HPV INFECTION IN THE ORAL CAVITY: EPIDEMIOLOGY, CLINICAL MANIFESTATIONS AND RELATIONSHIP WITH ORAL CANCER

V. CANDOTTO[1], D. LAURITANO[2], M. NARDONE[3], L. BAGGI[4], C. ARCURI[4], R. GATTO[5], R. M. GAUDIO[6], F. SPADARI[7], F. CARINCI[1]

[1] *Department of Morphology, Surgery and Experimental Medicine, University of Ferrara, Ferrara, Italy*
[2] *Department of Medicine and Surgery, University of Milan-Bicocca, Milan, Italy*
[3] *Ministry of Public Health, Rome, Italy*
[4] *Department of Clinical Sciences and Translational Medicine, University of Rome "Tor Vergata", Rome, Italy*
[5] *Department of Life, Health and Environmental Sciences, School of Dentistry, University of L'Aquila, L'Aquila, Italy*
[6] *Department of Medical Sciences, University of Ferrara, Ferrara, Italy*
[7] *Department of Biomedical, Surgical and Dental Sciences, University of Milan, "Ospedale Maggiore Policlinico Ca' Granda" Foundation, Unit of Oral Pathology and Medicine, Milan, Italy*

SUMMARY

Purpose. The aim of this paper is to describe epidemiology and clinical manifestations of oral infection by Human papillomavirus (HPV), with particular attention to potential malignant lesions.

Materials and methods. A systematic review of the literature was conducted to describe the state of the art about HPV infection in oropharyngeal mucosa and its modalities of transmission, with particular attention to possible simultaneous infection in multiple anatomic sites. The aspects of prevention and control of infection by administering vaccines and the diffusion of sexual education campaigns are discussed also. Therapeutic protocols are also described where necessary.

Results. In recent years there has been a growing interest in HPV oral medicine, suggesting a role of such a family of viruses in the development of neoplasms of the oropharyngeal district as well as of the uterine cervix. Even if the mass media have increasingly faced the problem, causing frequent alarming among patients, the dentist therefore needs a complete and up-to-date knowledge of this infectious condition that is one of the most common causes of sexually transmitted mucous membrane infections (e.g. genital, anal and oral).

Conclusions. Recent studies about HPV infection are a basic requirement in order to promote the health of patients and provide them with the most exhaustive indications from dentists.

Key words: human papillomavirus, oropharynx carcinoma, sexual transmission, oral infections, anti-HPV vaccines, HPV test.

Introduction

Human Papillomavirus (HPV) infection is considered the most common sexually transmitted infection (STI) (1). About 6 million people are diagnosed each year and approximately 9.0-13.0% of the world population (630 million people) is already infected with the disease (2). HPV plays a role in the pathogenesis of Head and Neck Squamous Cell Carcinomas (HNSCCs) and Oropharyngeal Squamous Cell Carcinomas (OPSCCs) (3, 4).

The etiologic role of human papillomavirus infection in the development of Squamous Cell Carcinomas (SCC) of the uterine cervix has been widely demonstrated, so that this tumour is caused, in almost of the cases, by the persistent infectious status by high-risk atypical genotypes. There is also strong evidence to support a causal role of these viruses in the etiopathogenesis of SCC, both in the anogenital area and in the head area (for example, pharynx, larynx and oral cavity) (5). Genital HPV infection is one of

the most commonly sexually transmitted infections (STIs) in the world population: in the United States, it was estimated that the prevalence of infection in women aged 19 to 54 years is 40% and is strongly associated with risk factors related to sexual behaviour (6); in Europe, the prevalence of high-risk HPV cervical cancer infection is 3-15% in women between the ages of 20 and 60, but is significantly higher (29-45%) in young women (20-24 years) (7).

Due to changes in sexual habits in the general population in recent decades (e.g. reduction in the age of onset of sexual activity, increase in number of partners, orogenital sexual habits), the epidemiological characteristics of HPV infections in both the genital and oropharynx districts, with a greater exposure to infection of patients at early age (8), make HPV an endemic infection.

This phenomenon would explain the increase in HPV-related SCC frequency in target districts. In particular, it was found that the detection of HPV 16 in the epithelial cells of tonsillary crypts and the lingual base is significantly associated with the presence of SCC (the increased risk of infection is 13 times more in subjects with carcinoma than healthy controls) (9). In the oral cavity, the risk of HPV infection in SCCs would be lower, though it was almost quadrupled compared to patients with healthy mucous membranes (10). The knowledge of the natural history of HPV infection, risk factors, clinical manifestations, and current preventive and therapeutic strategies, constitutes an indispensable prerequisite for the professional upgrade of the dentist, which is increasingly involved in the management of the patient at risk of infection or with clinical manifestations of the infection.

This review intends to describe the natural history of HPV infection in the oral and oropharyngeal mucosa, the transmission modalities, with particular attention to possible simultaneous infection in multiple anatomic sites (e.g. anogenital mucosa and oropharynx), the clinical manifestations and current issues in prevention and therapy.

The way of transmission and response of the host immune system

Mature viruses, excised from the surface layers of the cell by desquamation, are transmitted mainly for direct horizontal contact (e.g. sexual intercourse with genital and/or oral contact with the mucous membranes of an infected subject) or indirect (e.g. by medical instrument, contaminated utensils). In both cases the transmission occurs in the presence of epithelium microlesions that favour the entry of the virus into the receptive basal cells. The virus can also be transmitted vertically by maternal-fetal way (e.g., prenatal transplacental, during delivery or postnatal).

