Review Articles

High-grade cervical intraepithelial neoplasia, human papillomavirus and factors connected with recurrence following surgical treatment

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Summary

Although effective strategies for preventing cancer of the uterine cervix exist, this disease continues to be a serious health problem worldwide, especially in developing countries. Today, the role of human papillomavirus (HPV) as a causal factor for the emergence of cervical cancer and its precursor lesions is well established, and prevention programs against cervical cancer are based on detecting cervical intraepithelial neoplasia (CIN). HPV present immunological evasion mechanisms that inhibit detection of the virus by the host, which may result in persistent chronic infection and irrevocably comprise the host defenses. Conization is the surgical technique most used for treating high-grade CIN, since it makes it possible to exclude invasive neoplasia, evaluate resection margins and preserve fertility. However, several factors have been considered to be indicators for residual disease. This review had the aim of covering some factors relating to persistence and recurrence of high-grade CIN following conization.

Key words: Cervical intraepithelial neoplasia; Human papillomavirus; Conization; Recurrence; Immunological response.

Introduction

Human papillomavirus (HPV) is an epitheliotropic deoxyribonucleic acid (DNA) virus belonging to the family *Papillomaviridae.* It is mostly transmitted sexually and is considered to be the principal factor in the pathogenesis of cervical cancer and its precursor lesions. Today, more than 100 types of HPV have been detected, of which 40 infect the human genital tract. Of these, 15 are associated with cervical carcinogenesis [1, 2]

Prevention of cervical cancer is potentially effective since several forms of intervention for combating the multiple manifestations of the disease now exist. Nonetheless, estimates indicate that this is a disease with high prevalence, incidence and mortality, especially in developing countries [2].

In Brazil, the National Cancer Institute (INCA) states that cancer of the uterine cervix is the third most common malignant neoplasia among women, only surpassed by skin cancer (non-melanoma) and breast cancer, and it constitutes the fourth most important cause of death due to cancer among women. It is also forecast that there will be around 18,680 new cases of this disease in Brazil in the year 2008, with a risk estimated as 19 cases for every 100,000 women [3].

HPV infects the basal layer of the epithelium in the metaplastic region of the transformation zone of the uterine cervix, where the cells present the greatest vulnerability [4]. The relationship between HPV and carcinogenesis depends fundamentally on the type of virus (high or low oncological risk), viral load, persistence and integration of the virus with the host cell, genetic constitution and the individual's immunological response [5].

In the host, according to the type of virus, HPV may remain in the episomal form or be incorporated into the DNA. In the episomal form, its activity accompanies cell differentiation by the layers of the epithelium, thus resulting in surface cells with copies of the HPV that are ready for transmission. When incorporated into the DNA, the viral DNA forms part of the host's cell genome, thereby inducing the production of certain proteins that modify cell activity through a sequence of reactions that result, after a varying period of time, in cell proliferation and inhibition of apoptosis [6, 7].

Cervical intraepithelial neoplasia (CIN) can be divided into mild dysplasia (CIN I), moderate dysplasia (CIN II) and severe dysplasia or in-situ carcinoma (CIN III), and this may progress to invasive cancer if it is not treated [8].

The low-risk types of HPV that only induce benign genital warts include HPV 6 and 11. The high-risk group includes HPV 16, 18, 31, 33, 45 and 56, and these types are associated with the development of anogenital cancer and can be detected in 99% of cervical cancer cases [9, 10].

The viral oncoproteins E6 and E7 interact with the tumor-suppressor proteins (p53 and pRB) to alter their cycles and functions [11-13]. However, there is evidence suggesting that, in cases of HPV infection, other addi-

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tional factors are involved in the progression of precursor lesions, such as starting sexual activity at an early age, use of oral hormonal contraceptives, parity, smoking, multiple sexual partners and immune system deficiency. Thus, although virtually all cases of CIN III or cervical cancer contain HPV of high oncogenic risk, it seems that for this virus to cause lesions, various cofactors are involved [14, 15].

The incidence of cervical cancer and precancerous lesions is greatly reduced by screening for precursor lesions that can be treated by exfoliative cytological procedures, even in women who undergo total hysterectomy because of the risk of vaginal intraepithelial neoplasia [16]. The diagnostic methods for evaluating morphological abnormalities in lesions induced by HPV include clinical examination, colposcopy, oncological colpocytological analysis and histological analysis [17].

Molecular biology techniques have modified the knowledge of the epidemiological profile of HPV infection through recognizing the different subtypes of the virus. These techniques are considered to be an essential prerequisite for clinical studies that associate HPV with carcinogenesis. The set of techniques for detecting the genetic material (DNA) of HPV in cervical samples consists of molecular hybridization of nucleic acids of Southern blot type, hybrid capture, in situ hybridization and the polymerase chain reaction (PCR). HPV DNA analysis may improve clinical sensitivity in detecting precancerous lesions in cervical cancer cases. It is also used for monitoring after surgical procedures to treat CIN III [18].

