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Translational and transdisciplinary approach to the human papilloma virus – Preliminary evidence from the Italian "HPV board: a future without papilloma virus" project

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Abstract. – Human papillomavirus (HPV) is considered to be one of the viral infections associated with cancers and other diseases. HPV is detected asymptomatically in the oral mucosa. The presence of human papillomavirus in the oral mucosa appears to be closely associated with a series of benign and malign oral lesions.

The aim of this paper is to report the Italian experience in applying translational protocols, using new technologies and multidisciplinary strategies in Human Papilloma virus detection and treatment.

The "HPV board: a future without papilloma virus" project was born, promoted by CNEL (Italian Council of Economics and Labor) with the collaboration of numerous scientific societies to commonly approach to public knowledge of HPV-related oral lesions and their clinical management.

The preliminary results are related to the assessment of the proof-of-concept of this new project. More in details, "HPV Board" is a project that plans the presence of a working group, made up of otolaryngologists, dentists, oral and maxillofacial surgeons, in close contact with gynecologists, oncologists and pediatricians; this working group manages to combine very transversal skills, in order to promote primary prevention projects, early diagnosis and adequate therapies.

The "HPV BOARD" project will give the opportunity to increase the attention of patients and doctors on the early diagnosis of oncological diseases dependent on infection by the infectious agent HPV. In this panorama, dentists will have the role of "first sentinel" of public health because oral health is an indicator, too often overlooked, for the prevention of numerous diseases.

Key Words:
Human papillomavirus (HPV), Oral HPV, Oropharyngeal squamous cell carcinoma (OPSCC), Oral disease, Oral cancer, Molecular tests.

Introduction

Human papillomavirus (HPV) is considered to be one of the viral infections associated with cancers and other diseases. HPVs are non-enveloped viruses with double-stranded circular DNA. The papillomavirus genome consists of three segments with early, late and genomic regions. Half genome is formed by the early regions: E1, E2, E4-E8. The proteins E1 and E2 work together, are used in the replication of viral DNA and are responsible for coding the proteins that regulate the transcription of viral DNA, in particular E2 helps the processes of cell transformation, initia-

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tion and inhibition of apoptosis. When inactive, it promotes the expression of E6 and E7 and influences the development of the tumor lesion, when active, it inhibits E6 and E7 transcription leading to an increase in p53 expression and apoptosis of infected cells[1,2]. E4 protein triggered effects in the formation of nodules by HPV-1 and may be involved in cell cycle regulation[3]. E5 is used in the transformation of viral DNA and in the replication of viral DNA, while E6 is involved in the malignant transformation directed by the virus and does so through the non-relegation and regulation of the cell cycle[4]. Protein E7 contributes to the genetic instability of HPV-infected cells by interfering with the normal replication of centrosomes; is involved in HPV-dependent malignant transformation through the disruption of cell cycle regulation control and cell maturation[4]. The late region (L) with L1 and L2 constitutes 40% of the genome and involves the structural proteins of the virus the remaining part of the virus is structured with the genomic region involved in the vital regulatory mechanisms therefore it does not code for any protein but contains the sites binding to many viral and cellular transcriptional repressors and activators; performs a regulatory function of the transcription and replication of the viral genome under the control of the host cell[5,6].

Papillomavirus Etiopathogenesis

The integration of viral DNA into the host cell genome plays a key role in the HPV-induced tumorigenesis process. During the integration process some regions of the viral DNA are deleted, while others, even if integrated, are silenced at the transcriptional level, while is observed an overexpression of the E6/E7 viral oncoproteins of the high-risk HPV strains. The latter interfere with cell metabolism and favor the neoplastic transformation of the host cell. The life cycle of the virus is strictly dependent on the differentiation process of the host epithelial cell or keratinocyte, while the natural history of the infection depends on the genotype involved. The progressive changes that the infected keratinocyte undergoes during its differentiation, starting from the basal layer up to the surface layers, create the microenvironmental conditions suitable for viral replication and the assembly of new virions[7-9].