HPV anogenital infections primarily recognize an infected partner's sexual transmission. The risk of infection is closely related to sexual activity and is increased by the principal factors in the STIs (e.g. a high number of sexual partners, early onset of sexual activity). The use of the condom does not seem to adequately preserve from exposure to the virus, as it can be transmitted through contact with non-preserved infected sites (11).

A systematic review of worldwide literature conducted by Kreimer et al. (12) in 2005 reported that HPV DNA was detected in 35.6% of oropharyngeal cancers, with HPV type 16 accounting for a vast majority (87%) of HPV-positive cases. In addition to investigating the presence of HPV DNA, several studies have evaluated HPV functionality in oropharyngeal tumours (1). These studies show specificity of HPV to tumor cell nuclei (2), integration of HPV DNA into the human genome (3,13), high HPV viral copy numbers, and high level expression of the HPV oncogenes (E6 and E7) in tumours (14), all of which underscore a causal association of HPV with oropharyngeal cancers. Several analytic epidemiologic studies have evaluated the association of HPV with risk of oropharyngeal cancers predominantly through case-control study designs (15).

Oropharynx transmission is still unclear. It is plausible that orogenital sexual practices may involve the spread of the virus from a mucosal area to the other (16, 17), while the possibility of autoinoculation, rather frequent in children, is very few investigated (18).
Immune response plays a very important role in HPV infection; it is well documented, for example, that in immunocompetent subjects, skin warts often regress spontaneously, while immunodeficiency states, either congenital or acquired (e.g. transplanted or AIDS patients), favour the development and persistence of HPV-induced lesions. However, compared to other viral infections, the antibody and cellular immune response to HPV is generally not very intense, as the viral replication cycle occurs at intracellular level, without cytolysis, therefore the presentation of viral antigens to the immune system is minimal and the infection can persist for months or even years.

Diagnostic procedures

HPV may be associated with lesions only in case of a productive infection, so the objective examination is not particularly useful for diagnosis. Other diagnostic methods used in the past (e.g. histopathology in optical or electronic microscopy, immunocytochemistry and serology) have been abandoned because they are not sensitive and specific. Histopathological analysis can only suggest HPV infection in the presence of tissue alterations such as dyskeratosis, acanthosis, basal hyperplasia, and in particular coilocytosis, or the presence of cellular elements called coilocytes, characterized by globular form, large clear cytoplasm, picnotic nucleus surrounded by a clear vacuolization area. This finding is pathognomonic of HPV infection, however the sensitivity of detecting these alterations is low and very operator-dependent. Viral particles can be highlighted in the nuclei of infected epithelial cells by electronic microscopy, but the method is limited only to productive infections. Immunohistochemistry investigations are not very useful, as they aim at the identification of capsid proteins expressed in the late stage of HPV life cycle. The only immunohistochemical marker with recognized diagnostic significance is the p16 (INK4) protein, whose hyperexpression in oral or cervical displastic/neoplastic histologic samples is strongly associated with High Risk-HPV (HR-HPV) infection, although it is not possible to exclude the simultaneous presence of other genotypes (19).
Serologic tests are dubious in the diagnosis of active or past infection. Serumconversion, when performed, is followed by several months (on average 8-12 months) of viral DNA identification through HPV testing, and antibody response is not consistent throughout the individuals, sometimes falling within a short period of time, sometimes persisting despite the negativity of molecular tests (20). At present, the diagnosis of infection is based on nuclear biology techniques, aimed at detecting the virus DNA within the cells, and the most sensitive and most suitable biomolecular method to identify and to make the genome of HPV is the PCR (Polimerase Chain Reaction: Polymerase Chain Reaction). PCR is a technique that allows the amplification of *in vitro* DNA, starting from a specific fragment, an unlimited number of DNA molecules identical to the starting fragment; the advantage of the method is that it can also be used to test biological samples containing extremely small amounts of nucleic acids. Recently, the SPF10-LiPA system has been introduced, enabling successive PCR method amplification and genotype identification. Studies conducted so far reveal many advantages over other techniques, such as high sensitivity and specificity, ease to use, and finally the ability to detect multiple and persistent infections. However, as opposed to what happens in gynaecology, in oral medicine, standard diagnostic protocols have not yet been established, either in terms of the type of sample (e.g. histological samples *vs* exfoliated cells), method of cytological sampling (e.g. scraping by spatula or brush *vs* oral rinses) and preservation of sample (e.g. fresh frozen *vs* fixed in formalin

and paraffin). A diagnostic approach recommended as a screening method in subjects with clinically normal mucosa is, in current state of knowledge, cytological sample by mouthwash, to be performed with 10 ml of cetylpyridinium chloride solution or, alternatively, sterile saline solution. The sample thus harvested should be examined freshly at a laboratory by the molecular biology techniques (e.g. SPF10-LiPA), to identify the type or types of viral species (21). This procedure, in addition to the high sensitivity, is easily executable by the operator and the patient, is minimally invasive and therefore repeatable to control the patient in case of persistent infection. This technique also provides for bleeding cells from the entire epithelial oral mucosa and tonsillary crypts. HPV-DNA research kits have recently been introduced for saliva samples, though still unanimously validated by scientific literature. In the presence of oral lesions suggestive of viral infection, the use of a fragile, fresh and frozen tissue fragment, taken during the diagnostic incision biopsy is recommended (22). The advantage, in addition to the possibility of using the same sample for histopathological and virological investigations, is to obtain a diagnosis of site-specific HPV infection; however, in the case of negativity, it is not possible to exclude the presence of the virus in the other oropharyngeal epithelial sites.