In cases of high-grade CIN, the choice of therapeutic method depends on various factors, such as lesion severity, age, patient's clinical state, patient's reproductive desires, extent of the colposcopic image (grade, location and number of lesions), type of HPV and the technological capacity of the clinic. Although regression of CIN may occur [19, 20], excision methods and particularly classical cold conization, high frequency loop surgery (LEEP, loop electrosurgical excision procedure) and laser surgery are the techniques most used for treating high-grade CIN. These techniques make it possible to exclude invasive neoplasia, evaluate resection margins and preserve fertility. Clinical follow-up for women who undergo conization has the aim of detecting persistence or recurrence of CIN [21].

Factors relating to persistence and recurrence of high-grade cervical intraepithelial neoplasia following conization

Lesions classified as high-grade CIN are lesions with a greater likelihood of persistence and progression. Patients with high-grade CIN need to be divided into two treatment groups: those among whom the presence of an invasive lesion has been securely ruled out, and those among whom doubts persist. The latter group is related to recurrences and persistence of the disease [22].

Various factors have been considered to be indicators for residual disease, such as age, skin color, CIN grade, lesion severity, presence of lesions in several quadrants (extensive or multifocal lesions), smoking, margin involvement, gland involvement, number of mitoses, state of endocervical curettage, pregnancy and delivery, socioeconomic status, marital status, immunosuppression, type of therapy and oncogenicity of the HPV involved [23].

The presence of remaining dysplastic cells after the apparent complete excision of the lesion can, in the first instance, be explained by supposing that the histopathological result was incorrect because of an insufficient number of thin sections cut from the surgical specimen. A second hypothesis would be that the dysplastic epithelium was friable and could easily be separated from the stroma, such that damage occurred while removing the surgical specimen. Alternatively, there is the possibility that the intraepithelial lesions might be multifocal within the transformation zone. A further hypothesis that can always be borne in mind is that the appearance of a new lesion is unrelated to the one diagnosed previously [24].

Even if HPV infection is directly connected to CIN, it may spontaneously regress without leaving any histological mark [25]. The great majority of women present transitory infections due to this virus that are eliminated naturally without lesion development. CIN, and especially low-grade CIN, also regresses spontaneously in most women, and particularly in younger women [26]. These characteristics of HPV infection and the natural history of the neoplasia are complicating factors for clinicians following-up women who have undergone conization. Tests performed after the procedure that may be positive for HPV theoretically may not represent unfavorable evolution but, rather, transitory reinfection without clinical repercussions. In the following, some factors relating to persistence and recurrence of CIN following conization are presented.

Human papillomavirus

Certain risk factors predisposing towards persistence of HPV in CIN cases following conization have been suggested. However, variable and sometimes controversial results have been reported. Age, preventive Papanicolaou smears, HPV type, lesion grade from colposcopy-guided biopsy, lesion grade from the cone, state of the resection margin and conization with positive margins have been described as risk factors for persistence of HPV [27].

It is rare to detect HPV in morphologically normal squamous tissue surrounding the sites where CIN is present. This does not rule out occult infection within the natural history of CIN, but it indicates that when HPV-induced cervical lesions occur, the occult infection does not spread into the surrounding normal epithelium [28].

The viral load may be influenced by various factors, among which the sample quality, site from which it was collected (endocervix, ectocervix or pouch base), size of the histological lesion and, especially, the number of epithelial cells obtained [29].

Persistence of HPV infection after conization indicates

that the cervix is still exposed to a major oncogenic factor, with increased risk of recurrence and progression [30, 31]. After CIN excision, the HPV DNA detection rate is lower and, in women with HPV still detectable, the viral load tends to be lower than what was found before conization. This can be explained through the observation that abnormal epithelial cells of a preneoplastic or clearly invasive nature are rich in HPV genetic material. This material is integrated with the nucleus and promotes anarchical proliferation. Nevertheless, complete removal of the HPV-induced lesions does not ensure virus eradication [32].

Persistence of the virus following CIN excision, especially in cases of high viral load, may represent the existence of residual high-grade lesion or even recurrence, including in cases in which no abnormalities are found in oncological colpocytological tests. Infection with new types of HPV after conization may also be associated with recent exposure to risk through sexual relations with different partners, and thus constitutes new infection [33, 34]. Persistence of high viral loads of HPV of high oncogenic risk after conization, especially types 16 and 18, is strongly indicative of a risk of recurrence and even progression of CIN to invasive carcinoma or recurrence of CIN III [35]. In contrast, women in whom the viral load decreases to undetectable levels after conization present very low risk of developing cancer over the coming years.

