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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

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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 various 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-

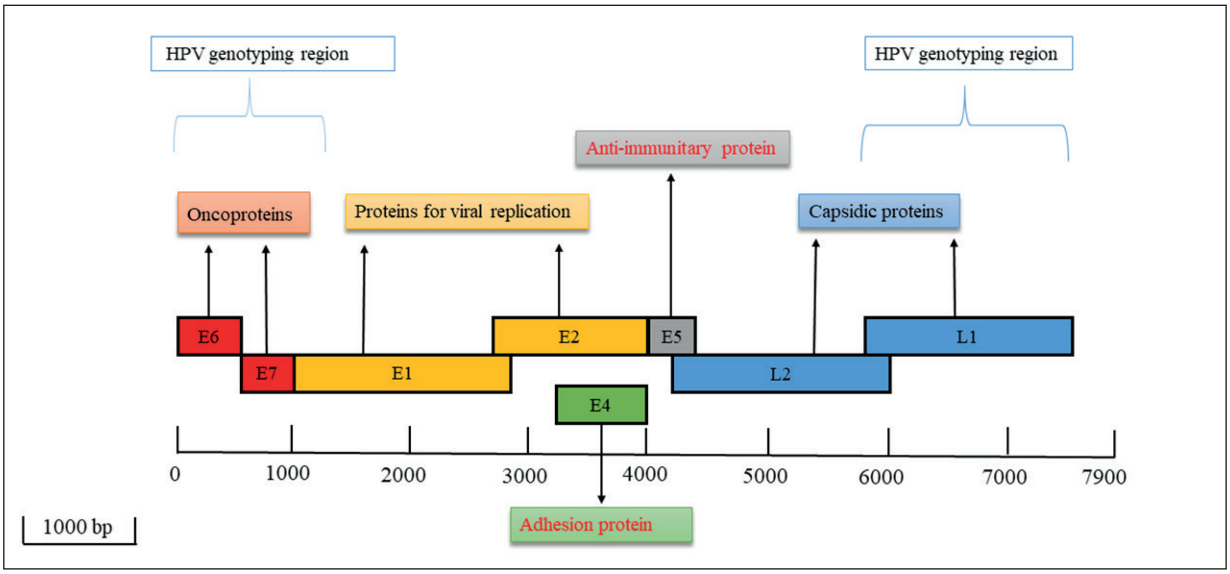

Figure 1. Schematic representation of the Human Papillomavirus genome. E6 and E7 are the oncogenic proteins that bind to p53 and pRb respectively; E1 and E2 involved in viral replication. More specifically, E1 is a helicase while E2 is a transactivator; E4 is expressed in later phases and are involved 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

