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Human Papilloma Virus Infection among Women Attending an STD Clinic Correlated to Reason for Attending, Presence of Clinical Signs, Concomitant Infections and Abnormal Cytology

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The purpose of this study was to demonstrate the prevalence of cervical human papilloma virus (HPV) infection correlated to reason for attending an STD clinic, presence of clinical signs of HPV infection, concomitant infection and abnormal cytology. Samples from the cervical canals of 588 consecutive women attending the STD clinic, Department of Dermato-Venereology, Sahlgrenska Hospital, Gothenburg, were taken with a Cytobrush for detection of HPV DNA with the dot blot/Southern-blot technique. Visible condylomata, i.e. filiform or papular condylomata, were registered. Acetic acid test and colposcopy were not routinely performed. Cytological examination was performed as well as isolation of *Chlamydia trachomatis* on Mc Coy's cells and culture on Sabouraud agar for *Candida albicans*. The prevalence of HPV DNA was 8% (48/588). In the group of 233 women attending because of concern about HPV infection, 94 (40%) had visible signs of HPV infection and 30 (13%) were positive for HPV DNA in the cervix. In 355 women attending for other reasons, such as discharge, pruritus or STD check-up, 4 (1%) had visible signs of HPV infection and 18 (5%) were HPV DNA positive. Of 98 women with visible signs of vulvar/vaginal HPV infection, 33 (34%) were HPV-positive in the cervix with a commercial Southern-blot test. Of 490 patients without visible signs of HPV infection, 15 (3%) were HPV-positive in the cervix. In the group of HPV-positive women a positive culture for *Candida* was demonstrated in 26% (11/43), compared to 16% (79/504) of the HPV-negative women. Abnormal cytology was seen in 23 (53%) of 43 HPV-positive women, compared to 8 (4%) of 188 HPV-negative women. Of the 23 HPV-positive women with abnormal cytology, 20 had HPV types 16, 18, 31 and/or 33. There was no significant correlation between high-risk HPV types and abnormal cytology within the HPV-positive group. In a follow-up of 21 women half had become HPV-negative within 3 months. Cytology in combination with clinical examination including colposcopy seems to be a relevant tool for HPV screening.

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In sexually active young women genital human papilloma virus (HPV) infection is the most frequent sexually transmitted disease (STD). The clinical appearance of this virus infection (1, 2) and the connection with the development of cervical cancer are well documented (3). The oncogenic potential for various genotypes of HPV differs. Types 16 and 18 have been shown to be high-risk types and 31, 33 and 35 to be intermediate types in this connection, whereas 6 and 11 are regarded as low-risk types (4). New HPV types are still being reported, so our knowledge concerning this virus is not complete (5–7).

The primary aim of this study was to determine the prevalence of HPV infection in a female population attending an STD clinic. Secondly, we wanted to investigate the correlation between a positive test for HPV DNA from the cervix and the reason for attending the STD clinic, as well as clinical signs of genital warts, abnormal cytology and coincident presence of *Chlamydia* and/or *Candida* infection. Lastly, we wanted to correlate the different HPV types to cervical cytology.

PATIENTS AND METHODS

During the period April 1990 – June 1991, 652 women attended for the first time the STD clinic, Department of Dermato-Venereology, Sahlgrenska Hospital, Gothenburg. These women were informed about the study and offered testing for cervical HPV infection.

Of 652 women attending, 52 did not want to participate and the remaining 600 patients (14–69 years old, mean age 26 years) were included after giving their consent. In samples from 12 women the amount of DNA was insufficient for dot blot/Southern blot analysis, which gave a final number of 588 women in the study. All patients were interviewed about their reason for attending. There were two groups: women attending because of discharge, pruritus or STD check-up ($n = 355$) or women attending because of "condyloma check-up", which meant that the woman had a recent partner with condylomata and/or had a suspicion of condylomata lesions ($n = 233$).

Gynaecological examination, with inspection of the cervix, vagina and vulva, was performed. Visible signs of HPV infection were registered. Acetic acid testing and colposcopy was not routinely performed. Filiform or papular condylomata were designated as "signs of HPV infection". A sample from the cervix and urethra was performed for isolation of *Chlamydia trachomatis* on Mc Coy's cells. A cotton-tipped swab from the wall of the vagina was taken for culture on Sabouraud agar for *Candida albicans*. Culture for *Candida albicans* was positive when abundant or moderate growth was found.

Samples for cytological smear (Papanicolau smear) was taken with an Ayres spatula from the fornix and the transformation zone of the cervix and by swabbing the endocervical canal with a Cytobrush (Medscand AB, Malmö).

Women without any signs of genital infection and who had not had cytology performed for one year prior to the study were offered cytological examination. In the HPV-positive group cytology was performed either at the screening visit ($n = 22$) or at the visit 1 month later ($n = 21$). Five of the 48 HPV-positive patients were not subjected to cytological examination at the first visit and did not return for follow-up. Altogether cytology was performed in 231 (39%) of the study group of 588 women.