HPV oral infection: oral manifestations

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 uterine cervix. Histological similarities between cervicovaginal and oropharyngeal districts, both coated with squamous or slightly keratinized epithelium (23), and the capacity of the virus to immortalize *in vitro* human oral keratinocytes, have allowed to transfer the concept of HPV-induced oncogenicity in the gynaecological area, to the oral cavity.

Subclinical infection

In the normal oral mucosa, the prevalence of HPV is contained in a wide range (0-81.1%), with an average value of 11% (24). In a large meta-analysis on a total population of over 4,500 subjects, Kreimer et al. (12) calculated a prevalence of subclinical HPV infection in 4.5% of subjects, in the majority of cases supported by HR genotypes. An overlapping result was obtained from Syrjanen et al. (25) who, in a systematic review of HPV frequency in oral SCCs and potentially malignant oral disorders with respect to healthy controls, found a 12% infection rate. Both groups of researchers have found that the remarkable discrepancy between the various studies analysed is the result of the lack of homogeneity and standardization of the parameters used, including: ethnological and sociodemographic variables, different sexual behaviours, sample size, finally, the variability of the diagnostic techniques employed and the different sensitivity of the biomolecular procedures.
However, based on these findings, it is believed that oral mucosa represents an infection reservoir, so that a primary involvement of the virus in the oral cancerogenesis process should be excluded. Then it is suggested that the virus can simply passes the oral cavity and sometimes stays in sites at more risk, such as tonsillary crypts, showing features similar to the cervical squamous cell junction, and in sites where undifferentiated basal keratinocytes, targeted by HPV infection, are more exposed to the environment.
In this perspective, it is desirable that future investigations will take the cytological sample using oral gargarisms as the gold standard for sampling, including the oropharyngeal and tonsillary region as well.

Orogenital infection

Various groups of researchers studied the concomitance of HPV orogenital infection in order

to check whether the presence of genital infection and the risk factors associated with sexual behaviour, may lead to a higher oral transmission probability. Some studies focused that oral transmission may occur following self-inoculation or direct contact with an infected partner (e.g. fellatio, cunnilingus, deep kisses). The percentages of patients with simultaneous orocervical infection are influenced by the sensitivity of diagnostic methods.

Early studies conducted in the 1990s with low sensitivity techniques and small samples, showed relatively high co-frequency rates (16-24%). More recently, other studies, sometimes including sexual partners, and with more sensitive diagnostic techniques, reported lower percentages of bifocal infection, in the absence of genotype concordance between the two districts and also among partners, suggesting the hypothesis of a different susceptibility to the virus of the two mucous membranes, greater in the genital area respect to oral mucosa. In fact, it has proposed a protective role-played by saliva in virtue of its cleansing action and also for the presence of salivary IgA.

A recent study conducted in Finland on the intra-family transmission dynamics of the virus found that 11% of women and 26% of their partners reported regular sexual orogenital behaviours (15). In another population (young university students) the infection was detected in 3% of cases and was associated with the total number of partners. The case-control study of De Souza et al. (10) suggested that sexual behaviour increases both the probability of HPV infection and of onset of SCC in oropharynx respect to controls. The only meta-analysis conducted in the literature, over a total of over 1,000 women, showed a prevalence of bifocal infection of 18.1% with genotype concordance in 27% of cases of orogenital infection (16). This study also found a significant increase in risk associated with HIV infection (HIV positive 27.2% *vs* HIV negative 15.5%). In the group of HIV positive patients, 47% of the cases was also identified by the same genotype in the two districts. These data suggest that although it is possible to transmit HPV from the cervical mucosa to the oral one, this infection is strongly conditioned by the immune system.

The most significant aspect emerging from these studies is related to the association with sexual behaviour, in terms of early onset of sexual activity and number of partners. Sanders et al. (17) calculated a 10-fold greater risk of oral HPV infection in patients with over 20 sex partners than individuals with less than 3 partners. Due to the remarkable change in sexual habits in the populations, which strongly influenced the spread of infidelity among young people, as well as the increase in oral sexual practices among teenagers, probably due to the conviction whether it is a form of risk-free sexual activity, an increase in cases of oral infection and with it a greater risk of HPV-related SCC incidence was observed in the last years.

Clinical Infection
Benign lesions

HPV is involved in the pathogenesis of several benign lesions that develop in the oral cavity, especially the verruca vulgaris, squamous papillomas, and the most rare focal epithelial hyperplasia. They are all esophytic growth-papillary lesions that can occur either single or multiple and with a smooth or verrucal surface of white, pink or red colour depending on the degree of keratinization of the lesion itself. These asymptomatic lesions can develop in every part of the oral mucosa but more frequently at the level of the tongue, the soft palate and the lips. In most cases, except for focal epithelial hyperplasia, they have a very similar clinical profile, so the diagnosis is based on histology. At the histological examination, coin-like aspects are characterized by polyclonal epithelial proliferation, coilocytosis – which corresponds to the extrinsection of the cytopathic effect of the virus against the host cell – and dyscheratosis. In HPV-related benign conditions, low-risk genotypes such as HPV 2, 4, 11, 13, 32 are most frequently found, although

high-risk genotypes have also been isolated in these lesions.

