REVIEW

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Human papillomavirus infection: etiopathogenesis, molecular biology and clinical manifestations *

Infecção pelo papilomavírus humano: etiopatogenia, biologia molecular e manifestações clínicas

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Abstract: Human papillomavirus (HPV) is a DNA virus that presents tropism for epithelial cells, causing infections of the skin and mucous membranes. Replication of HPV occurs in the nuclei of squamous cells and its life cycle is directly related to the differentiation program of the host cell. To date, nearly 100 different types of HPV have been characterized and there is a large number of other types that have not been sequenced yet. Besides being responsible for benign lesions of the skin and mucous membranes, HPV is also involved in the development of various mucocutaneous tumors: Bowen's disease, non-melanoma skin cancers and genital carcinomas. This review discusses the characteristics of HPV, malignant and benign mucous and skin manifestations caused by HPV, besides the main methods of detection and typing of the virus.
Keywords: DNA Viruses; Papillomavirus Infections; Warts

Resumo: O papilomavírus humano (HPV) é um vírus DNA que apresenta tropismo por células epiteliais, causando infecções na pele e nas mucosas. A replicação do HPV ocorre no núcleo das células escamosas e o seu ciclo de vida é diretamente relacionado ao programa de diferenciação da célula hospedeira. Até o momento, foram completamente caracterizados cerca de 100 tipos diferentes de HPVs e há um grande número adicional de tipos ainda não sequenciados. Além de ser o responsável por lesões benignas de pele e mucosas, o HPV também está envolvido no desenvolvimento de diversos tumores cutaneomucosos: doença de Bowen, cânceres de pele não melanoma e carcinomas genitais. Esta revisão aborda as características do HPV, quadros cutâneos e mucosos benignos e malignos causados por ele e os principais métodos empregados em sua detecção e tipagem.
Palavras-chave: Infecções por papilomavírus; Verrugas; Vírus DNA

INTRODUCTION

Papillomatous and verrucous lesions affecting the skin have been described since ancient Greece. Research on papillomavirus (PV) began in the early twentieth century. In 1933, a PV was isolated as a possible etiologic agent of warts in rabbits. Since then, this class of viruses has been considered natural viral infectious agents responsible for the development of warts in different groups of mammals, including man.

In 1935, Rous described the warts in rabbits as having the potential for malignant transformation. Strauss *et al.* reported the first visualization of PV particles in human warts by means of electron microscopy in 1949. In 1950, the carcinogenic potential of the human papillomavirus (HPV) was discovered in patients with epidermodysplasia verruciformis. The structure of the viral genome was only unveiled in 1963 by Crawford & Crawford.[1] However, in the subsequent years, research on HPV was discouraged due to the lack of a system of tissue culture and to the apparently benign nature of human warts.

In the 1970s, a gradual interest in these viruses started to arise. At that time, investigators described the diversity of this class of viruses, and Zur Hausen proposed the hypothesis that HPVs participated in the etiology of cervical cancers. [2]

In the early 1980s, there was a rapid increase in research on HPV; consequently, HPVs 16 and 18 were identified and their relationship with cervical cancer was established. The first epidemiological study on HPV and cervical cancer was published in 1987. [3] Since then, several epidemiological and molecular studies have confirmed that cervical infection by certain HPV types is a precursor of the genesis of cervical neoplasia.

CHARACTERISTICS OF THE VIRUSES

PVs are small DNA viruses (50-55 nm) belonging to the family Papovaviridae - genus Papillomavirus. They are non-enveloped viruses with icosahedral symmetry and present a genome of about 8000 base pairs (8Kb) of double-stranded, circular DNA. Despite their small size, the molecular biology of these viruses is quite complex. The viral DNA is associated with proteins similar to histones, wrapped in 72 capsomeres composed of two structural proteins, L1 and L2. These viruses can infect humans and a large number of animal species (cats, rabbits and nonhuman primates); man is the most extensively studied host [4].

PVs are highly species-specific and there is no report of a PV species causing productive infection in another species. They are viruses that exhibit tropism for epithelial cells causing infections of the skin and mucous membranes (genitals, mouth, larynx, esophagus), and their replication occurs in the nucleus of squamous epithelial cells. [5]

Their viral genome is divided into three regions based on their location and functional properties. The E (early) and L (late) regions, known as ORFs (Open Reading Frames or translation units), and a third region called LCR (long control region) or NCR (non-coding region) or URR (upstream regulatory region).

The E region contains up to eight genes (E1 to E8), which are responsible for replication of the HPV (E1 and E2), DNA transcription (E2), maturation and release of viral particles (E4), cell transformation (E5, E6, E7), and immortalization (E6 and E7). The E6/E7 genes also encode proteins associated with malignant lesions.[6] These proteins stimulate cell proliferation by interacting and suppressing the functions of cellular proteins p53 and pRb, involved in controlling cell proliferation. Only the E6/E7 proteins of high-risk HPVs are able to immortalize primary human keratinocytes, but not similar proteins of low-risk HPVs.

The genes of the L region (L1 and L2) encode a major and a minor capsid protein, respectively.[7] The late region L1 ORF is the most conserved among the HPVs. Its product, the L1 protein, represents 80% of the viral capsid proteins, being the most abundant protein and highly immunogenic. The L2 protein, together with the L1, contributes to the incorporation of the viral DNA into the virion.

The LCR region is between L1 and E6 and has between 500 and 1000 base pairs. In general, it is not well conserved among the HPVs and is involved in the gene expression and viral replication that occurs in the nucleus of the host cell.

Consistent evidence suggests that the genome of PVs is static and changes in their sequence by mutation or recombination are very rare events. Mutational changes seem to occur at a frequency similar to that occurring in the genome of the infected host. [4]

The life cycle of a PV is directly related to the cellular differentiation program of the host cell. The virus infects the epithelial basal cells that have the potential for differentiation. Vegetative viral functions, synthesis of DNA and capsid proteins, as well as assembly of new viruses occur exclusively in differentiated keratinocytes. [8]

PVs appear to remain in their host for long periods of life. A variety of different types of PVs can be detected in random sites of normal skin in humans and animals. This reinforces that a latent life cycle is often a characteristic of these viruses. [9]

PHYLOGENETICS

Unlike other viral groups, PVs are not mentioned by serotypes. Classification of these viruses is based on the species of origin and the degree of rela-

tionship between viral genomes.[8] They are classified into different types by comparing the nucleotide sequence of their viral genome.

