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HPV studies in sexual couples: prevalence, pathology and intervention

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2008

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citation for published version (APA)

Hogewoning, C. J. A. (2008). *HPV studies in sexual couples: prevalence, pathology and intervention*. [PhD-Thesis – Research external, graduation internal, Vrije Universiteit Amsterdam].

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Chapter 8

General Discussion



Here, we will discuss the differential diagnosis of penile lesions with special emphasis on flat penile lesions and their potential role in HPV-mediated carcinogenesis and HPV transmission. Furthermore, the relationship between viral load and the occurrence of flat penile lesions in men and CIN in women will be discussed.

1 Penile lesions

In search for genital HPV associated lesions in men Barasso *et al.* started in 1987 to use penoscopy on 8000 male partners of women with CIN or other HPV related lesions.¹ In more than 50% of these men HPV-associated genital lesions could be demonstrated, arguing for a causal involvement of sexually transmittable HPV infections in such lesions. Subsequent studies showed similar findings.^{2,3} However, the penile lesions observed represented various clinical manifestations and uniformity in their description was lacking. This not only hampered comparison of various studies, but also definitive conclusions to be made about what lesion type actually reflects an HPV origin and deserves more attention in the context of penile precancer and source for HPV transmission to sexual partners. In order to reach uniformity in the interpretation of the penoscopic findings we have used a more simple and transparent classification.⁴ The penile lesions observed in male sexual partners of women with CIN were classified as flat penile lesions, papular penile lesions, PPPs and condylomata acuminata. Amongst these, flat penile lesions, the characteristics of which are detailed in paragraph 1.2, were not only most common in these men (83%), but men with these lesions also displayed the most frequent association with hr-HPV presence in penile swabs (in about 70% of cases). As summarized in paragraph 1.2., sufficient evidence is collected in favour of these hr-HPVs being involved in these flat penile lesions. Given their potential importance as penile precancerous lesions and source of HPV, transmission to sexual partners HPV-associated flat penile lesions formed the focus of subsequent studies.

1.1 Differential diagnosis of hr-HPV-associated flat penile lesions

There are several characteristics on the basis of which hr-HPV-associated flat penile lesions can be differentiated from a number of other penile lesions. Firstly, flat penile lesions should be distinguished from other lesions based on clinical criteria. This requires repeated physical examination, combined with penoscopical inspection of the penis before and after acetic acid application (VIA, visual inspection after acetic acid). VIA is a relatively simple and inexpensive method to demonstrate flat penile lesions. It can be learned after a relatively short period of time. Lesions found can easily be documented by photography and when in doubt studied at a later moment with an experienced dermatologist or gynaecologist. Secondly, information

about the history of possible earlier penile lesions is important to exclude false positive scorings upon visual inspection. Thirdly, it is important to realize that flat penile lesions are barely visible with the naked eye. Since they stain white only after acetic acid application they are often referred to as acetowhite or subclinical lesions.

Although many penile lesions stain in the acetic acid test, most are already clearly visible before acetic whitening and therefore should be excluded as flat penile lesions.

After application of acetic acid flat penile lesions have distinct penoscopic properties. They show a white reaction on well-demarcated areas of 1 mm² to >2 cm². They are usually slightly elevated and show often punctuation or mosaicism as in cervical colposcopy (vascular patterns of capillary loops). Flat penile lesions are mainly found on the inner side of the foreskin, near or on the frenulum, and coronal sulcus. The number of flat penile lesions may vary from 1 to 10, but usually between 2 and 4 of these lesions are found per patient.

Finally it is necessary to demonstrate hrHPV in these lesions with a clinically validated test performed on a penile swab of the lesion.

Using the criteria described above flat penile lesions can be differentiated from:

a. Red plaques with papulo-squamous features.