Potentially malignant disorders

In the context of the malignant transformation of potentially malignant oral disorders (PMODs), the potential role of promoter of HPV is still debated (6). Several groups of researchers screened the hypothesis of an HR-HPV role in promoting carcinogenesis in the presence of PMODs, considering that oncogene proteins E6 and E7 act at a very early stage of carcinogenesis, significantly increasing the capacity of the epithelial cell of duplicate by blocking the apoptotic pathways. While the hypothesis that HPV contributes to the genesis of these lesions has been completely rejected, the hypothesis that PMDs HPV positive would have a better prognosis respect to HPV negative is still on debate.
The prevalence of PMOD infections is included in a wide range (0-85%) with a higher frequency of HPV 16 and 18. In addition considering the over-emphasized criticisms of non-uniform diagnostic procedures, these studies differ in terms of inclusion criteria and classification of lesions, sometimes not histologically diagnosed. The most common PMODs are oral leukoplakia (OL), proliferative verrucous leukoplakia (PVL), oral erythroplakia (OE) and oral lichen planus (OLP).
In relation to OL, the most frequent disorder among PMODs and therefore the most investigated in literature, current evidence suggests OL shows an increased risk of HPV infection with respect to clinically healthy mucosa, with a prevalence of around 20% (18, 19), with no significant differences in clinical presentation (20). More recently, Szarka et al. (21) published an increasing percentage, with HPV positive prevalence of 40.9%. PVL, previously considered a rare variant of OL, today is considered a distinct entity, characterized by multifocality, a strong tendency for recurrence and neoplastic transformation (22). In the past, due to the clinical presentation (white plaque with irregular surface and verrucous surface) and the first virological studies indicating a high frequency of HPV 16, it was strongly associated with infection, so to be defined as "viroplasia". However, subsequent studies (20, 23) provided others results: as the sensitivity and specificity of the diagnostic tests used increase, the frequency of infection rate is reduced or, however, does not increase significantly with respect to OL.
The OE is a rare PMOD characterized by a major neoplastic risk. Due to its very low frequency, references to viral infections are very rare in literature. The most recent data published by Syrjanen et al. (25) reported that of the 11 OE tested for HPV, 54.5% was found to be HPV 16 positive.
OLP was also investigated in relation to viral infection, with frequency of infection ranging from 27 to 65% (25). Some Authors hypothesized the influence of the erosive OLP in increasing the risk of HPV infection, although this hypothesis has not been confirmed by subsequent studies (19). In the review of Syrjanen et al. (25) the prevalence of HPV infection in OLP was 5.12%, with genotype 16 being most commonly involved.
The same Authors calculated the risk of HPV infection in the presence of a PMOD, which is significantly increased by almost 4 times in OL and OLP *versus* healthy oral mucous membranes, while the data is not significant for PVL. However, answering the question of whether HPV infection plays a role in the PMOD onset or has a negative prognostic role, still the scientific evidence to support is rather limited and controversial, not being able to exclude the hypothesis that the presence of the lesion facilitates the entry of the virus. Yang et al. (24) have also evaluated the percentage of OL undergoing neoplastic evolution and various risk factors, including HPV infection, highlighting a lack of genotypic correlation between the infection in OL and the risk SCC. Therefore, the Authors concluded that the etiologic role in HPV in the early stages of oral carcinogenesis seems rather modest.

Malignant lesions

The most common malignant neoplasm of the oral cavity is SCC. Recent estimates, both the annual incidence rates and the mortality rates of SCC, have not diminished in recent years, so SCC is still a serious problem of public health. This disease is characterized by strong local aggression and a poor prognosis, with a five-year survival ranging from 59.4 to 67%. The following evidence subsequently supported initial hypotheses about a possible etiologic role of the virus in oral and oropharyngeal carcinogenesis, dating back to the early 1980s:

- Epithelial tropism of virus;
- The confirmed etiologic role of HR-HPP in the almost total SCCs of the uterine cervix and in approximately 40% of the cases of other SCCs;
- The histological similarities between the epithelial of the two mucous membranes.

Despite the histological similarities of the two epithelial, it must be stressed that the environment of the uterine cervix is much more receptive to the presence of the virus than all the other anatomic sites.

The role of the virus in carcinogenesis of the oral cavity is very controversial. Syrjanen et al. (6) reported that HPV infection was almost 13 times more frequent in SCC oropharynx and 4 times more frequent in oral SCC than in healthy mucous membranes, especially with reference to HPV 16. Again, the prevalence range of infection is very wide (0-100%) and is influenced by the demographic and ethnic differences of the populations investigated, the different diagnostic procedures, but above all the topographical classification of the SCCs of the head-neck district for single sites. In fact it was noted that the prevalence of viral infection is particularly high in oropharyngeal SCCs (about 50% of SCC cases lingual and tonsils), which is therefore the second target site of the infection (26). Over the past five years, scientific literature has increasingly distinguishing two different tumour subtypes for population distribution, risk factors, anatomical site and biological behaviour, suggesting two different carcinogenic patterns. The first model is associated with known risk factors (e.g. smoking and alcohol), manifested mainly in older men (e.g. > 60 years), affecting the entire oral cavity and having a decreasing epidemiological trend, though without substantial reductions in mortality.