PVs are grouped into different genera, which in turn are divided into different species containing one or more genotypes. Each genotype is grouped into subtypes and variants depending on the similarity of the sequence in the L1 region. [4]

To date, about 100 different types of HPVs have been fully characterized. [4] In addition to all of these HPVs that have been fully sequenced, there is a large number of additional types whose genetic sequence has not been obtained through conventional methods yet.[8]

Different genera of HPVs share less than 60% similarity in the nucleotide sequence of the major capsid of the protein L1 ORF. Different virus species within the same genus share about 60% to 70% similarity. It is considered a new HPV type when its genome shows variations greater than 10% in L1, E6 and E7 genes, and when compared with any previously known type of HPV. Differences between 2 and 10% represent new subtypes and variations under 2% are variants of types. [4]

HPVs are grouped into the following genera: *Alpha-, Beta-, Gamma-, Mu- and Nu-papillomavirus.* The other genera include PVs isolated in mammals and birds. The phylogenetic grouping sometimes reflects biological and pathological similarities, but often there are differences. For example, various types and species of the same genus can display completely different characteristics and still belong to the same genus. [4,8]

Alpha-papillomavirus (Supergroup A)

Those HPVs with tropism for genital epithelium are part of this group. However, some types belonging to this genus cause common warts. This genus includes the types of HPV that present high risk for cervical cancer such as HPVs 16 and 18, respectively allocated in species 9 and 7 of this genus, as well as low-risk types of HPV such as HPVs 6 and 11, both in species 10. At the same time, this same genus includes non-mucosal HPV types, such as HPV 7 - associated with cutaneous warts in butchers and handlers of meat, poultry and fish, HPVs found in species 4 (HPVs 2, 27 and 57), and those found in species 2 (HPVs 3 and 10), which cause common warts on the skin. [4,8,10]

Beta-papillomavirus (Supergroup B - Subgroup B1)

They are divided into five different species. HPVs 5 and 8, which belong to species 1 of this genus, are the most commonly identified in the skin of individuals with epidermodysplasia verruciformis (EV). This genus also involves cutaneous HPVs detected in the skin of the population in general without skin lesions, demonstrating the ubiquity and high incidence of asymptomatic infections. [4,5,8,10]

Gamma-papillomavirus (Supergroup B - Subgroup B2)

It covers five different species with seven different types that cause skin lesions: HPVs 4, 48, 50, 60, 88, 65, and 95. [4,5,8]

Mu-papillomavirus (Supergroup E)

It includes HPVs 1 and 63. HPV 1 is the most studied member of this group and causes common and palmar warts. [4,5,8]

Nu-papillomavirus (Supergroup E)

It has only one species: HPV 41. [4,5,8]

CLASSIFICATION

Historically, HPVs are grouped considering their tissue tropism for certain types of epithelium and according to the location where they were first isolated. Based on these characteristics, there are three main groups of HPV: cutaneous, mucosal, and associated with EV (Table 1). Mucosal HPVs are divided into low and high risk according to their oncogenic potential.

All types of HPV have tropism for cells of the stratified squamous epithelium, but there are variations in terms of affinity for different anatomical sites. For example, HPV-1 is a skin type with a high rate of replication in keratinized epithelium of the palmar and plantar regions. HPV 16 is a mucosal HPV type with preference for genital areas, and HPV 11, also mucosal, presents replication in laryngeal and genital epithelium. [11]

This classification is not entirely correct, because genital HPV types can be detected in the skin and the opposite is also possible.

TECHNIQUES FOR DETECTION OF HPV

HPV does not grow on conventional culture media and serological diagnostic methods have limited accuracy. The diagnosis of HPV infection is made by histopathology of lesions[11] or detection of viral DNA in infected cells.[12]

Hybridization techniques and polymerase chain reaction (PCR) are methods used for HPV detection.

Among the hybridization techniques used are the following:

1) *Southern blot* has high specificity and sensitivity. It allows estimates of the amount of DNA in the lesion. It has limitations due to the high diversity of types of HPV, since it does not detect the DNA of unknown viral sequences. [13]

2) *Dot blot and reverse blot* are laborious tech-

TABLE 1: Classification of HPV types into cutaneous, mucosal (genital), cutaneous and / or mucosal and cutaneous associated with epidermodysplasia verruciformis according to the location of the lesion that it most often causes

Location	HPV types
Cutaneous	1, 4, 41, 48, 60, 63, 65, 76, 77, 88, 95
Mucosal	6, 11, 13, 16, 18, 26, 30, 31, 32, 33, 34, 35, 39, 42, 44, 45, 51, 52, 53, 54, 55, 56, 58, 59, 64, 66, 67, 68, 69, 70, 72, 73, 74, 81, 82, 83, 84, 86, 87, 89
Cutaneous and/or mucosal	2, 3, 7, 10, 27, 28, 29, 40, 43, 57, 61, 62, 78, 91, 94, 101, 103
Cutaneous associated with Epidermodysplasia Verruciformis	5, 8, 9, 12, 14, 15, 17, 19, 20/46*, 21, 22, 23, 24, 25, 36, 37, 38, 47, 49, 50, 80, 75, 92, 93, 96, 107

* HPV 46 is a subtype of HPV 20
DE VILLIERS modified TABLE, 2004

niques that present similar sensitivity and good accuracy. [12,13]

3) *In situ* hybridization uses radiolabeled probes and allows for topographic localization of viral DNA in cells and tissues. Although the sensitivity of this technique is limited, it is the best method to assess the distribution of HPV in lesions and it allows for viral localization by means of other markers. [13]

4) Non-radioactive hybrid capture: this technique is safe, easy to perform and reproduce. It presents good accuracy for mucosal lesions. [13]

Polymerase chain reaction (PCR) is the most sensitive method. It is also the most widely used for viral detection and its main application is related to situations where the amount of DNA available is limited. First, it is necessary to extract the genetic material to be used. After extracting the DNA, a mixture (premix) containing deoxyribonucleotide triphosphates (dATP, dCPT, dGTP, dTTP), primers (oligonucleotides), the DNA polymerase enzyme, and a buffer solution is added. This whole mix is sent to the thermal cycler, which runs in pre-defined temperature cycles, with specific time periods for each step of the reaction (denaturation, annealing, extension). The PCR result is visualized as a band of molecular weight specific for the DNA fragment amplified by electrophoresis on polyacrylamide or agarose gel, using staining with ethidium bromide.