These lesions can also be manifestations of inflammatory diseases such as Psoriasis, Lichen Planus, some forms of balanitis or of premalignant diseases such as Erythroplasia of Querec, Bowen's Disease or Bowenoid papulosis. However, a history of earlier skin disease, the general aspect of the lesions, other than genital localisations of the lesions and visibility before acetic acid application will give sufficient help in differentiating.

b. Skin-coloured and white lesions.

These lesions are probably the most difficult in the differential diagnosis of flat penile lesions. They consist among others of some forms of anogenital warts ie flat or inverted condylomata. Especially when small, these lesions, are very difficult to differentiate from flat penile lesions, because they also react on acetoacid colouring and are well demarcated. They are also slightly elevated and although their location is less specific around the sulcus and frenulum of the inner skin of the penis and most of the times also present on the shaft, they can mimic FPL very nearly. Hr-HPV detection and follow-up will help to differentiate FPL from these lesions.

Most other skin-coloured lesions, like small skintags, papules and PPP's are far more elevated and thus more easily to recognise.

c. White patches and plaques.

Lichen Sclerosis, Vitiligo, Hyperkeratosis overlying eczematous and (pre-)malignant disease or other diseases, particularly if they are pruritic enough to result in scratching and therefore hyperkeratosis should be carefully differentiated. When small and located around the sulcus and frenulum they could easily be regarded as FPL, however they are visible before acetic acid application, and

often located on other surfaces than FPI and will mostly react on local therapy with corticoids. When in doubt a small punch biopsy can always be of help in all the above mentioned lesions

1.2 Association of hr-HPV and flat penile lesions

The first indication for an involvement of hr-HPV in flat penile lesions came from DNA in situ hybridisation (DISH) analyses on biopsies taken from these lesions, showing the frequent presence of HPV DNA.^{2,3} In addition, it has been demonstrated by us that men with FPL reveal the highest association with hr-HPV as determined on penile swabs compared to other penile lesions. Absence or barely detectable hr-HPV signals were mostly seen in men with non-FPL penile lesions such as papular penile lesions, PPPs and penile condylomata acuminata and are due to very low HPV copy numbers.⁴ Therefore if hr-HPV is found in these non-FPL lesions it is only detected with very sensitive PCR techniques but not by a relative insensitive HPV detection technique such as ISH.⁵ In addition, real-time PCR for quantitation of HPV 16 revealed that the presence of FPL was associated with relatively high HPV 16 copy numbers in penile swabs, suggesting that these viral infections are clinically meaningful. Moreover we demonstrated that the larger the size of the FPL the higher the hrHPV copy number was.

Another finding in favour of an etiological relationship between hr-HPV and flat penile lesions is the location of FPL on the penis. The preferred sites of FPL on the penis (ie. inner side of the foreskin, near or on the frenulum, and coronal sulcus) are also known as the preferred primary sites of hr-HPV infection in men.^{6,7,8} Finally, as a circumstantial argument we found that in male partners of women with HPV associated CIN more and larger FPL's with higher HPV copy numbers were found than in male sexual partners of women without CIN.⁹ All these findings strongly argue that FPL constitute the HPV reservoir in men.

1.3 hr-HPV-associated flat penile lesions as penile cancer precursor

Many penile cancers arise at sites of infection, chronic irritation or injury. Inflammation, caused by poor penile hygiene, smegma retention, phimosis or some other local penile conditions like urethral stricture and scarring play an essential role in the development of penile cancer.^{8,10} Other risk factors for developing penile cancer, like number of sex partners, genital warts and other sexual transmitted diseases are clearly related to hr-HPV infection. Indeed several studies have shown that hr-HPV is associated with a substantial part of penile carcinomas. Although hr-HPV DNA has been demonstrated initially in 30-100% of penile carcinomas depending on the method of HPV-detection and the histological subtype, it has now been established that

hrHPV is etiologically involved in 35-45% of all penile carcinomas.^{10,11,12} The large majority of these hr-HPV infections concern HPV 16.^{10,11,12}