Identification of Human Papillomavirus Sub-Types

On the basis of DNA sequencing, have been identified so far more than 130 genotypes of HPV, defined for HPVs are divided into cutaneous types, which mainly infect hands and feet, and mucosal types, which target the mucosa of the oral cavity, of the upper pathways, air and digestive systems, the anogenital area, the urethra and the conjunctiva. Based on the biological behavior of the virus-induced lesions, they were also divided into three groups: HPV with high oncological risk (HR-HPV), such as HPV 16, 18, 45, 56, 59, 66, 67, 68, 70, 73, 82, associated with potentially and frankly malignant lesions (for example anogenital and head-neck CSCs); HPV with intermediate cancer risk such as HPV 31, 33, 35, 39, 51, 52, 53, 58; HPV with low cancer risk (LR-HPV) such as cutaneous HPV 2, 4, 27 and mucous types 6, 11, 13, 32, 42 most commonly associated with benign diseases (e.g., vulgar warts, warts and papillomas)[10]. Epidemiological and multidisciplinary studies have led to the demonstration of the etiological role of the Human Papilloma virus (HPV) in cervical cancer and other mucous membranes around the 1990s. Most (70-90%) HPV infections are transient and spontaneously regress within 18-24 months. A small percentage of viral infections persist beyond 2 years. Persistence is the necessary condition for the evolution towards cancer[11,12].

Oral Human Papillomavirus

HPV is detected asymptomatically in the oral mucosa, but the origin or reservoir of oral HPV is still unknown. HPV is known to contaminate the oral cavity of healthy individuals. Normal oral mucosa can act as a reservoir for new HPV infections and/or as a source of recurrent HPV-associated injury. According to recent epidemiological studies, the gingival pocket seems to be the only site of the oral mucosa in which basal cells, known targets of HPV in other sites of the mucosa, are normally exposed to HPV infection. Since the inflammation is almost always localized in the gingival pockets, epithelial cell division at this site seems to favor viral replication[13]. To support this view, the authors analyzed gum samples from 31 patients with periodontitis. 8 of 31 samples, after PCR analysis, showed high-risk HPV types. With in-situ hybridization it was possible to localize the viral DNA in the coronal part of the junctional epithelium of the gingival pocket[13]. There are scientific studies that state that in very rare cases the virus that is localized in some cells of the oral mucosa changes them and transforms them into malignant tumors. There are other scientific studies in which, however,

the high association of patients with oral cancer with papilloma virus infection was observed[14]. The presence of human papillomavirus in the oral mucosa appears to be closely associated with a series of benign papillomatous lesions. These include oral squamous papilloma, oral verruca vulgaris, oral condyloma acuminate, and focal epithelial hyperplasia[15]. Oral squamous papilloma: squamous papilloma (SP) of the oral cavity is an epithelial degeneration of the oral mucosa. It typically appears as an exophytic neoformation of about 1cm in diameter with a granular or cauliflower appearance and only rarely reaches dimensions greater than 5 mm. Squamous papilloma are generally pedunculated, with a color ranging from white to pink/ red, usually solitary. In a study[16] of 205 SPs, only four patients had two concurrent lesions. Verruca vulgaris (VV): oral verruca vulgaris (VV) or common verruca is the primary presentation of HPV skin infection and accounts for 70% of warts. It is estimated that around 10% of children and young adults are affected, with a peak incidence occurring in adolescents aged 12 to 16 years[17]. VV can be found anywhere on the skin but is most common in the periungual region of the hands[18]. Oral acuminate condyloma: acuminate condyloma is a very rare lesion, especially in the oral cavity. Although the presence of simultaneous lesions of the genitals and oral cavity suggests sexual transmission, additional routes of infection are possible, for example, via fomites. Adults are most commonly affected, with peaks in the third and fourth decades of life. Clinically, condyloma can present as a solitary lesion or in multiples, some of which may merge to form larger growths. In a study of 101 oral warts, 61% of patients experienced more than five lesions[19]. Focal epithelial hyperplasia: focal epithelial hyperplasia known as Heck's disease affects only the lining of the mouth, most commonly inside the lips, cheeks and sides of the tongue. However, the gums and tonsil area can also be affected. Characteristic is the presence of multiple lesions, of variable size and affecting different sites. In a study of 110 patients, only three patients (2.8%) had a single affected site, but multiple lesions were present at the site[20].