The second model, however, manifests itself in young people (for example <40 years), predominantly men, it is independent from traditional risk factors, and correlates with sexual behaviour (e.g. partner number, age of sexual intercourse, orogenital sexual practices), mainly affecting oropharyngeal sites (e.g. palatine pillars, tonsils, lingual base, lower face of the soft palate, pendulum veil). This second model is the type of SCC that is growing epidemiologically and shows a global prevalence of HR-HPV infection ranging from 37 to 72% (27). The scientific evidence available for oropharynx is therefore differentiated from those available for the oral cavity. Only the SCC of the oropharynx is most closely related to HR-HPV infection, while SCC of the mouth have not demonstrated a strong relationship, in fact HPV has a more marginal role in etiopathogenesis.

An interesting data reported by Kim et al. (28), found the HPV DNA integrated into 94.1% of the examined SCCs, and the significant correlation between HPV-DNA integration and cell cycle alteration is well known. The most alarming data is that, according to the latest epidemiological studies, due to the success of alcoholism and tobacco awareness campaigns, confirmed by the reduction of new cases of SCC oral and alcohol-related, the annual incidence of HPV verrucous carcinoma of oropharynx has increased exponentially over the last twenty years, so that it is estimated that by 2020 their frequency will be higher than SCCs in the uterine cervix and in the anogenital tract (29).

From a prognostic point of view, it has been observed that subjects with positive HPV-SCC of oropharynx have a more favourable prognosis than negative HPV. Yang et al. (30), in a review of randomized trials in SCC orpharyngeal pa-

tients in chemotherapy stage III and IV distinguished three categories with different prognosis (e.g. low, intermediate or high risk) in relation to some parameters (tumour extension, lymphnode involvement, smoking, and HPV infection). The statistical analysis confirmed that negative HPV tumours belonged to the worst prognosis, with a three-year survival of 46.2%, while for the HPV positive it was significantly higher (71% in the risk category intermediate and 93% in the low risk group) (30). The best prognosis would seem to be related to greater radio and chemosensibility, currently demonstrated for oropharingeal verrucous carcinomas HPV 16 positive. In the light of these evidences, National Comprehensive Cancer Network (NCCN) has updated the guidelines and diagnostic-therapeutic protocols of SCCs in the head and neck adding the search of HPV infection (for example by immunohistochemical detection of p16INK4 protein or biomolecular detection of HPV DNA) in the SCC of the oropharynx diagnostic protocol. However, since therapeutic protocols for HPV positive or negative HPV tumours have not been differentiated, it is likely that they will be defined in the near future with the aim of achieving individualized therapeutic protocols.

Verrucous carcinoma (VC) is a rare clinical-histological variant of SCC (<5% of cases of oral cancer) characterized by high degree of differentiation, reduced aggression, slow growth and poor tendency to metastatic diffusion often associated with a past PVL. Because of the non-simple differential diagnosis with the most common SCC, the HPV role in VC pathogenesis is not entirely clear: the few data in the literature indicate association with both LR genotypes (6 and 11), both with HR genotypes (16 and 18), mostly as multiple infections. However, the detection rate of the virus in the head-neck district is extremely low (7.7%), so there is insufficient data to demonstrate a causal relationship (31). A recent study reported prevalence rates of HPV-HR similar to SCCs and oral VCs, not suggesting a particular etiopathogenetic association of these infections (32).

Therapeutic protocols

Contrary to other viral infectious conditions that respond to pharmacological therapy, there are no active substances available for HPV infection that can eradicate the infection or induce regression of clinical lesions, if present. Some drugs have been tested for topical and/or systemic use for the treatment of genital lesions. For oral subclinical infections, similar to genital surgery, no treatment is provided; none of the antiviral drugs tested (e.g. acyclovir, ribavirin) have been effective in eliminating the infection. Management of HPV infection request follow-up and HPV testing every 8-12 months from first detection and periodically to verify the eradication of infection by the immune system. Of course, "persistent" is an infection always sustained by the same genotype that is maintained for more than 18-24 months (33).

In the presence of HPV-related lesions, their treatment is predominantly surgical, not being responsive to topical application or systemic administration of cytotoxic or immunomodulatory drugs. The surgical treatment is the excisional treatment with cold-bladed scalpels, quantum or laser resonance scales, which allow for the histological examination of the sample.

The management of suggestive lesions of PMOD or *in situ* SCC, wherether single or multifocal, is much more complex. It should be of relevance to a clinician with specific training in oral medicine, firstly diagnosing the lesions with incision biopsy and HPV test on the biopsy. The complete excision of the lesion should be confirmed by histologic diagnosis, depending on the possible presence of dysplasia of various degrees (low to high). In the presence of a diagnosis of carcinoma, the therapeutic approach is even more complex and depends on the stage of tumour according to TNM classification. However, it is good to keep in mind that because of the multifocality of HPV oropharyngeal infection, surgical removal of the lesion does not guarantee the eradication of infection, as HPV DNA may persist in the adjacent healthy mucous membrane.

The new therapeutic perspectives concern the possibility of using "therapeutic vaccines", namely real molecules capable of stopping cancer-related growth linked to vectors directed against certain viral antigens and therefore directed against positive VCs. In this case the challenge lies in the possibility of highly selectively targeting a specific tumour histotype, to a highly targeted therapy and with collateral effects extremely reduced, compared to the currently administered chemotherapy protocols, with a life-style benefit. However, therapeutic vaccine formulations are still under study and are not yet applicable.