Surgical margins

The frequency of findings of compromised margins in the surgical specimen from conization depends on several factors, such as the degree of CIN severity, involvement of the endocervix, dimensions of the cone and conization technique. Thus, there is a correlation between compromised surgical margins in the cone and the residual disease rate [36].

After conization, compromised surgical resection margins due to CIN or carcinoma may occur in 10 to 45% of the cases. The variability in this percentage is mainly due to the surgeon's level of experience and inability to view the endocervical resection margin. This latter is of importance for patient follow-up [37].

Anatomopathological findings of involvement of the surgical margins due to CIN are frequently used to clinically predict the presence of residual lesion. When this occurs, one of the treatments proposed is total hysterectomy. Some studies have shown a direct correlation between involvement of the cone margins and the presence of residual CIN in the hysterectomy specimen [37].

Studies have shown that there is no difference in the recurrence rate between cases using classical conization and LEEP. In both situations, more than 95% of the recurrences occur within the first five years. Extensive endocervical involvement of the cone margin following LEEP for treating CIN II and III strongly predisposes towards residual disease [38].

Recurrence or persistence of the disease may not be directly related to the state of the cone margins. More than 70% of the women with compromised margins are found not to present residual disease when undergoing hysterectomy. These women may present compromised margins because of the surgical trauma, which through the regenerative process may give rise to an immunological response. Nevertheless, women with cones presenting free margins may present recurrence due to multifocal disease, inadequate evaluation of the surgical specimen or persistence of HPV [39].

Extension to glands

In addition to positive margins, gland involvement has also been considered valuable as a prognostic factor predisposing towards lesion recurrence. Dysplastic cells may remain in the endocervical glands that are covered with normal epithelium, and they may progress to more advanced degrees of dysplasia or even to invasion of the cervical stroma, without altering the cytological and colposcopic findings. This phenomenon may explain cases of detection of invasive carcinoma in patients who previously underwent conization and who, during postoperative follow-up, presented normal results from cytological tests [40]. However, results obtained by our research group have not shown that extension to glands is associated with recurrence of CIN III [41].

Number of mitoses

In cases of high-grade CIN, the cell organization is altered over almost the whole thickness of the epithelium and the cells present a high degree of nuclear and cytoplasmic abnormalities, with typical and atypical mitoses. In atypical mitoses, it is common for parts of chromosomes or whole chromosomes to be missing, thereby leading to polyploidy, aneuploidy or other anomalies of greater complexity. This may be lethal to daughter cells, thus inducing apoptosis, but it may also cause the appearance of cell closes that are more aggressive, because of the deletion of other cell growth-regulating genes [42].

The presence of tripolar atypical mitoses in compromised margins can be considered to be a morphological criterion for progression of CIN I to CIN III. Greater numbers of mitoses are related to a greater CIN recurrence rate [43].

Human immunodeficiency virus

The incidence of CIN recurrence recorded among individuals with the human immunodeficiency virus (HIV) is very high and the literature indicates that, in such cases, conization should be performed for all grades of CIN. Clinical and laboratory control for HIV-infected patients, by means of $CD4^+$ cell counts and quantification of the viral load, seems to be closely related to persistence and/or recurrence of the lesion. High recurrence

rates have been observed after all types of treatment implemented among immunosuppressed patients, even after hysterectomy [44, 45].

Genetic abnormalities

When HPV infects cells, there may be an interaction between the HPV genome and that of immature host cells, thereby impeding cell differentiation and maturation. Thus, transformed cells contain viral DNA and infections persisting for 10-20 years enable the development of additional genetic abnormalities, with progression of low, moderate and high-grade lesions to invasive cancer [46].

Infection with HPV of high oncogenic risk seems to be important with regard to the occurrence of high-grade CIN. However, recurrence of CIN has only been observed in cases with hypermethylation of three or more of the eight genes studied (p16, RARBeta, GSTP1, MGMT, p14, TIMP3, E cad and DAPk), thus showing the importance of genetic abnormalities in cases of cervical carcinogenesis [47].

Viruses, together with chemical substances and radiation, appear to be causes of cancer. Viral carcinogenic action is associated with genetic abnormalities in control processes for the cell cycle and cell differentiation. In cancerous cells, the genetic control is faulty and these cells reproduce in an uncontrolled manner to form tumors instead of the normal cells that, over the course of the natural process of the life cycle, replicate, differentiate into different types and then die [48].