Like vulvar carcinoma and cervical carcinoma the vast majority of penile squamous cell carcinoma is likely to develop from progression of precursor lesions. It is plausible to assume that hr-HPV positive penile carcinoma develops from high grade hr-HPV containing precursor lesions, histologically classified as PIN3. Clinically these lesions manifest themselves as Erythroplasia of Queyrat (EQ), M.Bowen or Bowenoid Papulosis (BP) of which EQ has the highest (40%) and BP the lowest (<1%) risk to develop in penile carcinoma.⁴⁴ Like in the cervical cancer model in women, hr-HPV bearing high-grade intraepithelial neoplasias of the penis (PIN3) may develop from persistent hr-HPV infections. However development of persistent penile hr-HPV infections is very rare, since, as shown in our studies, penile hr-HPV infections display high spontaneous clearance rates. Hr-HPV containing FPL, histologically characterised by squamous hyperplasia and PIN1 are considered the clinical manifestation of a productive HPV infection which only rarely converts into a persistent hrHPV infection associated with a hr-HPV-related high grade precursor lesions (PIN3) of penile cancer. Thus although we assume that hr-HPV bearing FPL may be the precursor lesions of hr-HPV containing PIN3 this transition is much more rare than the transition of CIN1 to CIN3 in cervical carcinoma. Although there is no direct evidence we speculate that at least the hr-HPV bearing subset of the well-known clinical manifestations of high-grade PIN, ie. Erythroplasia of Querat, Morbus Bowen and Bowenoid papulosis may develop from hr-HPV containing flat penile lesions.^{14,15} Support for this concept was obtained from our studies showing that hr-HPV-containing flat penile lesions mainly showed the histological equivalent of a productive infection *i.e.* squamous hyperplasia/low-grade PIN, whereas hr-HPV bearing high-grade PIN being the histological sign of a persistent, transforming HPV infection was only observed in less than 3% of FPL.

This low prevalence of high-grade PIN in FPL corroborates with the high regression rate of FPL. More than 90% cumulative regression was shown after 5 years, and the median regression time was 12.4 months. Of 118 men studied only 5 were HPV positive after this period, all of them containing HPV 16 (Hogewoning, unpublished data).

A remark should be made here about lichen sclerosus (LS). In men, like in women the association of LS and SCC is also well established, LS preceding in 30% of all penile carcinomas.^{28,29,30,31,32,33,34} Although the presence of hr-HPV in men with LS is higher then in controls, this development seems independent from the presence of hr-HPV.^{34,45}

1.4 Putative role of flat penile lesions in HPV transmission

There is sufficient evidence that HPV is sexually transmitted both via men and women.⁴⁶ From the partner study we have now collected circumstantial evidence that hr-HPV-associated flat penile lesions form the reservoir of hr-HPV infections in men and are therefore relevant for HPV transmission to their sexual partners.¹⁶ Firstly, as mentioned earlier, the location of flat penile lesions (ie. the inner aspect of the foreskin, the frenulum and around the coronal sulcus) is also the preferred side where hr-HPV is demonstrated.^{6,7,8} Secondly the histology of the large majority of FPL is squamous metaplasia/PIN1, the histological equivalent of a productive HPV infection. Moreover, these lesions do not only show a high association with hr-HPV, as determined by PCR on penile scrapes of these lesion sites, but also display high viral copy numbers. They are therefore considered as the main cause of viral spread.

In addition, amongst sexual partners that were HPV positive, HPV type-specific concordance was higher than expected by chance.¹⁷ Interestingly, next to the presence of flat penile lesions also the presence of concordant hr-HPV types was associated with higher penile viral loads. It is particularly this strong relationship between increased viral loads and both HPV type-concordance and flat penile lesions that argue for these lesions being involved in viral transmission.

Conclusively, in combination, a positive test result of a penile scrape with a clinically validated hr-HPV test and the presence of flat penile lesions reflect a high likelihood of a productive viral infection that may trigger viral transmission to sexual partners.