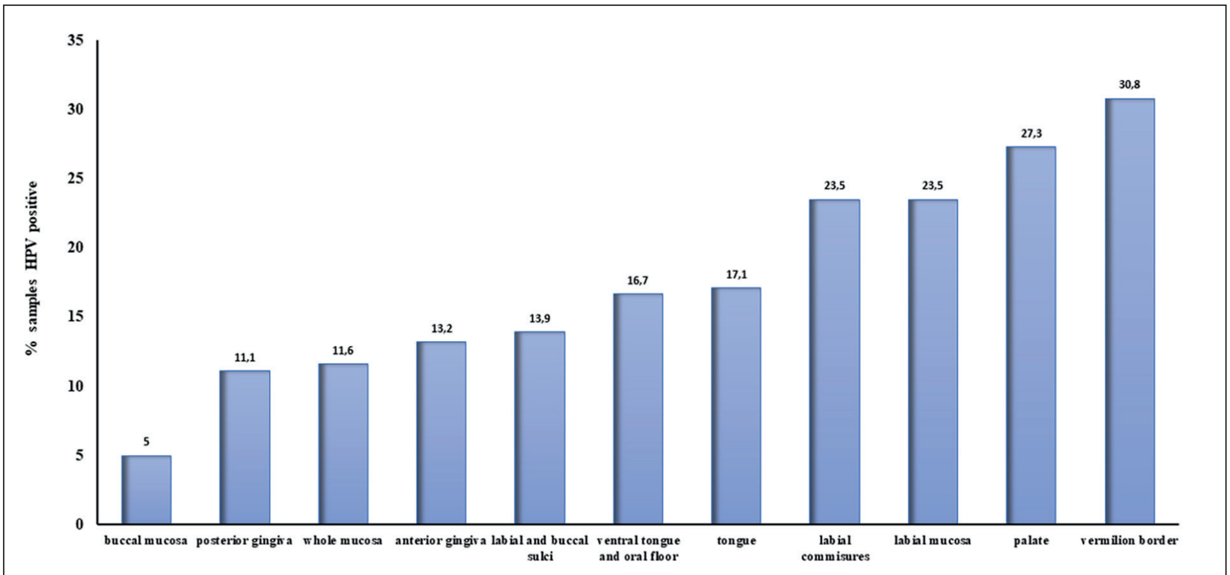

Figure 2. Percentage of lesions related to HPV in different oral sites[31].

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 resolution 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

References

1) Marschang RE. Viruses infecting reptiles. Viruses 2011; 3: 2087-2126.
2) Bharti AH, Chotaliya K, Marfatia YS. An update on oral human papillomavirus infection. Indian J Sex Transm Dis AIDS 2013; 34: 77-82.
3) Diaz ML. Human papilloma virus: prevention and treatment. Obstet Gynecol Clin North Am 2008; 35: 199-217, vii-viii.
4) Colon-López V, Quiñones-Avila V, Del Toro-Mejías LM, Reyes K, Rivera ME, Nieves K, Sánchez-Vazquez MM, Martínez-Ferrer M, Ortiz AP. Oral HPV infection in a clinic-based sample of Hispanic men. BMC Oral Health 2014; 14: 7.
5) Conway DI, Robertson C, Gray H, Young L, McDaid LM, Winter AJ, Campbell C, Pan J, Kavanagh K, Kean S, Bhatia R, Cubie H, Clarkson JE, Bagg J, Pollock KG, Cuschieri K. Human papilloma virus (HPV) oral prevalence in Scotland (HOPSCOTCH): a feasibility study in dental settings. PLoS One 2016; 11: e0165847.
6) Shah A, Malik A, Garg A, Mair M, Nair S, Chaturvedi P. Oral sex and human papilloma virus-related head and neck squamous cell cancer: a review of the literature. Postgrad Med J 2017; 93: 704-709.
7) Moktar A, Ravoori S, Vadhanam MV, Gairola CG, Gupta RC. Cigarette smoke-induced DNA damage and repair detected by the comet assay in HPV-transformed cervical cells. Int J Oncol 2009; 35: 1297-1304.
8) Kumar R, Rai AK, Das D, Das R, Kumar RS, Sarma A, Sharma S, Kataki AC, Ramteke A. Alcohol and tobacco

increases risk of high risk HPV infection in head and neck cancer patients: study from North-East region of India. PLoS One 2015; 10: e0140700.

9) Serrano-Villar S, Vasquez-Dominguez E, Perez-Molina JA, Sainz T, de Benito A, Latorre A, Moya A, Gosalbes MJ, Moreno S. HIV, HPV, and microbiota: partners in crime? AIDS 2017; 31: 591-594.

10) Doorbar J, Egawa N, Griffin H, Kranjec C, Murakami I. Human papillomavirus molecular biology and disease association. Rev Med Virol 2015; 25 Suppl 1: 2-23.

11) Popović B, Jekić B, Novaković I, Luković L, Konstantinović V, Babić M, Milasin J. Cancer genes alterations and HPV infection in oral squamous cell carcinoma. Int J Oral Maxillofac Surg 2010; 39: 909-915.

12) Badaracco G, Venuti A, Bartolazzi A, Morello R, Marzetti F, Marcante ML. Overexpression of p53 and bcl-2 proteins and the presence of HPV infection are independent events in head and neck cancer. J Oral Pathol Med 2000; 29: 173-179.