Smoking is considered to be one of the principal factors associated with persistence of viral activity, thereby increasing the risk of progression or recurrence of lesions in patients with CIN associated with HPV infection [49].

The two main mechanisms through which the smoking habit contributes towards cervical oncogenesis include direct exposure of the DNA of cervical epithelial cells to nicotine and cotidine. These have been found at high concentrations in the cervical mucosa and may induce mutations and abnormalities during gene activity. Indirectly, these substances inhibit the cell response of the immunological system and enable viral replication and infection of adjacent cells, thus increasing the possibility of incorporating the virus into the cell genome [49].

Follow-up for high-grade cervical intraepithelial neoplasia after conization

Independent of the surgical margins, the follow-up for CIN subsequent to conization should include oncological colpocytological tests, colposcopy and also the test to detect HPV DNA. The latter test has high predictive value for post-treatment follow-up, since HPV acts as a marker for undetected residual disease. It is observed that women with cytological abnormalities or persistence of HPV following conization are at greater risk of presenting such recurrence, although the positive predictive value of these tests is low. On the other hand, the HPV test has high negative predictive value, i.e., when HPV is not detected six months to one year after conization due to CIN II or III, it very unlikely that the woman will suffer recurrence [50, 51].

The potential for recurrence depends not only on whether the dysplasia is completely removed, but also on the individual's sexual habits and immune response and the oncogenicity of the HPV involved [52].

Local and systemic inflammatory response in patients with CIN III

Each individual's immunological response seems to be the principal determinant for occurrence, progression and recurrence of HPV infection. However, the exact mechanisms that trigger an efficient immune response against HPV-related lesions may relate to activation of the immunological system or the host's genetic composition [53].

Data on the natural history of HPV infection suggest that, soon after the initial infection, the viral DNA is transitorily detectable (within two years), with spontaneous resolution in up to 80% of such cases. Most women are able to develop an affective immune response that avoids the development of cervical carcinoma. However, it is not known whether carcinomas appear only in women who maintain detectable levels of viral DNA over the course of decades, or whether some carcinomas develop from late-stage reactivation of transitory infections after a long period of time [54].

HPV efficiently evades the innate immunological response and delays the activation of the adaptive immunological response. The host dendritic cells are exposed to low levels of protein in a non-inflammatory environment for a period of time and, as a result, an absence of local immunological response may become established in the infected mucosa. In this environment that is operationally tolerant to the HPV antigen, the host defenses become irrevocably compromised and HPV or non-HPV antigen-specific effector cells are recruited to the infected area, or their activity is under-regulated. Thus, if there is a lack of regulation of high-risk HPV E6 and E7 with high protein expression, in the presence of persistent HPV infection, and this does not result in an immunological response mediated by activated effector cells, evolution to high-grade intraepithelial squamous lesions and invasive carcinoma mediated by HPV will be unimpeded [55].

There is controversy regarding studies on inflammatory responses in CIN cases. Decreased numbers of CD4-positive T cells and an inverted CD4/CD8 ratio have been reported in cases of cervical infection due to HPV, at all grades of CIN. Conversely, increased total numbers of lymphocytes have been reported in 18 cases of CIN II and III. In addition to this, studies using different markers have shown decreases in numbers of macrophages, in comparison with normal controls [56, 57].

There are changes in the numbers and functional capacity of neutrophils circulating in patients with neoplasia of the uterine cervix, and these changes are asso-

ciated with invasive or preinvasive staging. This suggests that even CIN III is a systemic disease, thus indicating that these cells participate in the immune response to the tumor [58].

In patients with neoplasia of the uterine cervix of different staging, changes in the number and function of circulating mononuclear cells have been observed. Following surgical treatment, there are changes in the number and functional capacity of mononuclear cells only in cases with advanced staging, thus suggesting that circulating factors are produced by the neoplastic cells [59].

In CIN III patients presenting recurrence following conization, greater positivity (expression) of CD3 T lymphocytes has been found, in comparison with patients without recurrence. This suggests that strong positivity for this antibody is a factor indicative of worse evolution [60].

Conclusion

HPV presents evasion mechanisms that block efficient immunological responses and cause persistent infection to become established. This may evolve to high-grade CIN or invasive carcinoma if not treated.

In this review, some factors that contribute towards persistence and recurrence of high-grade CIN following surgical treatment have been discussed. It is essential for such patients to be aware of the importance of continuing with medical care for the remainder of their lives, since it is possible for the disease to occur or reoccur even several years after conization. In this respect, it is emphasized that there is a greater chance of recurrence in cases with compromised surgical margins, greater numbers of mitoses in the lesion, presence of HPV 18, greater numbers of methylated genes and presence of local CD3 T lymphocytes.

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