Prevention of infection

There are currently three prophylactic vaccines already evaluated by randomized clinical trials, but only the last two are available: monovalent vaccine against HPV 16; direct quadrilateral equivalent vaccine against HPV 6, 11, 16 and 18; bivalent vaccine against HPV 16 and 18.
The quadrivalent vaccine was approved by the Food and Drug Administration (FDA) in 2006 for the prevention of precancerous lesions and anogenital SCCs (cervical, vaginal and vulvar) in girls ages 9 to 26 (28); it was later extended to males of the same age group for the prevention of penile and anal SCC (28). The indication of this vaccine, which also protects against genotypes LR 6 and 11, also includes the prevention of condyloma and anogenital warts. In clinical trials so far, quadrivivalent vaccine has proven to be very effective in preventing SCC and premalignant lesions both in native patients (efficacy> 98%) and in previously infected individuals (efficacy between 50 and 78%).
The bivalent vaccine was only tested for cervical SCC and not for other HPV-related neoplasms and was recommended for girls aged 10 to 26 years. The percentages of efficacy in the prevention of premalignant and malignant lesions of this mucosal region are also extremely high (> 97% for native subjects) (34). However, it is important to note that the bivalent vaccine, structurally and functionally similar to quadrivalent, is also effective in preventing HPV 16 and 18-related lesions in other mucosal areas. In both cases, vaccination is tridose and currently most European countries (e.g. Sweden, Italy, France, the Netherlands, Germany, Switzerland, Denmark, Spain, Belgium) and over 70 countries around the world have free vaccination for girls aged 9-17, with a rate of about 50%. These vaccination campaigns, intended for young teenagers before sexual activity, are aimed at obtaining not only of the individual immunization of the vaccinated subjects but also of the so-called "flock immunity", which occurs when at least 80% of the population is vaccinated against a specific infection and involves a reduced circulation of the virus and consequently less likely that the unvaccinated ones may become ill. In order to reach this result, it is essential to extend vaccination to adolescent males, although the high cost of vaccinations is a limit, at least in developing or underdeveloped countries. Other crucial and still debated issues concern the ideal age of vaccination in the light of both the ever-early sexual intercourse among adolescents and the need to vaccinate native individuals to obtain higher efficacy rates, and the extending vaccination to homosexual males who are currently not protected either individually or indirectly as group immunity, and which are a vulnerable category for anogenital SCCs. Although there are still no data in the literature on the efficacy of HPV vaccines in reducing the incidence of SCC of oropharynx and oral cavity, it is reasonable to assume that in the long term the benefits will also be found for this category of tumours. Only in the future decades it will be possible to evaluate exactly the impact of vaccine programs on the epidemiology of positive HPV tumours. The oral cavity is populated by a large number of bacteria species that form polymicrobial communities called biofilm. The biofilm formed by the oral microbiota includes both symbiotic and potentially pathogenic species and viruses such as HPV. The advent of periodontal diseases and peri-implantitis appear associated with a microbial shift, more

commonly known as dysbiosis, that could be considered either a decrease in the number of beneficial symbionts and/or an increase in the number of pathogens (35-55).

Conclusions

In recent decades, the amount of information about HPV infection and its oncogenic potential has increased considerably, in line with advances in molecular biology techniques applied to diagnostic, preventive and therapeutic management of HPV-induced lesions. Understanding interactions between the viral agent and the host provided new targets for infection control and allow the formulation of new-targeted therapeutic protocols. It is important to emphasize that the general population knows very little about the transmission and clinical presentation of HPV infection, largely due to the lack of information campaigns, the implementation of which is desirable in order to gain better control of the infection.

In the expectation that such information and prevention programs are implemented, the role of the dentist is of great importance. Whatever the prevailing discipline to which he / she is referring, the knowledge of the notions relating to this frequent infectious condition, its natural history, clinical manifestations in the oropharyngeal district and other target sites, preventive and therapeutic measures is an important requirement in order to promote patient health and provide them with the most comprehensive indications.