13) Chung CH, Bagheri A, D'Souza G. Epidemiology of oral human papillomavirus infection. Oral Oncol 2014; 50: 364-369.

14) Saghravanian N, Ghazi N, Meshkat Z, Mohtasham N. Human papillomavirus in oral leukoplakia, verrucous carcinoma, squamous cell carcinoma, and normal mucous membrane. Oman Med J 2015; 30: 455-460.

15) Syrjanen S, Lodi G, von Bultzingslowen I, Aliko A, Arduino P, Campisi G, Challacombe S, Ficarra G, Flaitz C, Zhou HM, Maeda H, Miller C, Jontell M. Human papillomaviruses in oral carcinoma and oral potentially malignant disorders: a systematic review. Oral Dis 2011; 17 Suppl 1: 58-72.

16) Terenzi F, Saikia P, Sen GC. Interferon-inducible protein, P56, inhibits HPV DNA replication by binding to the viral protein E1. EMBO J 2008; 27: 3311-3321.

17) Montaldo C, Mastinu A, Zorco S, Santini N, Pisano E, Piras V, Denotti G, Peluffo C, Erriu M, Garau V, Orrù G. Distribution of human papillomavirus genotypes in sardinian patients with oral squamous cell carcinoma. Open Virol J 2010; 4: 163-168.

18) Tsakogiannis D, Papacharalampous M, Toska E, Kyriakopoulou Z, Dimitriou TG, Ruether IG, Komiotis D, Markoulatos P. Duplex real-time PCR assay and SYBR green I melting curve analysis for molecular identification of HPV genotypes 16, 18, 31, 35, 51 and 66. Mol Cell Probes 2015; 29: 13-18.

19) Lee SH. Melting profiles may affect detection of residual HPV L1 gene DNA fragments in Gardasil®. Curr Med Chem 2014; 21: 932-940.

20) Kim G, Cho H, Lee D, Park S, Lee J, Wang HY, Kim S, Park KH, Lee H. Comparison of FFPE histological versus LBP cytological samples for HPV detection and typing in cervical cancer. Exp Mol Pathol 2017; 102: 321-326.

21) Martinelli M, Mazza F, Frati ER, Fasolo MM, Colzani D, Bianchi S, Fasoli E, Amendola A, Orlando G, Tanzi E. HPV genotypes detected in the oropharyngeal mucosa of HIV-infected men who have sex with men in Northern Italy. Epidemiol Infect 2016; 144: 2641-2647.

22) Pugliese DB, Bruzzesi G, Montaldo C, Porcu L, Landi M, Mastinu A, Torri V, Licitra L, Locati LD. Oral prevalence and clearance of oncogenic human papilloma virus in a rehabilitation community for substance abusers in Italy: a case of behavioral correction? J Oral Pathol Med 2015; 44: 728-733.

23) Munoz N, Bosch FX, de Sanjose S, Herrero R, Castellsague X, Shah KV, Snijders PJ, Meijer CJ, International Agency for Research on Cancer Multicenter Cervical Cancer Study Group. Epidemiologic classification of human papillomavirus types associated with cervical cancer. N Engl J Med 2003; 348: 518-527.

24) Nagaraj M. Verruca vulgaris of the tongue. J Maxillofac Oral Surg 2013; 12: 329-332.

25) Jaju PP, Suvarna PV, Desai RS. Squamous papilloma: case report and review of literature. Int J Oral Sci 2010; 2: 222-225.

26) Grce M, Mravak-Stipetic M. Human papillomavirus-associated diseases. Clin Dermatol 2014; 32: 253-258.

27) Said AK, Leao JC, Fedele S, Porter SR. Focal epithelial hyperplasia – an update. J Oral Pathol Med 2013; 42: 435-442.

28) Grce M. [Molecular diagnosis of oral infections]. Acta Med Croatica 2013; 67: 425-432.