After amplification of viral DNA by PCR, the material needs to be subjected to a technique that permits identification of the HPV type. The techniques most often used for typing of the DNA amplified by means of PCR are: *Southern blot, dot blot,* reverse hybridization, restriction enzyme digestion, and sequencing. [12]

CLINICAL MANIFESTATIONS OF HPV INFECTION

I. Benign cutaneous lesions
CUTANEOUS WARTS

Warts are the most common and characteristic clinical manifestations of HPV infection. They are virus-induced pleomorphic tumors which affect various sites, particularly the skin of the extremities, mucosa, genital skin, and laryngeal and oral mucosas. [11]

Epidemiology, transmission and pathogenesis

HPV is a virus with worldwide distribution. Viral warts are very common viral infections, with an estimated incidence of 7 to 10% in the European population and 1% in the U.S. population.[14] These numbers increase 50 to 100 times in immunocompromised individuals; for example, in kidney-transplant recipients, reaching more than 90% 15 years after transplantation.[15] Warts occur at any age and incidence increases during the school age, with a peak in adolescence and early adulthood. [16]

HPV is transmitted through direct or indirect contact with an individual who has the lesion. Dysfunctions in the epithelial barrier by trauma, minor injuries or maceration cause loss of solution of continuity in the skin, thus allowing viral infection. After inoculation, the incubation period varies from 3 weeks to 8 months. [39] Spontaneous regression is observed in most cases. [17]

Cell-mediated immunity (CMI) seems to have a

more important role in the host response to HPV. A higher prevalence of warts and longer and persistent manifestations are observed in patients with CMI suppression, such as in kidney-transplant recipients, individuals with HIV (human immunodeficiency virus), and patients with EV. [5,11,17]

The life cycle of an HPV is directly linked to the cell differentiation program of the host cell. Infection begins when the HPV reaches the cells of the basal layer; there is no viral replication at this location and the virus just keeps its genome by amplification of a low number of copies. The replicative phase and protein synthesis occur in the suprabasal differentiated keratinocytes. [5,18] Progression time and type of lesion correlates with the quantity of viral particles detected. Younger warts present a higher viral amount when compared to old warts. Plantar warts have a higher viral load than common warts. The center of the lesion appears to be the main site of viral concentration. [18]

In benign lesions, replication of the viral genome is extracromossomal. In malignant lesions, the viral DNA is integrated into the chromosomes of the host cell and there is no viral replication. There is inactivation of expression of the E2 protein, which acts as a negative regulator of the expression of the E6 and E7 oncogenes. The last two promote cell immortalization by inhibiting cellular proteins that regulate the cell cycle (p53 and pRB), which are critical for tumor suppression. [5,18]

Histopathological characteristics of cutaneous warts

The histopathological characteristics of viral warts are papillomatosis, hyperkeratosis with prominent parakeratosis, hypergranulosis and acanthosis. [19]

The rete ridges of common warts are elongated and at the periphery point radially toward the center of the lesion (arborization). The most important characteristics in distinguishing common warts from other papillomas are: a) koilocytes (small vacuolated cells with small round strongly basophilic nucleus surrounded by a clear halo and pale cytoplasm, located in the outer stratum spinosum and stratum granulosum); they represent the viral cytopathic effect, b) vertical rows of parakeratosis, and c) foci of keratohyalin granules. These three changes are evident in young or active common warts. [19]

Some authors have suggested that certain histological features are specific to each type of HPV. Thus, histopathological examination would help in identifying different viral types. Other researchers do not agree that different HPVs determine different histologic patterns that are characteristic of each virus type; therefore, there would be no correlation between histology and HPV type. [17]

Flat warts show hyperkeratosis and acanthosis. Papillomatosis and areas of parakeratosis are not less prominent, with only a slight elongation of rete ridges being observed. There is diffuse vacuolation and increase in cell size with centralization of nuclei that become strongly basophilic and pyknotic in the spinous and granular layers. Superficial palmoplantar warts (mosaic) present histopathological aspects similar to common warts. As for deep palmoplantar warts (myrmecia), they are characterized by presenting, in the granular and spinous layers, abundant keratohyalin granules and eosinophils forming irregular inclusion bodies in the cytoplasm of keratinocytes. [17,19]

I. 1. COMMON WARTS

They are individualized papules or nodules with a rough surface. Lesions may be single or multiple, of varying sizes and are usually asymptomatic (Figure 1). Confluence of lesions can form large masses. They occur in any part of the integument, but are more common on the back of hands and fingers. [17] A frequent location in children is the knee. [11]

Isolated warts may remain unaltered for months or years, or a large number of new lesions may develop rapidly in a short period of time. The development of warts is not predictable. Approximately 65% of warts disappear spontaneously within two years. The patient's age and number of lesions do not seem to affect the prognosis. [20]

The types of HPV most involved in lesions of common warts or verruca vulgaris (VV) are: HPV 2[5,21], HPV 27, HPV 57 [21,22] (HPV types closely related to HPV 2), HPV 4 [5,23] and HPV 1. [24,25] HPV 7 is the most frequent type of warts in butchers and is also described in fish and poultry handlers. [26]