All women with a positive HPV test (presence of cervical HPV DNA detected as described below) were recalled for re-examination after 1 and 3 months, and a new sample for Southern-blot test was taken. Twenty-one of the 48 HPV-positive women were examined on both occasions.

Cytology

All smears taken from HPV-positive patients, as well as all abnormal smears taken from HPV-negative patients, were reexamined. All samples were examined by the same pathologist using the following 5 criterias:

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Table I. *Results of cytological examination in relation to HPV types in 231 women*

Cytological pattern	Low-risk HPV	Medium/ high-risk HPV	Neg	Total
Normal	6	14	180	200
Dysplasia	0	3	1	4
Uncharacteristic atypia	0	8	3	11
Uncharacteristic atypia, koilocytosis	1	5	1	7
Uncharacteristic atypia, suspicion of koilocytosis	0	2	1	3
Koilocytosis	2	2	2	6
Total	9	34	188	231

1. *Uncharacteristic atypia:* cells with nuclear atypia which are not obviously dysplastic but can be considered neither entirely inflammatory nor reactively caused.

2. *Koilocytosis:* cells showing nuclear hyperchromasia, a characteristic perinuclear halo and distinct, often amphophilic, condensation of the periphery of the cytoplasm. They may also contain dyskeratinocytes and show evidence of dys/parakeratosis, karyorrhexis or be multinuclear.

3. *Uncharacteristic atypia and koilocytosis:* cells showing atypia which in part are uncharacteristic but which also contain koilocytotic cells.

4. *Uncharacteristic atypia and suspicion of koilocytosis:* cells basically showing uncharacteristic atypia but where a number of the cells demonstrate alterations that suggest viral infection but are not totally convincing for koilocytosis.

5. *Dysplasia:* cells with mild or moderate precancerous abnormalities (mild or moderate dysplasia, CIN 1–2) or cancerous cells (severe dysplasia/CIS, CIN 3). This group also includes smears with atypical koilocytosis, i.e. cells that in addition to dysplastic features show evidence of koilocytosis.

Detection and typing of HPV DNA

Samples were taken from the endocervix and from the transformation zone with a Cytobrush (Medscand AB, Malmö) and transferred to the laboratory in tubes containing 1 ml of a lytic buffer (Oncor Inc. Gaithenburg, MD, USA), followed by digestion with proteinase K, final concentration 0.5 mg/ml, in the same buffer for 1 h at 60°C.

The digested samples were subsequently divided into two aliquots, 1/4 vol. for dot-blot hybridization and 3/4 vol. for Southern-blot analysis performed as described elsewhere (8,9). All probes were obtained from Oncor Inc, Gaithenburg, MD, and represented the HPV genotypes 6, 11, 16, 18, 31, 33 and 35.

Statistics

Fisher's exact 2 × 2 table test was used.

RESULTS

The presence of cervical HPV DNA in the total study group was demonstrated in 8% (48/588). Of 233 women attending because of suspicion of HPV infection, 30 (13%) were positive and in the group attending for other reasons 18 (5%).

In the subgroup attending because of specific concern about HPV infection, visible signs of HPV infection were seen in 40% (94/233), compared to 1% (4/355) of the women attending for other reasons. Of altogether 98 women with signs of vulvar/vaginal HPV infection, 33 (34%) were HPV-positive in the cervix, compared to 15 (3%) of 490 women without clinical signs of HPV infection. This difference was statistically significant, $p < 0.001$.

Of the HPV-positive patients 23% (11/48) had more than one HPV type. HPV 6 or 11 were the only types in 21% (10/48) patients; HPV 16 or 18 were detected in 44% (21/48) and HPV 31 and 33 in 53% (25/48) of the positive specimens. Altogether the prevalence if high/ intermediate-risk HPV types in the HPV-positive group was 80% (38/48), compared to low-risk HPV types in 21% (10/48). This difference was statistically significant, $p < 0.001$. In no woman was type 35 detected.

In patients with cervical HPV infection 26% (11/43) had a positive *Candida* culture, compared to 16% (79/504) of the patients without demonstrable HPV DNA. The prevalence of *Chlamydia* infection in the HPV-positive population was 6% (3/43), and in the HPV-negative population the prevalence was 12% (61/500). There was no statistically significant difference.

The correlation between HPV and cytological pattern is shown in Table I. Abnormal cytology was demonstrated in 23 (53%) of the HPV-positive women and in 8 (4%) of the HPV-negative. This corresponds to a sensitivity of 53%, a specificity of 96% and a positive predictive value of 53,5% for cytology as a measurement of HPV infection. Different histological patterns are seen in the different HPV types, as shown in Table I. Dysplasia was seen in 3 patients in the group with high/ intermediate-risk HPV types and in one woman negative for HPV. Table I shows that 3 (33%) of 9 patients with low risk types HPV, compared to 20 (59%) of 34 patients infected with high/intermediate-risk types HPV, had an abnormal cytology. There was no significant correlation between abnormal cytology and HPV types associated with cervical cancer.