29) Lübbe J, Kormann A, Adams V, Hassam S, Grätz KW, Panizzon RG, Burg G. HPV-11- and HPV-16-associated oral verrucous carcinoma. Dermatology 1996; 192: 217-221.

30) Villagómez-Ortíz VJ, Paz-Delgadillo DE, Marino-Martínez I, Ceseñas-Falcón LÁ, Sandoval-de la Fuente A, Reyes-Escobedo A. [Prevalence of human papillomavirus infection in squamous cell carcinoma of the oral cavity, oropharynx and larynx]. Cir Cir 2016; 84: 363-368.

31) Mravak-StipetiĐ M, Sabol I, KranjĐiĐ J, KneževiĐ M, Grce M. Human papillomavirus in the lesions of the oral mucosa according to topography. PLoS One 2013; 8: e69736.

32) Cleveland JL, Junger ML, Saraiya M, Markowitz LE, Dunne EF, Epstein JB. The connection between human papillomavirus and oropharyngeal squamous cell carcinomas in the United States: implications for dentistry. J Am Dent Assoc 2011; 142: 915-924.

33) Termine N, Giovannelli L, Rodolico V, Matranga D, Pannone G, Campisi G. Biopsy vs. brushing: comparison of two sampling methods for the detection of HPV-DNA in squamous cell carcinoma of the oral cavity. Oral Oncol 2012; 48: 870-875.

34) Termine N, Panzarella V, Falaschini S, Russo A, Matranga D, Lo Muzio L, Campisi G. HPV in oral squamous cell carcinoma vs head and neck squamous cell carcinoma biopsies: a meta-analysis (1988-2007). Ann Oncol 2008; 19: 1681-1690.

35) Hammer A, Mejlgaard E, Gravitt P, Hogdall E, Christiansen P, Steiniche T, Blaakaer J. HPV genotype distribution in older Danish women undergoing sur-

gery due to cervical cancer. Acta Obstet Gynecol Scand 2015; 94: 1262-1268.

36) Mazurek AM, Fiszer-Kierzkowska A, Rutkowski T, Skladowski K, Pierzyna M, Scieglinska D, Wozniak G, Glowacki G, Kawczynski R, Malusecka E. Optimization of circulating cell-free DNA recovery for KRAS mutation and HPV detection in plasma. Cancer Biomark 2013; 13: 385-394.

37) Dixon EP, Lenz KL, Doobay H, Brown CA, Malinowski DP, Fischer TJ. Recovery of DNA from BD SurePath cytology specimens and compatibility with the Roche AMPLICOR human papillomavirus (HPV) test. J Clin Virol 2010; 48: 31-35.

38) Campos EA, Simões JA, Rabelo-Santos SH, Sarian LO, Pitta DR, Levi JE, Derchain S. Recovery of DNA for the detection and genotyping of human papillomavirus from clinical cervical specimens stored for up to 2 years in a universal collection medium with denaturing reagent. J Virol Methods 2008; 147: 333-337.

39) Rabelo-Santos SH, Levi JE, Derchain SF, Sarian LO, Zeferino LC, Messias S, Moraes DL, Campos EA, Syrjanen KJ. DNA recovery from Hybrid Capture II samples stored in specimen transport medium with denaturing reagent, for the detection of human papillomavirus by PCR. J Virol Methods 2005; 126: 197-201.

40) Canfell K, Gray W, Snijders P, Murray C, Tipper S, Drinkwater K, Beral V. Factors predicting successful DNA recovery from archival cervical smear samples. Cytopathology 2004; 15: 276-282.

41) Burd EM. Human papillomavirus laboratory testing: the changing paradigm. Clin Microbiol Rev 2016; 29: 291-319.

42) Husain N, Neyaz A. Human papillomavirus associated head and neck squamous cell carcinoma: controversies and new concepts. J Oral Biol Craniofac Res 2017; 7: 198-205.

43) Kerr DA, Pitman MB, Sweeney B, Arpin RN 3rd, Wilbur DC, Faquin WC. Performance of the Roche cobas 4800 high-risk human papillomavirus test in cytologic preparations of squamous cell carcinoma of the head and neck. Cancer Cytopathol 2014; 122: 167-174.