FIGURE 1: Common wart on the back of the third right finger

I. 2. PLANTAR WART

Warts that occur on the plantar region. They may lie deep, and this presentation form is known as myrmecia. They are commonly painful and caused by HPV 1. When developed more superficially, forming hyperkeratotic plaques, they are called mosaic warts, which are less painful and usually caused by HPV 2. HPV 4 is also detected in lesions of plantar warts. [27]

I. 3. FLAT WARTS

Flat warts are slightly raised, of skin color or pigmented (brownish, slightly yellowish), with flat, smooth or slightly rough surface. They are rounded or polygonal and their size ranges from 1 to 5 mm in diameter or more (Figure 2). The face and back of hands are the most common locations. The warts may be numerous and there is often linear distribution of lesions corresponding to excoriated lesion or other trauma (Koebner phenomenon). Spontaneous regression is common, usually preceded by inflammation of the lesions. The HPV types most frequently detected in lesions of flat warts are HPV 3 and HPV 10. [11.17]

I.4. FILIFORM WARTS

These are pedunculated, spiculated lesions growing in a perpendicular or oblique way in relation to the skin surface (Figure 3). They appear as isolated or multiple lesions affecting mainly the face and neck. It is a morphological variation of the common wart and the HPV types found appear to be the same found in lesions of common warts, especially HPV 2. [11]

I.5. PIGMENTED WARTS

Clinically, pigmented warts present coloration varying from gray to blackish-brown, and histopathologically, they present specific homogeneous cytoplasmic inclusion bodies (Figure 4). The HPV types detected in these lesions are HPV 4, 60 and 65. [28]

II. EPIDERMODYSPLASIA VERRUCIFORMIS (EV)

It is a rare, usually autosomal recessive, genetic disorder where there is cellular immunity defect and high susceptibility to skin cancer induced by HPV. The association between HPV and cancer was first recognized in the 50s among patients with EV. Patients with EV are highly predisposed to infection by a specific group of HPVs and at a high risk of developing cutaneous malignant tumors, resulting from the oncogenic effects of the viruses. [29]

The skin lesions appear early in childhood and are polymorphic. They are indistinguishable from flat warts when present on the face and neck, and scaly hypo or hyper-pigmented erythematous macules, similar to versicolor, when present on the trunk and limbs. Thicker, pink or violet plaques, similar to seborrheic keratosis, are also found. Malignant transformations generally begin in the fourth and fifth decades of life and predominate in sun-exposed areas, suggesting an important role of ultraviolet radiation. Premalignant lesions such as actinic keratoses and malignant lesions such as Bowen's disease and invasive squamous cell carcinoma are observed. Basal cell carcinoma is rare in these patients. [30]

The HPV types found in lesions of patients with EV are referred to as HPVs associated with epidermodysplasia verruciformis (HPV-EV). The HPV-EV types most commonly found in lesions of patients with EV are 5, 8, 9, 12, 14, 15, 17, 19-25, 28, 29, 36-38, 47, 49 and 50. HPV 3 and 10 are detected in the flat warts of these patients, similar to the general population. HPV 5, followed by HPV 8, is the most commonly found in malignant lesions. HPV 14, 17, 20 and 47 are less frequently found. [13,30,31]

With the advancement of techniques for HPV identification and typing, HPV-EVs began to be detected not only in patients with the diagnosis of EV but also in normal skin and lesions of and immunocom-
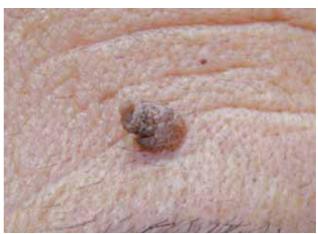

FIGURE 2: Flat wart on the ankle

FIGURE 3: Filiform wart in kidney-transplant recipient

FIGURE 4: Pigmented wart

FIGURE 5: Flat warts in epidermodysplasia verruciformis-simile in a kidney-transplant recipient

promised (Figure 5) and immunocompetent individuals - not commonly pathogenic in the latter, in patients with psoriasis and in patients with a disorder of keratinocyte proliferation such as autoimmune bullous diseases and burns. [4,9,32,33]

III. MALIGNANT SKIN LESIONS
III.1. BOWEN'S DISEASE

Bowen's disease (BD) is a squamous cell carcinoma *in situ* that occasionally progresses to invasive carcinoma.

According to the literature, HPV, particularly the high-risk mucosal types, are frequently found in lesions of extra-genital Bowen's disease (EGBD), especially in the periungual region, on hands and more rarely on feet. The detection of the virus in these locations suggests autoinoculation from genital lesions. [34]

The role of HPV is well established in genital BD, but is not fully clarified in its extra-genital forms. [35]

In EGBD, HPV detection is not restricted to the extremities (feet, hands, periungual region). High-risk HPVs are also found in lesions of EGBD in the absence of genital lesions. [35]

Other types of HPV have been detected in EGBD, such as HPV 2, low-risk mucosal HPVs 6 and 11, HPV 54, 58, 61, 62, 73, HPV 58 detected in EGBD located on elbows, fingers and toes associated with cervical and vulvar carcinoma, cutaneous HPVs such as HPV 27, HPV 76 and HPV-EV 20 and HP-EV 23.

In 2005, Zheng *et al.* [35] evaluated samples from 41 patients with EGBD and detected high-risk mucosal HPVs in 7% of the lesions (HPV 16 and 33) and cutaneous HPVs (HPV 27 and 76) in 5% of them. In lesions with high-risk HPV, the viral load was high and demonstration of viral DNA in the nucleus of cells of the spinous layer and part of the basal layer in the tissue affected by EGBD was easily detectable by the *in situ*

hybridization technique. These findings were not observed in lesions of cutaneous HPV types and in control samples of normal skin. Based on these results, the authors concluded that high-risk mucosal HPVs may play an important role in the pathogenesis of EGDB.