References

1. Suarez TP, Kelly JA, Pinkerton SD, et al. Influence of a partner's HIV serostatus, use of highly active antiretroviral therapy, and viral load on perceptions of sexual risk behavior in a community sample of men who have sex with men. J Acquir Immune Defic Syndr. 2001;28:471-477.
2. Pagliusi S. Vaccines against Human Papillomavirus: World Health Organization. 2013.
3. Gillison ML, Koch WM, Capone RB, et al. Evidence for a causal association between human papillomavirus and a subset of head and neck cancers. J Natl Cancer Inst. 2000;92:709-720.
4. Chaturvedi AK, Engels EA, Pfeiffer RM, et al. Human papillomavirus and rising oropharyngeal cancer incidence in the United States. J Clin Oncol. 2011;29:4294-4301.
5. Hariri S, Unger ER, Sternberg M, et al. Prevalence of genital human papillomavirus among females in the United States, the National Health And Nutrition Examination Survey, 2003-2006. J Infect Dis. 2011;204:566-573.
6. De Vuyst H, Clifford G, Li N, et al. HPV infection in Europe. Eur J Cancer. 2009;45:2632-2639.
7. Gillison ML, Broutian T, Pickard RK, et al. Prevalence of oral HPV infection in the United States, 2009-2010. JAMA. 2012;307:693-703.
8. D'Souza G, Kreimer AR, Viscidi R, et al. Case-control study of human papillomavirus and oropharyngeal cancer. N Engl J Med. 2007;356:1944-1956.
9. Syrjanen K, Syrjanen S, Lamberg M, et al. Morphological and immunohistochemical evidence suggesting human papillomavirus (HPV) involvement in oral squamous cell carcinogenesis. Int J Oral Surg. 1983;12:418-424.
10. Burd EM. Human papillomavirus and cervical cancer. Clin Microbiol Rev. 2003;16:1-17.
11. Longworth MS & Laimins LA. Pathogenesis of human papillomaviruses in differentiating epithelia. Microbiol Mol Biol Rev. 2004;68:362-372.
12. Kreimer AR, Bhatia RK, Messeguer AL, et al. Oral human papillomavirus in healthy individuals: a systematic review of the literature. Sex Transm Dis. 2010;37:386-391.
13. Baseman JG, Koutsky LA. The epidemiology of human papillomavirus infections. J Clin Virol. 2005;32 Suppl 1:S16-24.
14. Rintala MA, Grenman SE, Puranen MH, et al. Transmission of high-risk human papillomavirus (HPV) between parents and infant: a prospective study of HPV in families in Finland. J Clin Microbiol. 2005;43:376-381.
15. Grobe A, Hanken H, Kluwe L, et al. Immunohistochemical analysis of p16 expression, HPV infection and its prognostic utility in oral squamous cell carcinoma. J Oral Pathol Med. 2013;42:676-681.
16. Termine N, Panzarella V, Falaschini S, et al. 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.
17. D'Souza G, Sugar E, Ruby W, et al. Analysis of the effect of DNA purification on detection of human papillomavirus in oral rinse samples by PCR. J Clin Microbiol. 2005;43:5526-5535.

18. Termine N, Giovannelli L, Rodolico V, et al. 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.
19. Thompson IO, van der Bijl P, van Wyk CW, et al. A comparative light-microscopic, electron-microscopic and chemical study of human vaginal and buccal epithelium. Arch Oral Biol. 2001;46:1091-1098.
20. Smith EM, Ritchie JM, Yankowitz J, et al. HPV prevalence and concordance in the cervix and oral cavity of pregnant women. Infect Dis Obstet Gynecol. 2004;12: 45-56.
21. Rintala M, Grenman S, Puranen M, et al. Natural history of oral papillomavirus infections in spouses: a prospective Finnish HPV Family Study. J Clin Virol. 2006;35:89-94.
22. Termine N, Giovannelli L, Matranga D, et al. Oral human papillomavirus infection in women with cervical HPV infection: new data from an Italian cohort and a metanalysis of the literature. Oral Oncol. 2011;47:244-250.
23. Sanders AE, Slade GD, Patton LL. National prevalence of oral HPV infection and related risk factors in the US adult population reply. Oral Dis. 2013;19:106.
24. Feller L, Lemmer J. Oral leukoplakia as it relates to HPV infection: a review. Int J Dent Hyg. 2012;540-561.
25. Syrjanen S, Lodi G, von Bultzingslowen I, et al. Human papillomaviruses in oral carcinoma and oral potentially malignant disorders: a systematic review. Oral Dis. 2011;17 Suppl 1:58-72.
26. Campisi G, Giovannelli L, Arico P, et al. HPV DNA in clinically different variants of oral leukoplakia and lichen planus. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2004;98:705-711.
27. Szarka K, Tar I, Feher E, et al. Progressive increase of human papillomavirus carriage rates in potentially malignant and malignant oral disorders with increasing malignant potential. Oral Microbiol Immunol. 2009; 24:314-318.
28. Kim SH, Koo BS, Kang S, et al. HPV integration begins in the tonsillar crypt and leads to the alteration of p16, EGFR and c-myc during tumor formation. Int J Cancer. 2007;120:1418-1425.
29. Bagan JV, Jimenez Y, Murillo J, et al. Lack of association between proliferative verrucous leukoplakia and human papillomavirus infection. J Oral Maxillofac Surg. 2007;65:46-49.
30. Yang SW, Lee YS, Chen TA, et al. Human papillomavirus in oral leukoplakia is no prognostic indicator of malignant transformation. Cancer Epidemiol. 2009;33:118-122.
31. Nasman A, Attner P, Hammarstedt L, et al. Incidence of human papillomavirus (HPV) positive tonsillar carcinoma in Stockholm, Sweden: an epidemic of viral-induced carcinoma? Int J Cancer. 2009;125:362-366.
32. D'Souza G, Gross ND, Pai SI, et al. Oral human papillomavirus (HPV) infection in HPV-positive patients with oropharyngeal cancer and their partners. J Clin Oncol. 2014;32:2408-2415.
33. Andrews E, Seaman WT, Webster-Cyriaque J. Oropharyngeal carcinoma in non-smokers and non-drinkers: a role for HPV. Oral Oncol. 2009;45:486-491.
34. Marur S, D'Souza G, Westra WH, et al. HPV-associated head and neck cancer: a virus-related cancer epidemic. Lancet Oncol. 2010;11:781-789.
35. del Pino M, Bleeker MC, Quint WG, et al. Comprehensive analysis of human papillomavirus prevalence and the potential role of low-risk types in verrucous carcinoma. Mod Pathol. 2012;25:1354-1363.
36. Akrish S, Ben-Izhak O, Sabo E, et al. Oral squamous cell carcinoma associated with proliferative verrucous leukoplakia compared with conventional squamous cell carcinoma-a clinical, histologic and immunohistochemical study. Oral Surg Oral Med Oral Pathol Oral Radiol. 2015;119:318-325.
37. Syrjanen S, Termine N, Capra G, et al. Oral HPV infection: current strategies for prevention and therapy. Curr Pharm Des. 2012;18:5452-5469.
38. Lu B, Kumar A, Castellsague X, et al. Efficacy and safety of prophylactic vaccines against cervical HPV infection and diseases among women: a systematic review & meta-analysis. BMC Infect Dis. 2011;11:13.
39. Lopez MA, Andreasi Bassi M, Confalone L, et al. Clinical outcome of 215 transmucosal implants with a conical connection: a retrospective study after 5-year follow-up. J Biol Regul Homeost Agents. 2016;30:55-60.
40. Lopez MA, Andreasi Bassi M, Confalone L, et al. Retrospective study on bone-level and soft-tissue-level cylindrical implants. J Biol Regul Homeost Agents. 2016;30:43-48.
41. Lopez MA, Andreasi Bassi M, Confalone L, et al. The influence of conical plus octagonal internal connection on implant survival and success rate: a retrospective study of 66 fixtures. J Biol Regul Homeost Agents. 2016;30:49-54.
42. Andreasi Bassi M, Andrisani C, Lopez MA, et al. Endoscopically controlled hydraulic sinus lift in combination with rotary instruments: one-year follow-up of a case series. J Biol Regul Homeost Agents. 2016; 30(S2):21-28.
43. Carinci F, Lauritano D, Cura F, et al. Prevention of bacterial leakage at implant-Abutment connection level: An in vitro study of the efficacy of three different implant systems. Journal of Biological Regulators and Homeostatic Agents. 2016;30:69-73.
44. Scapoli L, Girardi A, Palmieri A, et al. Quantitative Analysis of Periodontal Pathogens in Periodontitis and Gingivitis. J Biol Regul Homeost Agents. 2015;29:101-110.
45. Lauritano D, Martinelli M, Mucchi D, et al. Bacterial load of periodontal pathogens among Italian patients with chronic periodontitis: A comparative study of three different areas. Journal of Biological Regulators