44) Kerr DA, Sweeney B, Arpin RN 3rd, Ring M, Pitman MB, Wilbur DC, Faquin WC. Automated extraction of formalin-fixed, paraffin-embedded tissue for high-risk human papillomavirus testing of head and neck squamous cell carcinomas using the Roche Cobas 4800 system. Arch Pathol Lab Med 2016; 140: 844-848.

45) Chaudhary AK, Pandya S, Mehrotra R, Bharti AC, Singh M, Singh M. Comparative study between the Hybrid Capture II test and PCR based assay for the detection of human papillomavirus DNA in oral submucous fibrosis and oral squamous cell carcinoma. Virol J 2010; 7: 253.

46) Ukpo OC, Flanagan JJ, Ma XJ, Luo Y, Thorstad WL, Lewis JS Jr. High-risk human papillomavirus E6/E7 mRNA detection by a novel in situ hybridization assay strongly correlates with p16 expression and patient outcomes in oropharyngeal squamous cell carcinoma. Am J Surg Pathol 2011; 35: 1343-1350.

47) Kelesidis T, Aish L, Steller MA, Aish IS, Shen J, Foukas P, Panayiotides J, Petrikkos G, Karakitsos P, Tsiodras S. Human papillomavirus (HPV) detection using in situ hybridization in histologic samples: correlations with cytologic changes and polymerase chain reaction HPV detection. Am J Clin Pathol 2011; 136: 119-127.

48) Jordan RC, Lingen MW, Perez-Ordonez B, He X, Pickard R, Koluder M, Jiang B, Wakely P, Xiao W, Gillison ML. Validation of methods for oropharyngeal cancer HPV status determination in US cooperative group trials. Am J Surg Pathol 2012; 36: 945-954.

49) Chandrani P, Kulkarni V, Iyer P, Upadhyay P, Chaubal R, Das P, Mulherkar R, Singh R, Dutt A. NGS-based approach to determine the presence of HPV and their sites of integration in human cancer genome. Br J Cancer 2015; 112: 1958-1965.

50) Tardif KD, Simmon KE, Kommedal O, Pyne MT, Schlaberg R. Sequencing-based genotyping of mixed human papillomavirus infections by use of RipSeq software. J Clin Microbiol 2013; 51: 1278-1280.

51) Singh V, Husain N, Akhtar N, Khan MY, Sonkar AA, Kumar V. p16 and p53 in HPV-positive versus HPV-negative oral squamous cell carcinoma: do pathways differ? J Oral Pathol Med 2017; 46: 744-751.

52) Wang Z, Sturgis EM, Zhang Y, Huang Z, Zhou Q, Wei Q, Li G. Combined p53-related genetic variants together with HPV infection increase oral cancer risk. Int J Cancer 2012; 131: E251-E258.

53) Tandon D, Dewangan J, Srivastava S, Garg VK, Rath SK. miRNA genetic variants: as potential diagnostic biomarkers for oral cancer. Pathol Res Pract 2018; 214: 281-289.

54) Erkek E, Basar H, Bozdogan O, Emeksiz MC. Giant condyloma acuminata of Buschke-Lowenstein: successful treatment with a combination of surgical excision, oral acitretin and topical imiquimod. Clin Exp Dermatol 2009; 34: 366-368.

55) Shira RB. Surgical treatment of benign soft tissue lesions of the oral cavity. J Am Dent Assoc 1958; 57: 1-17.

56) Pandey M, Thomas G, Somanathan T, Sankaranarayanan R, Abraham EK, Jacob BJ, Mathew B, Trivandrum Oral Cancer Screening Study Group. Evaluation of surgical excision of non-homogeneous oral leukoplakia in a screening intervention trial, Kerala, India. Oral Oncol 2001; 37: 103-109.