Table II. *Follow-up of 21 HPV-positive women after 1 and 3 months*

Screening visit HPV type	Visit 1 HPV type 1 month	Visit 2 HPV type 3 months
6	neg	6
6	neg	neg
6	6+18	neg
6+31	neg	neg
11	neg	neg
16	16	16
16	16	neg
16	neg	neg
16	16+31	neg
16	neg	neg
16	16+31	31
16+31	neg	neg
18	18	18
18	18	18
18+31	18+31	18+31
31	31	31
31	31	31
31	neg	neg
33	33	33
33	33	33
33	33	neg

In a follow-up study, where 21 patients were re-examined for HPV both after 1 and 3 months, we found that 11 had become negative: 4 of 5 positive for HPV 6/11, compared to 7 of 16 positive for high/intermediate risk-HPV types (Table II). This difference was not statistically significant.

DISCUSSION

HPV infections pose a serious clinical problem. Since the introduction of commercial kits for the detection of HPV DNA, a vast number of clinical studies have been published (9–17). Estimates of the prevalence of cervical HPV infection rely on the sensitivity and specificity of the technique used and on the population studied. The Southern-blot test is considered reliable and is by many considered as " the golden standard" among the hybridization tests for HPV. In the present study of a female STD population, 8% were dB positive in cervix, and in all cases the result was confirmed by Southern-blot. Using the Southern-blot technique in various populations, authors have reported a prevalence of HPV varying between 3% and 29% (12–16, 18). In a study from Sweden by Hjerpe et al. (10), the prevalence figures for healthy female controls without symptoms were 7.8% and for patients with symptoms of HPV infection 16%. In another Swedish study by Lindh and co-workers (11), HPV was found with the Southern-blot technique from the cervix in 8.8% in healthy women without signs of HPV infection (11). It seems that the figures in different parts of Sweden are in good correlation. A failure to detect HPV with Southern-blot does not exclude the presence of HPV DNA. The recently developed polymerase chain reaction (PCR) technique for detecting HPV DNA has a much higher sensitivity (19). Prevalence figures between 18 and 39% with this technique for the detection of HPV DNA from normal cervices have been reported (17, 19, 20, 22).

In 23% of the infected women more than one HPV type was detected. Types 16 and 18 were the HPV types most often detected (44%), but a concomitant dysplasia was diagnosed by cytological examination in only 2 of these patients. These findings are in agreement with other studies (23).

We could demonstrate that more than half of the HPV-positive group of women had an abnormal cytology, but there was no correlation between dysplasia and high-risk types. The criteria for the different cytological patterns were strictly defined (see Patients and Methods). Rosenfeld et al. (21) have shown 17% of an HPV-positive group of women examined to have cytological abnormalities. Kiviat et al. (23) and Hallam et al. (20) did not find any correlation between dysplasia and high-risk HPV types, which is in accordance with our findings. Among the women in our study we found 2 with HPV type 16, one with type 31 and one who was HPV-negative who had a dysplastic cytological examination. The women who were HPV-negative but had a dysplasia, could of course have an HPV type that we were not able to detect with our limited probes. Cytology as a tool in the measurement of HPV infection has in our study a sensitivity of 53%, a specificity of 96% and a positive predictive value of 53,5%.

It has been considered that koilocytosis, i.e. ballooned cells with a condensed nucleus and fluffy cytoplasm, is a pathognomonic sign of HPV (24). In a study of acetowhite lesions of vulva, we found koilocytosis histologically in lesions where EBV DNA but no HPV DNA could be demonstrated with the PCR technique (25). Obviously koilocytosis could be found in both EBV and HPV-affected lesions, and as we did not examine the presence of EBV DNA in this study, HPV-negative women with koilocytosis could perhaps be infected with EBV.

In the follow-up after 3 months 11 of 21 women had become HPV-negative. Though the group of women who were re-evaluated after 3 months was small, there was a tendency that those with HPV type 6/11 became negative more often than those with types 16, 18, 31 and 33. This pattern was also found in a study by Kataja et al. (26).

The importance of detecting other STDs in patients with HPV has earlier been pointed out (27). In our study a positive *Candida* culture was found more frequently in the HPV-positive group. Kinghorn studied a group of HPV infected women and found *Candida* infection in 25% (28). This is in agreement with our study. It is possible that a *Candida* infection might activate a latent HPV infection. A concomitant *Chlamydia* infection has been reported in 12–18% of females with HPV infection (29, 30). In our study *Chlamydia* was found in a lower frequency in HPV-positive females compared to HPV-negative, 6% versus 12%.

In our opinion a cytological test offered women attending an STD clinic seems relevant. Since the peak prevalence of HPV infection precedes the peak prevalence of CIN (31), women with signs of HPV infection are often recommended annual PAP smear, although this is controversial (32). As long as we do not have a cheaper and more exact HPV test, we probably have to rely on cytology in combination with clinical examination with colposcopy. Hybridization tests for the detection of HPV DNA have their place in researching programmes but are of limited value in clinical work today.

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