III.2. BASAL AND SQUAMOUS CELL CARCINOMAS

The exact role of HPV in the development of nonmelanoma skin cancer (NMSC) - squamous cell carcinoma (SCC) and basal cell carcinoma (BCC) - is not yet fully defined. [36] Growing evidence suggests that HPV has important potential in the process of skin carcinogenesis. [36,37,38]

The association between HPV and NMSC is observed both in immunocompetent patients and immunocompromised individuals. In the latter, positive detection of viral DNA in lesions is higher and presence of varied types of HPV in the same lesion is more frequent. [13,37,39] More than 90% of kidney-transplant recipients who had a transplant over 15 years ago will develop viral warts; and 40% will develop NMSC, that is, a risk of 50 to 100 times higher than the general population. In this group of patients, unlike what is observed in the general population, the most prevalent type of NMSC is the SCC, in an approximate ratio of 3:1, and lesions tend to be multiple and more aggressive. [15] In lesions of SCC of kidney-transplant recipients, HPV-EVs are the most commonly found. Detection of HPV in these lesions is high, reaching up to 80-88%. [32.37] Detection of new types of HPV-EV is common, as well as coinfection of the same lesion with more than one HPV type. In lesions of BCC, HPV detection is lower. [32,37,39]

There are few studies on HPV and NMSC lesions in immunocompetent individuals. In these individuals, HPV prevalence is lower, ranging from 35% to 55% in lesions of SCC and 43.5% in BCC. [37.39]

HPV-EVs are also the predominant types. Skin and mucosal types have also been detected; however, it can be observed in studies that show these types as the most common that the methods for detection of EV types are limited and unsatisfactory. [37.39]

In 2000, Harwood *et al.* [39] detected the presence of HPV in 84.1% of SCC lesions and in 75% of BCC lesions of immunocompromised patients who had undergone kidney transplantation, compared to 27.2% of SCC lesions and 36.7% of BCC lesions in the immunocompetent group. The prevalent HPV type in both groups was HPV-EV. Cutaneous types of HPV and infection by multiple HPV types were observed only in the group of immunocompromised. There were no statistically significant differences between BCC and SCC with respect to the HPV types present in the lesions.

In 2003, Iftner *et al.,* [24] conducted a case-control study to investigate the association between HPV and NMSC. HPV was detected in 59.7% of the 72 SCC samples analyzed and in 27.8% of the 18 BCC lesions. HPV 4 and HPV 33 were the most prevalent in the lesions of NMSC. The authors noted that high-risk mucosal HPVs may represent a risk factor for NMSC lesions in immunocompetent patients.

Forslund *et al.* , in 2007, [38] studied 349 immunocompetent patients with skin lesions: 82 lesions of SCC, 126 lesions of BCC, 49 lesions of actinic keratosis and 92 benign lesions (only one case of common wart). Paired biopsies of normal skin from the same patient were carried out, they were also submitted to PCR for detection of HPV DNA. 42 different types of HPV were identified and there was a strong association between HPV prevalence and solar exposure. HPVs from the beta-papillomavirus group, species 1 were more frequent in benign lesions, whereas HPV types of the genus beta-papillomavirus, species 2 predominated in lesions of SCC. The authors conclude that the HPV types of the genus beta-papillomavirus, species 2 are implicated in the etiology of skin SCC.

In 2008, Asgari *et al.* [36] conducted a case-control study in Caucasian immunocompetent individuals in order to analyze the role of HPV in skin lesions of NMSC. 132 patients with SCC and 95 individuals without lesions of NMSC were analyzed. Material from the lesion and the perilesional area were obtained from patients with NMSC lesions. In the control group, the material was obtained from exposed area and areas not exposed to sunlight. HPV prevalence was similar in both study groups, suggesting that HPV is widely distributed in the general population. When compared to the control group, HPV types of the genus beta-papillomavirus (HPV-EV) species 2 were more prevalent in lesions of NMSC, suggesting that certain types of HPV may be involved in the progression of NMSC; however, additional factors for cancer development would be necessary.

Detection and typing of HPV in NMSC lesions are hampered by the genomic variety of these viruses. Moreover, the techniques used by the early 1990s were less capable of meeting this viral complexity. Despite considerable progress in methods of detection and typing, the introduction of new *primers* and use of degenerate *primers*, discrepancies in the prevalence and spectrum of viral types found in these lesions in different studies are still observed.[37,39] Detection of mucosal HPVs in lesions of SCC is frequent, and the most commonly identified is HPV 16. [40] The possible association of genital carcinomas and ungual SCC was documented in several studies, suggesting autoinoculation from genital lesions. [38] More rarely, other types of HPV are also found in lesions of ungual SCC, such as HPV 2, 31, 34, 35, 58, 61 and 73. [38.40]

IV. BENIGN MUCOSAL LESIONS
IV.1. FOCAL EPITHELIAL HYPERPLASIA

Focal epithelial hyperplasia (FEH) or Heck's disease is a rare disease of the oral mucosa. It has a benign course and is associated with HPV 13 and 32. [41] It is more common in children and women and presents clear racial predominance, being more common among American Indians, Eskimos and some African communities. It is clinically characterized by multiple small papules, pinkish in color, individual or forming plaques (Figure 6). Its lesions are asymptomatic and prone to spontaneous regression. The most common location is the lower lip. Less frequently, FEH affects the upper lip, tongue, oral mucosa, oropharynx, palate and floor of mouth.