Blockage of viral transmission can be achieved by condom use.^{18,19,20} It is good to realise that many epidemiological studies concerning HPV infections in men so far did not take into account the presence of flat penile lesions and consequently firm conclusions about the clinical significance of these infections were lacking.^{20,47} The recognition of flat penile lesions, which so far have been neglected because of their poor visibility and high degree of spontaneous healing, probably will cause a change in this regard.

2 Prevention of flat penile lesions

2.1 Effect of condom use on HPV-associated flat penile lesions

Although evidence demonstrates that the use of condoms by men substantially reduces the risk of genital transmission of human immunodeficiency virus in women, data on the effectiveness of condoms in reducing the incidence of other sexually transmitted infections are more limited. In particular, some studies have found that condom use by men does not reduce the risk of HPV infection in women.¹⁸ However, most data on condom use and HPV infection are

from cross-sectional studies and the prospective studies reported to date were not explicitly designed to evaluate condom use.¹⁹ Winer *et al*²⁰ showed in a longitudinal prospective epidemiological study a protective effect against HPV infection of the cervix and the vulva of consistent condom use in young women, starting sexual activity.

This study is discussed more extensively in paragraph 3.1.1, prevention of female genital HPV infection with the use of condoms. Essential is that the consistent use of condoms did not prevent a HPV infection at all times, probably because of forms of non protected (nonpenetrative) genital contact. However, in women the effect was clear and the inverse association between the risk of HPV infection and the use of condoms was increasing with the frequency of the condom use itself. It is plausible that condoms will probably also work the other way around and will protect “virginal” men against a genital HPV infection.

Demonstrating an association between flat penile lesions and a high viral load of hr-HPV in our first study led to the assumption that consistent condom use by the male sex partners of women with CIN would possibly have an effect on the intraepithelial lesions of both sex partners. Indeed, we demonstrated an accelerated regression of hr-HPV-associated flat penile lesions and the clearance of HPV in time in case of condom use. In fact, the effect of condom use was particularly seen when HPV type concordance was found in both sex partners. From these findings it can be postulated that in HPV-type concordant men, re-infection of the penile skin probably not only hampers the elimination of the virus but also increases the risk of developing productive penile lesions. Blocking this process is a likely explanation for the positive effect of consistent condom use. However, it should be realized that individual susceptibility and immunological responses to type-specific HPV infections in sex couples have to be studied in more detail to fully understand this mechanism.^{16,17}

2.2 Prophylactic vaccination

Relatively new is the development and use of prophylactic vaccines. Two vaccines have been approved in the 27 countries of the EC, *i.e.* Cervarix[®] and Gardasil[®], and are being proposed by the national health organisations to the governments of these states for use in the prophylaxis of cervical cancer. Both vaccines induce high titres of neutralizing antibodies against hr-HPV types 16 and 18 that persist for more than 5 years and prevent the development of HPV 16 and 18 related CIN3 lesions.²¹ It is expected that these vaccines will prevent in the future at least 70% of cervical carcinomas. Moreover, Gardasil[®], a quadrivalent vaccine, gives also protection against condylomata acuminata by inducing antibodies against HPV 6 and 11.

Three situations can be recognised when prophylactic HPV vaccination is used:

2.2.1 Influence of male vaccination on FPL and penile and anal carcinomas

Since HPV 16 is the most predominant hr-HPV type in hr-HPV related vulvar and penile cancer it is plausible that HPV 16 related precursors of vulvar and penile carcinoma will also be prevented by these vaccines.²³ However, the long period of time for these carcinomas to develop and their rarity make that it will be a long-lasting effort to prove the effectiveness in preventing these invasive cancers. Therefore PIN3 lesions would be the most ideal surrogate marker to evaluate the effect of these prophylactic HPV 16/18 L1 VLP vaccines on hr-HPV positive penile carcinomas. However the rarity of these lesions makes evaluation of the effectiveness of these vaccines difficult. Conversely, by evaluating the influence of vaccination of males on the development of hr-HPV associated flat penile lesions in a double blind study, results on the potential effectiveness of these vaccines could be obtained in a relative short period of time. Only in a few countries HPV vaccines are presently approved for use in young, HPV naïve, boys aged 9-14 years.^{51,52} This advice has been based on the assumption that HPV associated penile and anal cancers develop from persistent HPV infections as described above. The need for such a HPV vaccin should not be underestimated. In regions with a large population of homosexual men the incidence of anal carcinoma is steeply rising.³⁵ These carcinomas are present in relative young homosexual men and almost all related to hr-HPV, mainly HPV 16. For these men too vaccination would probably have been effective, if given at an early (presexual) age.