HPV Diagnosis and Detection Methods

The detection rate of HPV in normal oral mucosa shows variations depending on whether buccal curettage, biopsy, or mouthwash samples are collected and which of the molecular detection methods are used. HPV detection methods in use include immunoperoxidase, immunofluorescence, in situ hybridization, Southern blot, Dot blot, Reverse blot hybridization, and polymerase chain reaction (PCR). PCR is considered the highest sensitivity and can detect even a single copy of the viral DNA per infected cell. PCR is a technique that allows the in vitro amplification of DNA starting from a specific fragment, obtaining, by means of a DNA-polymerase, 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 quantities of nucleic acids[21]. Currently, HPV infection can only be reliably identified by detecting viral DNA or RNA in samples[22]. HPV can be associated with clinically objective lesions only in the case of a productive infection; therefore, the physical examination is not particularly useful for diagnosis. Other diagnostic methods used in the past (for example histopathology in light or electron microscopy, immunohistochemistry and serology have been abandoned because they are not very sensitive and specific[23]. However, the human papillomavirus test indicates the presence of the virus in the sampled area but nothing about the stage of HPV infection or previous HPV infections or their outcome. Histopathological analysis can only suggest HPV infection, in the presence of tissue alterations such as dyskeratosis, acanthosis, basal hyperplasia and in particular coilocytosis, i.e., the presence of cellular elements, called koilocyte, characterized by a globular shape large clear cytoplasm, pycnotic nucleus surrounded by a clear halo of vacuolation[24]. This finding is pathognomonic of HPV infection; however, the detection sensitivity of these changes is low and very operator dependent. Viral particles can be detected in the nuclei of infected epithelial cells with electron microscopy, but the method is limited to productive infections only. Immunohistochemical investigations are equally of little clinical utility as they are aimed at identifying the capsid proteins expressed in the late phase of the HPV life cycle. The only immunohistochemical marker with recognized diagnostic significance is the protein p16 (INK4), the overexpression of which in dysplastic or frankly neoplastic oral and cervical histological specimens is strongly associated with HR-HPV infection, constituting a surrogate marker, although it is not possible. exclude the simultaneous presence of other genotypes[25]. Serological tests are of doubtful

reliability in the diagnosis of active or previous infection. When seroconversion occurs, it follows the detection of viral DNA by HPV tests for several months (on average 8-12 months) and the antibody response does not have a constant trend in all individuals, sometimes ending in a short interval of time. at other times persisting despite the negativity of molecular tests. There are only a few studies in which the concordance between oral HPV infection and the serology of HPV itself has been evaluated. In a cohort study of HPV in a Finnish family, they found an association between oral HPV and the L1 protein among men but not among women. It was therefore hypothesized that this was due to the fact that in the male genitalia the mucosa is scarce and therefore the oral mucosa could be the site for immune recognition[26]. Cytology combined with HPV DNA testing has been discussed as a sensitive method for detecting patients at risk for HPV pre-cancers and malignancies in the head and neck region, but there is no consensus so far. Over 200 commercial kits are available for detecting HPV DNA or RNA. Hybridization methods are specific because HPV positive signals can be localized, and tissue morphology is visualized simultaneously. Currently, the diagnosis of certainty of the infection makes use of nuclear biology techniques, aimed at detecting the DNA of the virus inside the cells by means of labeled gene probes, and the most sensitive and most appropriate biomolecular method to identify and carry out the typing of the HPV genome is PCR[27,28]. Although molecular tests are currently mandatory and represent the gold standard for disease clinic, new diagnostic tests are starting to be used[29]. For example, in the United States, a non-invasive saliva test is commercially available. The tests designed for genital HPV infection have also been validated for oral lesions. Most of these tests are designed as real-time protocol, some as Hybrid-capture non-PCR methods, others use sequencing or microarray systems[30,31]. According to an Italian study published in Cancer, a valid alternative to molecular tests could be cytobrushing, an easy collection of cells using a swab, similar to what is already done on the female uterine cervix to carry out the Pap test or the HPV test. The Italian authors analyzed the data relating to 164 people: some had a neoplastic lesion of the oral cavity or oropharynx, others a non-cancerous lesion, others still did not show any clinically evident signs of disease in those areas. All the participants underwent both a cytological sampling (cytobrushing, in fact, performed with a swab in the back of the throat), then analyzed to verify the presence or absence of the virus, and the more traditional biopsy: the results of the two tests are then compared to evaluate the concordance between the results in the two types of sampling. The results of the Papilloma virus test conducted on the cells taken with the swab agree in 90% of cases with those obtained by analyzing the biopsies. Furthermore, it was found that patients with atypical cytology have an almost 10 times higher risk of having histologically ascertained squamous carcinoma of the head and neck area and that the presence of Papillomavirus infection on the oropharyngeal cytology sample is associated with a 5 times higher risk of have cytological atypia as well as a diagnosis of oropharyngeal cancer. Defining after extensive evaluation studies, cytological sampling as a useful screening tool on a population at risk to evaluate oral and oropharyngeal lesions, also limiting the undesirable effects of a biopsy and decreasing patient discomfort and also costs[32-34]. An alternative test to biopsy, the Cobas HPV test, validated on oral rinse samples collected from 187 patients (45 with OPSCC, 61 with oral SCC (OCSCC) and 81 control patients with benign or malignant thyroid nodules, a highly specific and potentially sensitive test for oropharyngeal cancer and may be a potentially useful screening test for early stage oropharyngeal cancer[35]. However, contrary to what happens in gynecology, in oral medicine a standard diagnostic protocol has not yet been established both in terms of the type of sample to be subjected to biomolecular investigation, of cytological sampling method (for example scraping using a spatula or toothbrush vs oral rinse) and the conservation procedure of the histological sampling, both in terms of the specific biomolecular technique to be used. The fallout of this lack of standardization unfortunately consists in obtaining non-univocal results in the various studies published in the literature and in the difficulty of comparing them[36], therefore it is hoped that this methodological gap will be filled as soon as possible by international scientific societies[37].