IV.2. CONDYLOMATA ACUMINATA

The most common manifestations of HPV in the genital area are anogenital warts or condylomata acuminata.[13] These lesions present as papules, nodules or soft, filiform, pinkish, sessile or pedunculated growths. They may present exophytic growth similar to a cauliflower and are usually asymptomatic. The low-risk HPVs, HPV 6 and HPV 11, are the most detected ones in lesions of condylomata acuminata. High-risk HPVs such as HPV 16 and 18 and other HPV types can be found isolated or more often coinfected with HPV 6 and 11. [42]

The Buschke-Loewenstein tumor (giant condyloma acuminatum or verrucous carcinoma of the anogenital region) is a clinically aggressive tumor, with ulcerated cauliflower-like lesions, often associated with fistulas and abscesses. They present exophytic and endophytic growth, local invasion and high recurrence rates. Metastases are very rare, and histo-

FIGURE 6: Focal epithelial hyperplasia or Heck's disease in an indigenous child of the Xingu Indigenous Park

logically, it is benign. This lesion is associated with HPV 6 and 11. [43]

IV.3. BOWENOID PAPULOSIS

The term bowenoid papulosis (BP) refers to multifocal papular lesions on the genitalia with histological features similar to SCC *in situ* or BD. Its clinical manifestation is characterized by multiple brownish or erythematous papules located in the anogenital region, affecting mostly young adults with an active sex life. Clinically, it must be differentiated from seborrheic keratosis, melanocytic nevus and common warts. BP is strongly associated with HPV 16. [13]

Despite the histological atypia and association with high-risk HPV, the course of BP in males and in young individuals is usually benign, with spontaneous regression occurring in many cases. In females, the association with cancer of the cervix suggests a less benign course, both in women who have lesions and in the partners of individuals with BP. In the elderly and immunocompromised patients, its evolution also tends to be more aggressive. [13] Other types of HPV such as HPV 18, 31-35, 39-42, 48 and 51-54 have been detected in lesions of BP. [44]

V. MALIGNANT MUCUSAL LESIONS
V.1. BOWEN'S DISEASE OF THE GENITALIA

Carcinoma *in situ* or BD of the genitalia is associated with high-risk HPVs, especially HPV 16.[45] Clinically, it presents as a plaque, usually single, without tendency to spontaneous regression and with potential to progress to SCC. Some authors consider that mucosal BD corresponds to erythroplasia of Queyrat (EQ). However, other researchers believe that they are entities with dif-

ferent histological patterns. The characteristic lesions of EQ are erythematous, velvety, bright plaques with or without infiltration, which may affect the glans, prepuce, urethra, vulva, oral mucosa, tongue and conjunctiva. EQ progression to SCC occurs in more than 30% of the cases and is higher than that observed in relation to BD. Studies on detection of HPV types in lesions of EQ are scarce. HPV 16 is the most commonly found and HPV-EV 8 has also been detected. [46]

V.2. VULVAR CANCER

Invasive vulvar cancer is usually preceded by vulvar intraepithelial neoplasia (VIN) or cervical carcinoma and often develops from long-course genital warts. The detection of HPV in vulvar SCC lesions ranges from 30% to 70%.[47] Detection of HPV in vulvar cancer is much lower than that of cervical carcinoma. This may be due to the sensitivity of the detection methods employed or the presence of new HPV types not yet identified that may be present in the lesions. [13]

HPV 16 is the most observed type in vulvar carcinomas. HPVs 18, 21, 31, 33 and 34 have also been detected in these lesions.

V.3.PENILE CANCER

Clinically, the lesions are hardened, nodular, ulcerated or erosive and may present verrucous surface. The detection of HPV in lesions of penile cancer reaches 40-70% positivity and the most frequent type is HPV 16. [48]

V.4. ANAL CANCER

HPV is detected in approximately 80 to 96% of anal cancer lesions. The most frequent type is HPV 16. Other HPVs such as HPVs 18 and 33 are also detected. [47]

V.5. CERVICAL CANCER

A large number of lesions of the cervical region are associated with HPV, from incipient cytologic abnormalities and dysplasia of varying degrees to cervical cancer. A causal relationship between HPV and cervical cancer is observed in about 90% to 100% of the cases. [49] Cervical infection by some types of HPV is a precursor in the genesis of cervical neoplasia, although other co-factors contribute to the development of neoplasia.

HPVs 16 and 18 are the two most important carcinogenic types and account for about 70% of cervical carcinomas and 50% of intraepithelial neoplasia grade 3. HPVs 31, 33, 35, 39, 45, 51, 52 and 58 have also been detected in lesions of cervical cancer. [50]

Table 2 shows a summary of the clinical manifestations most often associated with each type of HPV.

CONCLUSION

The group of cutaneous HPVs presents a significant number of different types, with great ubiquity and many regional and racial differences. The study and better understanding of HPV are necessary because these viruses are implicated in skin diseases that are very common in the practice of dermatologies as well as they are involved in the etiology of several cancers. ❑

TABLE 2: Major clinical manifestations caused by different types of HPV

HPV type	Associated clinical manifestations	HPV type	Associated clinical manifestations
1	Deep plantar wart, palmar warts, common warts	34	Orogenital warts, cutaneous Bowen's disease
2	Common warts	35	Anogenital warts, CIN, cervical cancer
3	Flat warts	36-38	EV
4	Common warts, endophytic plantar warts	39	Anogenital warts, CIN, cervical cancer
5	EV, SCC in EV	40	Anogenital warts, CIN, VIN, PIN, cutaneous lesions (rare)
6	Anogenital warts, laryngeal papillomas, Buschke-Lowenstein tumor, CIN	41	Flat warts, SCC
7	Butcher's wart	42, 43	Anogenital warts
8	EV, SCC in EV	44	Orogenital warts
9	EV	45	Anogenital warts, CIN, cervical cancer
10	Flat warts	46	Reclassified as HPV-20b, EV
11	Anogenital warts, CIN, laryngeal papillomas	47	EV, SCC in EV
12	EV	48	Cutaneous warts (rare), SCC in immunocompromised individuals
13	Focal epithelial hyperplasia	49	EV, flat warts in immunocompromised individuals
14	EV, SCC in EV	50	EV
15	EV	51	Anogenital warts, CIN, cervical cancer
16	Anogenital warts, CIN, VIN, PIN, cervical carcinoma	52, 53	Anogenital warts, CIN, cervical cancer
17	EV, SCC in EV	54	Anogenital warts, Buschke-Lowenstein tumor (rare)
18	Genital warts, CIN, cervical carcinoma	55	Orogenital warts, Bowenoid papulosis
19	EV	56	Anogenital warts, CIN, cervical cancer
20	EV, SCC in EV	57	Orogenital warts, cutaneous warts
21-25	EV	58	Anogenital warts, CIN, cervical cancer
26	Cutaneous lesions in immunocompromised patients, rarely genital lesions	59	Orogenital warts
27	Common warts	60	Plantar epidermoid cyst
2	Flat and common warts	61, 62	VIN
29	Common warts (rare)	63	Cutaneous warts (rare), plantar warts
30	Anogenital lesions, laryngeal cancer	64	Orogenital warts, VIN
31	Anogenital warts, CIN, cervical carcinoma	65	Pigmented flat warts
32	Focal epithelial hyperplasia, laryngeal papilloma	66-68	Anogenital warts, CIN, cervical cancer
		69	CIN
		70	Anogenital warts
33	CIN, VIN, cervical cancer	72	Cervical lesions