2.2.2 Influence of male vaccination on prevalence cervical carcinoma in women

Strictly taken it can be argued to vaccinate only the boys to prevent cervical cancer. This will result in "herd immunity" in men and effectively blocks HPV transmission to women and thus cervical cancer. This approach is not advised because at present the effect of male vaccination is not well evaluated, studies are however underway.

Cost-effectiveness studies have shown that the influence of prophylactic vaccination of women is effective in cervical cancer prevention as long as the percentage of vaccinated women will not be lower than 70%. In that situation no additional effect of male vaccination of men can be expected.⁵³ The high degree of vaccination is thought to result in "herd immunity" of the women and probably blocks HPV transmission in the population. Lower percentages of vaccination result in lower efficacy of the vaccines and make it necessary to vaccinate male to remain effective in preventing cervical cancer.²¹

2.2.3 Influence of female vaccination on prevalence of penile and anal cancer and their precursor lesions

Vaccination of young preadult women will not only have a beneficial effect on the prevalence

of cervical cancer but will also have a preventive effect on HPV infection and HPV associated FPL, and penile- and anal cancer. Using this approach it might be expected that a high percentage of vaccinated women will also block HPV transmission to men and thus may prevent HPV associated lesions in men. However no CE studies have been published to evaluate this effect. Of course this only regards heterosexual men and bisexual men who started their sexual career as heterosexual; men having sex with men only will miss any effect of female vaccination.

2.3 Circumcision

Male circumcision prevents effectively the development of phimosis and Lichen Sclerosus.⁴⁸ So it is plausible that it indirectly reduces progression to penile cancer as well.²⁵ Circumcision is also likely to be a preventive measure against flat penile lesions as circumcision is associated with a reduced risk of penile HPV infection, an important risk factor for penile carcinoma.^{24,18,49} Hernandez *et al* compared the HPV prevalence on the glans penis and coronal sulcus, penile shaft, scrotum, semen, and urine in circumcised and non-circumcised heterosexual men. The prevalence of any HPV infection in the glans/corona was significantly higher in uncircumcised men than in circumcised men.³⁹ Moreover the number of male sexual partners of women with CIN having PIN lesions was lower when circumcised.⁴⁰ In line with this observation we found that FPL in circumcised men with a female sexual partner not having CIN had a lower rate of both PIN and flat penile lesions compared to uncircumcised men.⁹ Male circumcision therefore prevents both HPV-related and non-HPV related penile cancer, as well as other sexually transmitted diseases and the transmission of HIV.

3 CIN

3.1 Women and condom use

3.1.1 Prevention of genital HPV infection by condom use

There is ample evidence that condom use helps preventing transmission of STI's in women, including HIV. Older publications regarding HPV and condom use were cross-sectional and could not demonstrate a significant effect of condom use on the transmission of hr-HPV.^{18,19} However, the first prospective study, from Winer *et al* (2006)²⁰, with a rigid enrollment and follow-up scheme, showed a clear effect on HPV transmission in young females, starting their sexual careers when entering college. Restricting their analyses of risk factors to women who first had intercourse within two weeks before enrollment or during the study ensured that the

cohort was susceptible to new infections and that infections detected during follow-up were incident. Informing by computer assisted questionnaires every two weeks measured condom use more precise than in other studies. The study showed that women whose partners used condoms at all times of vaginal intercourse during a follow up of eight months were 70% percent less likely to acquire a new infection than were women whose partners used condoms less than 5% of the time. Even the use of condoms more than half the time led to a 50% risk reduction. In conclusion this study demonstrated a strong inverse association between the frequency of condom use by male partners and the risk of HPV infection in women. The association increased with the increasing frequency of condom use, suggesting a causal, protective effect. Imperfect condom use and transmission of HPV by non protected non penetrative genital contact can well explain the detection of some infections in women who reported consistent condom use.