The "HPV Board Project"

Nowadays, diagnostic tests are required to be quick, easy to carry out, with high sensitivity and specificity, as well as providing biological

materials that reduce the invasiveness of the sample. With these premises, the possibility of having the same diagnostic information has been explored for some time, also using biological materials other than the traditional ones; specifically, saliva seems to be a very good and promising candidate. In the field of "virology", some applications have proved to be not only promising, but also increasingly improved overtime: an example is the story of the tests used to detect the SARS-CoV-2 infection. The first test was carried out by taking materials from rhino-pharyngeal mucosa. Various technologies have been tested to analyze the biological materials: the rapid lateral-flow antigenic tests, the immunofluorescence analysis, up to the molecular tests obtained thanks to the use of Real-Time PCR (RT-PCR). All these tests were very useful and have allowed to control the pandemic in its early stages; but now, the challenge is to monitor the population that cannot undergo frequent swabs. The salivary tests allow to perform tests daily, thus monitoring a great number of patients without any invasiveness. Numerous tests have now been validated to be applied to the saliva material with success, with a sensitivity comparable to the well investigated nasopharyngeal swabs. Another example is the investigation of the presence of human Papilloma virus (HPV) in the oropharyngeal cavity. The use of salivary tests may allow monitoring all such subjects who undergo dental visit. In particular, some companies have developed systems able to rapidly identify the HPV16 in saliva samples. It is, in fact, well known in the scientific literature that the genotype of interest in the oral cavity is the HPV16. The colorimetric test, based on colloidal gold technology, allows you to detect the presence of HPV infection in less than ten minutes. A simple variation of the colorimetric peak at a specific wavelength read by a small spectrophotometer signals the presence of the virus. As for the molecular test, the challenge is twofold, as the saliva sample must be processed without requiring specific training regarding the execution of the test, and must be carried out quickly, therefore it must be a test POCT (Point of Care). In practice, the test is carried out automatically by inserting the saliva into a cartridge that contains the reagents and processed using a small instrument without the intervention of the operator, even providing the interpretation of the result automatically. Molecular testing has a second advantage that can also identify it as a confirmatory test. Everyone knows the great sensitivity of these systems, which in any case require longer times and higher costs. However, the advantage lies in the fact that those oncogenic genes that provide an indication not only of the presence can be used as target genes for the test, remember that HPV can spontaneously disappear after a short time without consequence for the host, but also of the danger of the same, because it contains genes that can predispose to degeneration with even serious clinical consequences. Raising awareness among the population on the value of vaccination and on new diagnostic tools to defeat related HPV tumors, which also affect the head / neck district, is a need that is highlighted by numerous scientists and institutions in Italy. In fact, every year in Italy there are more than 6,000 new diagnoses linked to the presence of the Papilloma Virus, with a significant socio-economic impact. WHO and the European Cancer Organization have launched a call to action to eradicate HPV-related cancers through immunization, early diagnosis with state-of-the-art testing and timely treatment. For this, the "HPV board: a future without papilloma virus" project was born, promoted by CNEL (National Council of Economics and Labor) with the collaboration of numerous scientific societies. The project has a high social value, but also an economic one; in fact, the "HPV board" aims to increase the prevention of pathologies which, when not intercepted in time, have repercussions both on the patient and on the national health system. Recently, the Covid19 pandemic has highlighted the strategic importance for European Union countries such as Italy to strengthen the efficiency of the social and health system. The "HPV Board" project was born from the national collaboration of numerous doctors, communication experts, institutions, researchers, and research institutes. The approach is based on the simplification of diagnostic procedures with simple, quick, and reliable tools to be introduced into daily clinical practice, in dental and ENT practices. This systematic approach could represent a real revolution: in fact, it will contribute, together with vaccine-related immunization, and the innovation of current therapies, to the decrease of the incidence curve of HPV-related tumors. "HPV Board" is a project that plans the presence of a working group, made up of otolaryngologists, dentists, oral and maxillofacial surgeons, in close contact with gynecologists, oncologists