HPV type	Associated clinical manifestations	HPV type	Associated clinical manifestations
73	Anogenital warts	88	Cutaneous lesions
75-77	Cutaneous lesions in immunocompromised individuals	89	Low-risk mucosal lesions
		90	low-risk mucosal lesions
78	Cutaneous lesions, mucosal lesions (rare)	91	Low-risk mucosal lesions and cutaneous lesions
80	EV, SCC		
81	Low-risk mucosal lesions	92	EV, pre-malignant cutaneous lesions, SCC
82	High-risk mucosal lesions, benign lesions	93	EV, pre-malignant cutaneous lesions, SCC
83	Low-risk mucosal lesions	94	Cutaneous lesions, mucosal lesions (rare)
84	Low-risk mucosal lesions	95	Cutaneous lesions
85	High-risk mucous lesions	96	EV, pre-malignant cutaneous lesions, SCC
86	Low-risk mucosal lesions	107	EV, SCC
87	Low-risk mucosal lesions		

REFERENCES

1. Crawford LV, Crawford EM. A comparative study of polyoma viruses. Virology. 1963;21:258-63.
2. zur Hausen H. Human Papillomaviruses and their possible role in squamous cell carcinomas. Curr Top Microbiol Immunol. 1977;78:1-30.
3. de Villiers EM, Wagner D, Schneider A, Wesch H, Miklaw H, Wahrendorf J, et al. Human papillomavirus infections in women with and without abnormal cervical cytology. Lancet. 1987;2: 703- 6.
4. de Villiers EM, Fauquet C, Broker TR, Bernard HU, zur Hausen H. Classification of papillomaviruses. Virology. 2004;324:17-27.
5. Doorbar J. The papillomaviruses life cycle. J Clin Virol. 2005;32:Suppl1:S7- 15.
6. Syrjanen SM, Syrjanen KJ. New concepts on the role of human papillomaviruses in cell cycle regulation. Ann Med. 1999;31:175-87.
7. Orth G, Favre M. Human papillomaviruses. Biochemical and biologic properties. Clin Dermatol. 1985;3:27-42.
8. Hazard K. Cutaneous Human Papillomaviruses [Thesis]. Malmo: Lund University; 2007.
9. Antonsson A, Forslund O, Ekberg H, Sterner G, Hanson BG. The ubiquity and impressive genomic diversity of human skin papillomaviruses suggest a commensalic nature of theses viruses. J Virol. 2000;74:11636-41.
10. Bernard HU. The clinical importance of the nomenclature, evolution and taxonomy of human papillomaviruses. J Clin Virol. 2005;32S:S1-S6.
11. Sterling, JC. Viral infections. In: Burns T, Breathnach S, Cox N, Griffiyths C, ed. Textbook of Dermatology. 7 ed. Oxford: Blackwell Science; 2004. p. 25.37- 60.
12. Molijn A, Kleter B, Quint W, Van Doorn LJ. Molecular diagnosis of human papillomavirus (HPV) infections. J Clin Virol. 2005;32S:S43-51.
13. Majewski S, Jablonska S. Human papillomaviruses-associated tumors of the skin and mucosa. J Am Acad Dermatol. 1997;36:658-9.
14. Hengge UR. Papillomavirus diseases. Hautarzt. 2004;55:841-51.
15. Lindelof B, Sigurgeirsson B, Gabel H, Stern RS. Incidence of skin cancer in 5356 patients following organ transplantation. Br J Dermatol. 2000;143:614-8.
16. Kilkenny M, Marks R. The descriptive epidemiology of warts in the community. Aust J Dermatol. 1996;37:80-6.
17. Jablonska S, Majewski S, Obalek S, Orth, G. Cutaneous wart. Clin Dermatol. 1997;15:309-19.
18. Tyring SK. Human papillomavirus infections: epidemiology, pathogenesis, and host immune response. J Am Acad Dermatol. 2000;43:S18-26.
19. Xu X, Erickson L, Chen L, Elder DE. Diseases caused by viruses - In: Elder DE, Elenitsas R, Johnson Jr BL, Murphy GF, Xu X. Ed - Lever's Histopathology of the skin. 10 ed. Philadelphia: Lippincott-Williams & Wilkins; 2008. p. 649-52.
20. Berman A, Winkelmann RK. Involuting common warts. J Am Acad Dermatol. 1980;3:356-62.
21. Rubben A, Krones R, Schwetschenau B, Grussendorf-Conen E-I. Common warts from immunocompetent patients show the same distribution of human papilloma-viruses types as common warts from immunocompromised patients. Br J Dermatol. 1993;128:264-70.
22. Rubben A, Kalka K, Spelten B, Grussendorf-Conen E-I. Clinical features and age distribution of patients with HPV 2/27/57- induced common warts. Arch Dermatol Res. 1997;289:337-40.
23. Wilson CAB, Holmes SC, Campo MS, White SI, Tillman D, Mackie RM, et al. Novel variants of human papillomavirus type 2 in warts from immunocompromised individuals. Br J Dermatol. 1989;121:571-6.
24. Iftner A, Klug SJ, Garbe C, Blum A, Stancu A, Wilczynski SP, et al. The prevalence of human papillomavirus genotypes in nonmelanoma skin cancers of nonimmuno-suppressed individuals identifies high-risk genital types as possible risk factors. Cancer Res. 2003;63:7515-9.
25. Hagiwara K, Uezato H, Arakaki H, Nonaka S, Nonaka K, Nonaka H, et al. A genotype distribution of human papillomaviruses detected by polymerase chain reaction and direct sequencing analysis in a large sample of common warts in Japan. J Med Virol. 2005;77:107-12.