and Homeostatic Agents. 2016;30:149-154.
46. Lauritano D, Cura F, Candotto V, et al. Periodontal Pockets as a Reservoir of Helicobacter Pylori Causing Relapse of Gastric Ulcer: A Review of the Literature. J Biol Regul Homeost Agents. 2015;29:123-126.
47. Lauritano D, Muzio LLO, Gaudio RM, et al. The ecological catastrophe of oral diseases: A possible link between periodontitis and protozoa. Journal of Biological Regulators and Homeostatic Agents. 2016;30: 143-147.
48. Lauritano D, Muzio LL, Gaudio RM, et al. Why should patients with systemic disease and tobacco smokers go to the dentist? Journal of Biological Regulators and Homeostatic Agents. 2016;30:135-141.
49. Scapoli L, Girardi A, Palmieri A, et al. Interleukin-6 Gene Polymorphism Modulates the Risk of Periodontal Diseases. J Biol Regul Homeost Agents. 2015 ;29:111-116.
50. Lauritano D, Scapoli L, Mucchi D, et al. Infectogenomics: Lack of association between vdr, il6, il10 polymorphisms and "red Complex" bacterial load in a group of Italian adults with chronic periodontal disease. Journal of Biological Regulators and Homeostatic Agents. 2016;30:155-160.
51. Lauritano D, Pazzi D, Iapichino A, et al. Evaluation of the efficacy of a new oral gel containing carvacrol and thymol for home oral care in the management of chronic periodontitis using PCR analysis: a microbiological pilot study. J Biol Regul Homeost Agents. 2016;30:129-134.
52. Lauritano D, Bignozzi CA, Pazzi D, et al. Evaluation of the efficacy of a new oral gel as an adjunct to home oral hygiene in the management of chronic periodontitis. A microbiological study using PCR analysis. Journal of Biological Regulators and Homeostatic Agents. 2016;30:123-128.
53. Lauritano D, Cura F, Candotto V, et al. Evaluation of the Efficacy of Titanium Dioxide with Monovalent Silver Ions Covalently Linked (Tiab) as an Adjunct to Scaling and Root Planing in the Management of Chronic Periodontitis Using Pcr Analysis: A Microbiological Study. J Biol Regul Homeost Agents. 2015; 29:127-130.
54. Lauritano D, Cura F, Gaudio RM, et al. Polymerase Chain Reaction to Evaluate the Efficacy of Silica Dioxide Colloidal Solutions in the Treatment of Chronic Periodontitis: A Case Control Study. J Biol Regul Homeost Agents. 2015;29:131-135.
55. Lauritano D, Bignozzi CA, Pazzi D, et al. Efficacy of a new coating of implantabutment connections in reducing bacterial loading: An in vitro study. ORAL and Implantology. 2017;10:1-10.

Correspondence to:
Prof. Francesco Carinci, MD
Department of Morphology, Surgery and Experimental Medicine
University of Ferrara
Via Luigi Borsari 46
44121 Ferrara, Italy
Phone: +39.0532.455874
Fax: +39.0532.455876
E-mail: crc@unife.it