and pediatricians; this working group manages to combine very transversal skills, in order to promote primary prevention projects, early diagnosis and adequate therapies. A multidisciplinary approach is essential for an early diagnosis and adequate treatment of all malignancies of the head and neck. This innovative and multidisciplinary approach is necessary in Italy and in the rest of European countries, since the epidemiological picture has drastically changed in Western countries in the last 20 years precisely due to the enormous spread of the Papillomavirus in the young-adult population. This epidemiological data has determined a net increase in the incidence of HPV-dependent oropharyngeal carcinomas, which have clear peculiarities from a clinical point of view and, fortunately, a much better prognosis than in smoking and alcohol-induced carcinomas, which in in the meantime, thanks also to awareness campaigns, they have decreased. Therefore, the awareness of patients and clinicians, together with vaccination, will be essential to reduce the incidence of induced HPV carcinomas and to treat them in the most appropriate way.

Conclusions

In recent years, the amount of information regarding HPV infection and its oncogenic potential has grown significantly. Many epidemiological studies have suggested that human papillomavirus, especially type 16, is involved in the genesis of squamous cell carcinoma of the oral cavity and oropharynx, especially in young non-smoking patients; therefore, its detection in lesions in this region is important. In the same way, interest in molecular biology techniques applied to the diagnostic, preventive and therapeutic process of HPV-induced lesions has grown at the same time. In fact, understanding the interactions between the viral agent and the host has provided new targets for the control of the infection and laid the foundations for the formulation of new targeted therapeutic protocols. The investigations to be requested for the virus search after surgical removal of the lesion are the immunohistochemical research of HPV-related viral proteins, the immunohistochemical evaluation of the p16 protein, whose positivity suggests the presence of the Papillomavirus but nevertheless the typing of the viral DNA (HPV- DNA test) indicates with certainty the presence of HPV and also allows to identify its type (high or low cancer risk). To date, new diagnostic strategies that aim not only at therapy but above all at prevention are being tested. The "HPV BOARD" project will give the opportunity to increase the attention of patients and doctors on the early diagnosis of oncological diseases dependent on infection by the infectious agent HPV. In this panorama, dentists will have the role of "first sentinel" of public health because oral health is an indicator, too often overlooked, for the prevention of numerous diseases, in all the age groups.

Conflict of Interest
The Authors declare that they have no conflict of interests.

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Ethics Approval and Consent to Participate
Not applicable.

Consent for Publication
All the authors approve the publication.

Availability of Data and Materials
The data supporting the findings of the article is available in the CNEL website.

Founding
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