems. Some are able to identify the HPV genotype at the DNA level by using different complementary primers for different HPV sequences, while others are designed to analyze E6/E7 RNA expression[46]. When a biopsy is available, P16 immunohistochemistry or *in situ* hybridization analysis may also be useful and new diagnostic strategies are under experimentation[47,48]. New molecular tests, such as ones designed using new generation sequencing, are also necessary in order to determine the impact of vaccination on oral HPV infections evidencing new genotypes[49,50].

Genetic Markers in Full-Blown Oral Cancer

Different oncogenes as well as some microRNAs (miR) are proposed as valid molecular markers in oral carcinoma prognosis. For example, the mutant-type p53 (TRP53) protein expression is involved in oral HPV cancer progression. In the physiological status, the wild type p53 protein prevents cancer formation, and thus it functions as a tumor suppressor, playing a role in conserving the stability by preventing the genome mutation. Some mutations belonging to the mutated P53 gene are associated with an increased risk of oral cancer[51,52].

Recently, microRNA family has been indicated as promising marker in the prognosis of tumor diseases. They comprise a class of small (21-40 bp) non-coding RNAs that have a role in various cell regulatory activities. An increase in cell expression of these molecules (e.g., miR-149 and miR-499) is associated with oral cancer differentiation and diffusion[53].

Treatment of HPV Lesions in Dental-Oral Care Units

Malignant neoplastic lesions often require a second level clinical approach involving a specific medical network including, for example, maxilla facial surgery, oncologists, and laboratory analysis. Moreover, apart from tumor abscission, the treatments could require appropriate chemotherapy and/or radiotherapy. In these cases, the laboratory course also results as being essential, i.e., the evaluation of the patient's clinical parameters or the oncogene mutation profile, but this description is beyond the scope of this review. In fact, the treatment of oral no-malignant lesions could be applicable in an oral-dental unit following precise lesion characterization by means of laboratory diagnosis, which includes procedures for lesion ablation using mechanical devices or lesion tissue destruction by physical approaches. Now, surgical scalpels, electrocautery, cryosurgery, laser therapy, and photodynamic therapy (PDT) could be used. The choice of method depends on different parameters, such as clinical features, operator skills, and patient compliance.

Cold Knife Surgical Excision

Surgical excision is the main treatment for oral lesions and consists in the complete removal of the latter[54]. It is very useful for diagnostic purposes because the blade can leave the lesion intact, thereby permitting the pathologist to formulate a proper diagnosis[55]. The main disadvantages of this technique are the need for anaesthesia, bleeding, and the formation of scars and, in the case of large lesions, there are also cosmetic implications and problems in-patient rehabilitation[56,57]. The frequency of recurrence of oral lesions is uncommon and more frequent in HIV patients in cases of incomplete removal or of infection of the epithelium surrounding the lesion or again in the case of multiple lesions[58-60]. The overall percentage of recurrence for benign lesions could be as high as 30%[61]. As far as premalignant lesions are concerned, surgical excision with a proper margin width may be resolutive; however, treating oral precancerous does not exclude the possibility

that an OSCC or an OVC may still develop[62,63]. The recurrence rate varies according to the type of premalignant lesion: in oral leukoplakia, after surgical excision, the average recurrence value is 30%[64]. In malignant lesions, the recurrence rate for OVC and for OSCC depends on the staging of the lesions and ranges from 0% to 68%[65] and from 64% to 84%, respectively[66].

Quantic Molecular Resonance (QMR)

A new possible method of surgical excision is the use of a quantic molecular resonance (QMR) scalpel[67]. QMR is a scalpel that produces a stream of quanta able to break the molecular bonds through a resonance effect without increasing kinetic energy and temperature, which remains lower than 45°C[67]. It has many advantages since it offers three working modalities (cut, coagulation, and cut-coagulation), high precision, a higher speed of incision than a cold knife, low intervention time, and a sample usable in the histological exam with minimal artefacts risk. The main disadvantage is the need for specific training, in fact, the movement and power of the device must be calibrated to avoid any thermal damage[67,68].

Laser Therapy

Lasers are a source of electromagnetic radiation emitted by different mediums that identify the type of laser: CO_2, Diode, Erbium: Yttrium/Aluminum/Garnet (Er:YAG), Neodymium: YAG (Nd:YAG) and Potassium-Titanyl-Phosphate (KTP). Of these different types, the CO_2 laser remains the most used and validated laser in oral surgery[69]. Compared to surgical excision with a scalpel, laser therapy is more accurate in the excision; there is no/minimal pain during surgery; healing occurs with minimal scar formation; there is a lower risk of disease transmission due to its sterilizing effect and the selective removal of affected epithelium and minimal damage to the surrounding tissue gives excellent healing[70,71]. Laser can be used for excision but also for vaporization of the lesions and, in cases of multiple and/or larger lesions, vaporization may be a better option avoiding tissue retraction, functional problems and postoperative pain[72]. Its use for biopsies remains controversial because the high temperature can burn and make the margins of the bioptic sample unreadable. However, Romeo et al[73] described a successful use of two types of lasers (KTP, diode laser) for biopsies, but a distinction could be made between non-suspicious lesions where lasers are fundamental for surgical reso-