57) Sadasivan A, Thankappan K, Rajapurkar M, Shetty S, Sreehari S, Iyer S. Verrucous lesions of the oral cavity treated with surgery: analysis of clinico-pathologic features and outcome. Contemp Clin Dent 2012; 3: 60-63.

58) Babaji P, Singh V, Chaurasia VR, Masamatti VS, Sharma AM. Squamous papilloma of the hard palate. Indian J Dent 2014; 5: 211-213.

59) Pringle GA. The role of human papillomavirus in oral disease. Dent Clin North Am 2014; 58: 385-399.

60) Carneiro TE, Marinho SA, Verli FD, Mesquita AT, Lima NL, Miranda JL. Oral squamous papilloma: clinical, histologic and immunohistochemical analyses. J Oral Sci 2009; 51: 367-372.

61) Lipke MM. An armamentarium of wart treatments. Clin Med Res 2006; 4: 273-293.

62) Lodi G, Franchini R, Warnakulasuriya S, Varoni EM, Sardella A, Kerr AR, Carrassi A, MacDonald LC, Worthington HV. Interventions for treating oral leukoplakia to prevent oral cancer. Cochrane Database Syst Rev 2016; 7: CD001829.

63) Villa A, Woo SB. Leukoplakia-A diagnostic and management algorithm. J Oral Maxillofac Surg 2017; 75: 723-734.

64) Feller L, Lemmer J. Oral leukoplakia as it relates to HPV infection: a review. Int J Dent 2012; 2012: 540561.

65) Peng Q, Wang Y, Quan H, Li Y, Tang Z. Oral verrucous carcinoma: from multifactorial etiology to diverse treatment regimens (review). Int J Oncol 2016; 49: 59-73.

66) Ajila V, Shetty H, Babu S, Shetty V, Hegde S. Human papilloma virus associated squamous cell carcinoma of the head and neck. J Sex Transm Dis 2015; 2015: 791024.

67) Vescovi P, Manfredi M, Merigo E, Fornaini C, Rocca JP, Nammour S, Bonanini M. Quantic molecular resonance scalpel and its potential applications in oral surgery. Br J Oral Maxillofac Surg 2008; 46: 355-357.

68) Ilaria G, Marco M, Elisabetta M, Giovanni M, Carlo F, Maddalena M, Mauro B, Paolo V. Advantages of new technologies in oral mucosal surgery: an intraoperative comparison among Nd:YAG laser, quantic molecular resonance scalpel, and cold blade. Lasers Med Sci 2015; 30: 1903-1910.

69) Neukam FW, Stelze F. Laser tumor treatment in oral and maxillofacial surgery. Physics Procedia 2010; 5: 91-100.

70) Rakhewar PS, Patil HP, Thorat M. Diode laser treatment of an oral squamous papilloma of soft palate. J Dent Lasers 2015; 9: 114-117.

71) van der Hem PS, Nauta JM, van der Wal JE, Roodenburg JLN. The results of CO2 laser surgery in patients with oral leukoplakia: a 25 year follow up. Oral Oncol 2005; 41: 31-37.

72) Mogedas-Vegara A, Hueto-Madrid JA, Chimenos-Küstner E, Bescós-Atín C. Oral leukoplakia treatment with the carbon dioxide laser: a systematic review of the literature. J Craniomaxillofac Surg 2016; 44: 331-336.

73) Romeo U, Palaia G, Del Vecchio A, Tenore G, Gambarini G, Gutknecht N, De Luca M. Effects of KTP laser on oral soft tissues. An in vitro study. Lasers Med Sci 2010; 25: 539-543.

74) Sterling JC, Handfield-Jones S, Hudson PM; British Association of Dermatologists. Guidelines for the management of cutaneous warts. Br J Dermatol 2001; 144: 4-11.

75) Fornaini C, Merigo E, Rocca JP, Lagori G, Raybaud H, Selleri S, Cucinotta A. 450 nm Blue Laser and Oral Surgery: preliminary ex vivo study. J Contemp Dent Pract 2016; 17: 795-800.