26. Keefe M, Al-Ghamdi A, Coggon D, Maitland NJ, Egger P, Keefe CJ, et al. Cutaneous warts in butchers [see coments]. Br J Dermatol, 1994;130:9-14.
27. Lai JY, Doyle RJ, Bluhm JM, Johnson JC. Multiplexed PCR genotyping of HPVs from plantaris verrucae. J Clin Virol. 2006;35:435-41.
28. Egawa K, Honda Y, Inaba Y, Ono T. Pigmented viral warts: a clinical and histopathological study including human papillomavirus type. Br J Dermatol. 1998; 138: 381-9.
29. Orth G. Human Papillomaviruses Associated with Epidermodisplasia Verruciformis in Non-Melanoma Skin Cancers: Guilty or Innocent? J Invest Dermatol. 2005;125: XII-XIII.
30. de Oliveira WRP, Festa Neto C, Rady PL, Tyring SK. Clinical aspects of epidermodysplasia verruciformis. J Eur Acad Dermatol Venereal. 2003;17:394-8.
31. Majewski S, Jablonska S. Do epidermodysplasia verruciformis human papilloma viruses contribute to malignant and benign epidermal proligerations? Arch Dermatol. 2002;138:629-54.
32. Berkhout RT, Bouwes-Bavinck JN, Ter-Schegget T. Persistence of human papillomavirus DNA in benign and (pre)malignant skin lesions from renal transplant recipients. J Clin Microbiol. 2000;38:2087-96.
33. Sterling JC. Human papillomaviruses and skin cancer. J Clin Virol. 2005;32S:S67- 71.
34. Derancourt. C, Mougin C, Chopard-Lallier M, Coumes-Marquet S, Drobacheff C, et al. Oncogenic human papillomaviruses in extra-genital Bowen disease revealed by in situ hybridization. Ann Dermatol Venereol. 2001; 128: 715-8.
35. Zheng S, Adachi A, Shimizu M, Shibata SI, Yasue S, Sakakibara A, et al. Human papillomaviruses of the mucosal type are present in some cases of extragenital Bowen's disease. Br J Dermatol. 2005;152:1243-7.
36. Asgari MM, Kiviat NB, Critchlow CW, Stern JE, Argenyi ZB, Raugi GJ, et al. Detection of human papillomavirus DNA in cutaneous squamous cell carcinoma among immunocompetent individuals. J Invest Dermatol. 2008;128:1409-17.
37. Harwood CA, Proby CM. Human papillomavirus and non-melanoma skin cancer. Curr Opin Infect Dis. 2002;15:101-14.
38. Forslund O, Ifner T, Andersson K, Lindelo B, Hradil E, Nordin P, et al. Cutaneous human papillomaviruses found in sun-exposed skin: beta-papillomavirus species 2 predominates in squamous cell carcinoma. J Infect Dis. 2007;196:876-83.
39. Harwood CA, Surentheran T, Mcgregor JM, Spink PJ, Leigth IM, Breuer J, et al. Human papillomavirus infection and non-melanoma skin cancer in immunosup-pressed and immunocompetent individuals. J Med Virol. 2000;61:289-97.
40. Alam M, Caldwell J, Eliezri Y. Human papillomavirus associated digital squamous cell carcinoma. Literature review and report of 21 new cases. J Am Acad Dermatol. 2003;48:385-93.
41. Vera-Iglesias E, García-Arpa M, Sánchez-Caminero P, Romero-Aguilera G, De La Calle C. Focal epithelial hyperplasia. Actas Dermosifiliogr. 2007;98:621-3.
42. Chan PKS, Luk ACS, Luk TNM, Lee KF, Cheung JLK, Ho KM, et al. Distribiuion of human papillomavirus types in anogenital warts of men. J Clin Virol. 2009;44:111-4.
43. Asato Y, Taira K, Yamamota Y, Uezato H. Detection of human papillomavirus type 11 in a case of Buschke-Löwenstein tumor. Eur J Dermatol. 2008;18:329-31.
44. Hama N, Ohtsuka T, Yamazaki S. Detection of mucosal human papilloma virus DNA in bowenoid papulosis, Bowen's disease and squamous cell carcinoma of the skin. J Dermatol. 2006; 33:331-7.
45. Bonvicini F, Venturoli S, Ambretti S, Paterini P, Santini D, Ceccarelli C, Zerbini M, Musiani M. Presence and type of oncogenic papillomavirus in classic and in diffe-rentiated vulvar intraepithelial neoplasia and keratinizing vulvar squamous cell carcinoma. J Med Virol. 2005;77: 102-6.
46. Wieland U, Jurk S, We,enborn S, Krieg T, Pfister H, Ritzkowsky A. Erythroplasia of Queyrat: coinfection with cutaneous carcinogenic human papillomavirus type 8 and genital papillomaviruses in a carcinoma in situ. J Inv Dermatol. 2000; 115: 396-401.
47. de Vuyst H, Clifford GM, Nascimento MC, Madeleine MM, Franceschi S. Prevalence and type distribution of human papillomavirus in carcinoma and intraepithelial neoplasia of the vulva, vagina and anus: A meta-analysis. Int J Cancer. 2009;124:1626-36.
48. Heideman DAM, Waterboer T, Pawlita M, Van Diemen PD, Nindl I, Leijte JA, et al. Human papillomavirus-16 is the predominant type etiologically involved in penile squamous cell carcinoma. J Clin Oncol. 2007;25:4550-6.
49. Muñoz N, Bosch FX, De Sanjosé S, Herrero R, Castellsagué X, Shah KV, et al. Epidemiologic classification of human papillomavirus types associated with cervical cancer. N Engl J Med. 2003;348:518-27.
50. zur Hausen H. Papillomaviruses and cancer: from basic studies to clinical application. Nat Rev Cancer. 2002;2:342-50.

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