3.1.2 Healing of CIN lesions by condom use

Regarding CIN as the female equivalent of PIN we expected also an effect of condom use on the clinical course of CIN lesions. In our partner study we demonstrated that intervention with condom use for at least 3 months promotes CIN regression and HPV clearance. This effect was mainly seen in women with CIN1 and small CIN2 lesions, lesions that are likely to represent productive infections. It should be realised that for ethical reasons we could not test the effect of condoms on large CIN2 and CIN3 lesions, which were treated according to standard protocols because of the risk of invasive cancer. Whether the clinical course of \geq CIN2 lesions will be affected by condom use remains open since the large majority of these lesions represent transforming HPV infections with no viral spread.

3.2 Prevention of CIN / cervical carcinoma by male circumcision

Male circumcision (MC) is significantly associated with lower cervical cancer incidence and lower HIV prevalence in sub-Saharan Africa, independent of Muslim and Christian religion.⁴¹ Male circumcision is also strongly associated with lower HIV and HPV prevalence among countries with primarily heterosexual HIV transmission, but not among countries with primarily homosexual or injection drug use HIV transmission.⁵⁰ These findings strengthen the reported biological link between MC and some sexually transmitted infectious diseases, including HPV and HIV. Studies on the effect of MC on prevalence of FPL are presently ongoing and a reduced prevalence of FPL in circumcised men is expected (preliminary results of our study in the Kisumu region in Kenya, Smith, Hogewoning, Bleeker en Meijer).

In an important study Castelsague *et al* demonstrated that circumcised men were less likely

than uncircumcised men to have HPV infection.²⁴ Monogamous women whose male partners had six or more sexual partners and were circumcised had a lower risk of cervical cancer than women whose partners were uncircumcised. In this study male circumcision was associated with a reduced risk of penile HPV infection and, in the case of men with a history of multiple sexual partners, a reduced risk of cervical cancer in their current female partners.

3.2 Viral load and CIN

In previous paragraphs it was mentioned that low viral loads in men do not reflect productive hr-HPV infections and their clinical manifestations (ie. flat penile lesions). Similarly, in their female counterpart there is evidence that the amount of viral DNA also has clinical implications. In fact, an increased hr-HPV viral load in cervical scrapings has been proposed as a determinant for high-grade cervical intraepithelial neoplasia and cervical cancer (\geq CIN2). However, data for HPV types different from HPV 16 were limited and inconsistent. In addition, a viral load threshold to distinguish hr-HPV positive women without \geq CIN 2 still has not been defined. We analyzed in a cross-sectional study the potential value of using viral load thresholds for HPV 16, 18, 31, and 33 to distinguish women with a single infection with these types without underlying high-grade CIN. The findings of this study support the idea that increased viral loads are generally associated with an increased risk of prevalent high-grade CIN and that application of a viral load threshold will increase the specificity of hr-HPV testing for high-grade CIN and cervical cancer without affecting the sensitivity. However, a more recent longitudinal study (ie 18 months follow-up) using a population-based cervical screening cohort (POBASCAM) revealed that the use of viral load thresholds had a negative influence on the longitudinal sensitivity for high-grade CIN. In relation to cytology, viral load testing showed a higher sensitivity for high-grade CIN2, but at the cost of a marked decrease in specificity. In conclusion, in a cervical screening setting viral load assessment alone has no additive value to stratify high-risk HPV-positive women for risk of high-grade CIN.

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