76) Gobbo M, Bussani R, Perinetti G, Rupel K, Bevilaqua L, Ottaviani G, Biasotto M. Blue diode laser versus traditional infrared diode laser and quantic molecular resonance scalpel: clinical and histological findings after excisional biopsy of benign oral lesions. J Biomed Opt 2017; 22: 121602.

77) Farah CS, Savage NW. Cryotherapy for treatment of oral lesions. Aust Dent J 2006; 51: 2-5.

78) Lin HP, Chen HM, Cheng SJ, Yu CH, Chiang CP. Cryogun cryotherapy for oral leukoplakia. Head Neck 2012; 34: 1306-1311.

79) Yu CH, Lin HP, Cheng SJ, Sun A, Chen HM. Cryotherapy for oral precancers and cancers. J Formos Med Assoc 2014; 113: 272-277.

80) Rezende KM, Moraes PdE C, Oliveira LB, Thomaz LA, Junqueira JL, Bonecker M. Cryosurgery as an effective alternative for treatment of oral lesions in children. Braz Dent J 2014; 25: 352-356.

81) Bansal A, Jain S, Gupta S. Cryosurgery in the treatment of oro-facial lesions. Indian J Dent Res 2012; 23: 297.

82) Yeh CJ. Treatment of verrucous hyperplasia and verrucous carcinoma by shave excision and simple cryosurgery. Int J Oral Maxillofac Surg 2003; 32: 280-283.

83) Ishida CE, Ramos-e-Silva M. Cryosurgery in oral lesions. Int J Dermatol 1998; 37: 283-285.

84) Tambuwala A, Sangle A, Khan A, Sayed A. Excision of oral leukoplakia by CO2 lasers versus traditional scalpel: a comparative study. J Maxillofac Oral Surg 2014; 13: 320-327.

85) Konopka K, Goslinski T. Photodynamic therapy in dentistry. J Dent Res 2007; 86: 694-707.

86) Garcia-Zuazaga J, Cooper KD, Baron ED. Photodynamic therapy in dermatology: current concepts in the treatment of skin cancer. Expert Rev Anticancer Ther 2005; 5: 791-800.

87) Jerjes W, Hamdoon Z, Hopper C. Photodynamic therapy in the management of potentially malignant and malignant oral disorders. Head Neck Oncol 2012; 4: 16.

88) Vohra F, Al-Kheraif AA, Qadri T, Hassan MI, Ahmed A, Warnakulasuriya S, Javed F. Efficacy of photodynamic therapy in the management of oral premalignant lesions. A systematic review. Photodiagnosis Photodyn Ther 2015; 12: 150-159.

89) Taibi R, Lleshi A, Barzan L, Fiorica F, Leghissa M, Vaccher E, De Paoli P, Franchin G, Berretta M, Tirelli U. Head and neck cancer survivors patients and late effects related to oncologic treatment: update of literature. Eur Rev Med Pharmacol Sci 2014; 18: 1473-1481.

90) Gloghini AVCC, Gualeni AV, Cortellazzi B, Perrone F, Pilotti S. Defining the better algorithm for the accurate identification of HPV status among oropharyngeal squamous-cell carcinoma. Results from a pilot study. WCRJ 2015; 2: e497.

91) Perri F, Cavaliere C, Russo F, Pisconti S, Facchini G, Taibi R, Della Vittoria Scarpati G. Role of traslational research in squamous cell carcinoma of the head and neck: is it possible to improve the therapeutic scenario? WCRJ 2015; 2: e520.

92) D'Andrea F, Ceccarelli M, Venanzi Rullo E, Facciolà A, Marino A, Cacopardo B, Pellicanò GF, Nunnari G. Vaccines against HPV in people living with HIV: a review. WCRJ 2019; 6: e1348.

93) Visalli G, Facciolà A, D'Aleo F, Pinzone MR, Condorelli F, Picerno I, Nunnari G, Pellicanò GF, Ceccarelli M, E. VR. HPV and urinary bladder carcinoma: a review of the literature. WCRJ 2018; 5: e1038.