lution and precancerous/malignant lesions where thermal damage may lead to a mis-diagnosis or under-diagnosis. In this case, they suggested enlarging the incision by 0.5 mm to ensure a definite histological diagnosis[73]. The main reported complications are post-operative pain, mental and lingual nerve paraesthesia, headaches, sialadenitis, granuloma formation, and bleeding[72]. In the case of oral benign lesions, lasers can be resolutive with a high success rate, 64% to 100%[74]. In cases of premalignant lesions, some authors have described a recurrence rate similar to other surgical treatments, namely 7.7-38.1% with possible malignant transformation in 0.13-17.5% of cases[69]. However, these data do not refer specifically to oral lesions caused by HPV and, to the best of our knowledge, any retrospective studies focusing specifically on oral HPV lesions have yet to be carried out. Recently, blue diode laser technology has been introduced in oral surgery[75]. This laser, whose wavelength is absorbed by melanin and haemoglobin chromophores, may perform better than other lasers because of the high quality cutting, low temperature increases, its increased antiseptic and photobiomodulation properties and the reduced risk of histological artefacts[75,76]. However, no studies about its use for HPV oral lesion currently exist.

Cryotherapy

Cryotherapy consists in the application of nitrogen oxide at very low temperatures to the lesion causing cell death. The temperature can vary according to the type of lesions and goes from -20°C to -30/-50°C (small cancers or aggressive cancers)[77]. Cryosurgery can be "open" with the application of nitrogen oxide to the lesion by means of a cotton swab or spray or "closed" with the application of a cryoprobe to the lesion[78]. Cryosurgery is indicated for the treatment of benign, premalignant and even malignant lesions and is used for palliative measures in intractable patients, for metastasis, and obstructive lesions[79]. Compared with simple surgical excision, it is bloodless, with no need for sutures and it is simple to use; the procedure is also quite quick (depending on the grade of malignancy and the dimension of the lesions). Due to these advantages, cryosurgery may be used for infants, anxious patients, and any subjects for whom other treatment is contraindicated[80]. The main disadvantages lie in the difficulty in judging the extent of the cryolesion, which may lead to recurrence; moreover, large lesions may need more cycles of treatment and extensive cryosurgery

procedures may produce considerable scars[81]. In case of malignant lesions, since cryosurgery does not involve tissue removal, a shave excision is also needed for complete regression of the lesions[82]. The complications of cryosurgery include pain, bullae and vesicles, headaches, post-surgical infection, fever and neuropathy[83]. The success rate for benign lesions depends on the practitioner and ranges from 47% to 92.5%[61] while for premalignant lesions, the recurrence rate is 13-25%[84].

Photodynamic Therapy

Photodynamic therapy (PDT) consists in an emission of a wavelength absorbed by a specific photosensitizing molecule, (5-aminoavulenic acid, temoporfin, benzoporphyrin derivates, tinethyletiopurpurin, talaporfin sodium)[85], administered to the target tissue causing the formation of reactive oxygen species and free radicals that damage cell structures[86]. The photosensitizing agent may be administered orally, intravenously or topically[85]. PDT has many advantages such as low costs, simple administration and low side effects. It represents a minimally invasive procedure, with none or a minimal presence of scars and has a low recurrence rate. It is also painless and can be used for large lesions. PDT may also be used in conjunction with other conventional therapy in the treatment of oral carcinoma[87]. There is a wide range of literature describing the response to therapy for premalignant lesions: complete response in 27-100%, partial response in 5-50% and no response in 0-25%. The recurrence rate is 0% to 36%. This probably depends on other factors, such as the thickness of the lesion, dysplasia grade, the type of lesion, and its extension[63,88-93]{Taibi, 2014 #66}.

Conclusions

More than 200 genotypes of HPV have been detected in human tissues. These are transmitted by skin or mucosa contact and some of these genotypes can cause oral warts. Persistent/silent infection with high-risk HPVs may progress to precancerous lesions and invasive cancer. Oral infection involves the transmission from infected to non-infected tissues in the same subject or between two different people. Different pathogenicity and host genetic-habit profiles can cause problems for a clinical evaluation, especially in the initial period of oral HPV infection. For this reason, this review has discussed the main aspects in a clinical approach for oral HPV infection and has given an ordered description of the procedures/methods already described in the literature. Our analysis has shown the inadequacy in the initial evaluation of infection in the oral mucosa, especially for the clinicians who most often are the first to meet the problem, namely, dentists. The initial laboratory diagnosis is crucial to manage the subsequent treatment and clinical follow-up in patient, for example, between high risk and low risk HPV in initial lesions, or oncogene profiles in full-blown oral cancer.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Links-Footnotes

HPV CDC report 2014: https://www.cdc.gov/mmwr/preview/mmwrhtml/rr6305a1.htm
Molecular HPV test: https://www.oraldna.com/oral-hpv-testing.htm

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