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Human Papillomavirus, Cytomegalovirus, and Adeno-Associated Virus Infections in Pregnant and Nonpregnant Women with Cervical Intraepithelial Neoplasia

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Two hundred eight cervical specimens from two groups of subjects, 165 nonpregnant women and 53 pregnant women with cervical intraepithelial neoplasia (CIN) of grades I to III, were positive by PCR analyses for human papillomaviruses (HPVs), adeno-associated virus type 2 (AAV 2), and human cytomegalovirus (HCMV) in 67, 6, and 4.1% of the cases, respectively. The presence of AAV 2 infection was more frequently associated with pregnancy (17 versus 2.4%) and HPV-positive cervices (odds ratio = 6.358) than HCMV was. Increased HPV infection was strongly associated ($P < 0.001$) with a higher CIN grade, but there is no evidence that AAV 2 and HCMV infections have any impact on CIN development.

The causal role of human papillomavirus (HPV) infections in cervical cancer development has been well established in the last 2 decades (2). More than 40 HPV genotypes infect the genital tract; however, only a subset of high-risk HPVs is involved in cervical cancer pathogenesis, most notably HPV type 16 (HPV 16), found in more than 50% of cervical cancer cases worldwide.

Latent HPV infection is found in approximately 40% of young women, but only 1% of high-risk HPV-positive cervices (or fewer) will develop cervical cancer (2). Since only a small fraction of HPV-infected women develop cervical cancer, additional risk factors are likely to be important determinants of disease development. These factors could include hormonal factors, other sexually transmitted infections, tobacco smoking, and dietary factors (4).

It is still unclear whether other infectious agents contribute to increased cervical cancer risk. A history of multiple genital infections is more common in women with cervical cancer (8), suggesting that these agents might increase the risk of developing cancer. However, the possibility that their presence may be an indicator of sexual activity and hence a surrogate marker of oncogenic HPV infection cannot be excluded.

Most adults (80 to 90%) are infected with adeno-associated virus (AAV) (9), which is commonly carried in the genital region (11). AAV is a human helper virus-dependent parvovirus with the ability to suppress the oncogenic phenotype of a variety of viruses and oncogenes. From in vitro studies, AAV has been shown to prevent the replication of HPVs and therefore may protect infected patients from cervical intraepithelial neoplasia (CIN) (13). However, in vivo studies of women with cervical HPV infection or CIN gave inconsistent results regarding the role of AAV in cervical carcinogenesis (16, 20).

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Human cytomegalovirus (HCMV), a widespread human herpesvirus, has been shown to induce cervical neoplasia in mice (12), to morphologically transform human cells in culture (15), and to cause persistent infections of the genitourinary tracts of women (6). However, HCMV DNA is not detected in most transformants and the mechanism by which HCMV might contribute to oncogenesis is still unclear.

The aim of this retrospective study was to determine whether infection with AAV and/or HCMV influences the already established oncogenic impact of HPV infection on CIN development in general and during pregnancy, which is often associated with the reactivation of latent viral infections.

Two groups of young women with CIN grades I to III (cytological diagnosis) were enrolled in this study: 165 nonpregnant women (mean age, 26.7 years; 37 women with CIN I, 65 with CIN II, and 63 with CIN III) and 53 pregnant women (mean age, 25.8 years; 23 women with CIN I, 14 with CIN II, and 16 with CIN III). For nonpregnant women, cervical specimens were collected during regular gynecological examinations with the standard cyto-brush, and for pregnant women, a cotton swab was used. Samples were placed in sterile phosphate-buffered saline (pH 7.2) and frozen at −20°C until DNA extraction was carried out (10). PCR conditions for HPV amplification have previously been described (10). A nested-PCR approach with two sets of primers complementary to the early regulatory region of the HCMV genome was applied for the detection of HCMV (3). AAV type 2 (AAV 2) was detected by two different approaches: one-step PCR (11) and nested-PCR amplification (18).

HPV, AAV 2, and HCMV were found in, respectively, 67, 6, and 4.1% of the 218 women (Table 1). For nonpregnant women with CIN I to III, 35.1, 64.6, and 81% of the samples, respectively, were HPV positive. Increased HPV infection was strongly associated ($P < 0.001$) with a higher CIN grade in the group of nonpregnant women. For pregnant women with CIN I to III, 60.9, 92.9, and 81.2% of the samples, respectively, were HPV positive. There was no statistically significant correlation

TABLE 1. Prevalences of different viral infections among nonpregnant and pregnant women and correlations between viral infections and CIN status in different study groups

Group and CIN status	No. of women	No. (%) of women with result for:					
		HPV		AAV 2		HCMV	
		−	+	−	+	−	+
Nonpregnant women[a]	165	59 (35.8)	106 (64.2)	161 (97.6)	4 (2.4)	156 (94.5)	9 (5.5)
CIN I	37	24 (64.9)	13 (35.1)	37 (100)	0 (0)	34 (91.9)	3 (8.1)
CIN II	65	23 (35.4)	42 (64.6)	64 (98.5)	1 (1.5)	62 (95.4)	3 (4.6)
CIN III	63	12 (19.0)	51 (81.0)	60 (95.2)	3 (4.8)	60 (95.2)	3 (4.8)
Pregnant women[b]	53	13 (24.5)	40 (75.5)	44 (83.0)	9 (17.0)	53 (100)	0 (0)
CIN I	23	9 (39.1)	14 (60.9)	18 (78.3)	5 (21.7)	23 (100)	0 (0)
CIN II	14	1 (7.1)	13 (92.9)	13 (92.9)	1 (7.1)	14 (100)	0 (0)
CIN III	16	3 (18.8)	13 (81.2)	13 (81.2)	3 (18.8)	16 (100)	0 (0)
All women[c]	218	72 (33.0)	146 (67.0)	205 (94.0)	13 (6.0)	209 (95.9)	9 (4.1)

[a] For nonpregnant women, the association between HPV and CIN grade was significant ($P < 0.001$) and the associations between AAV 2 and CIN grade and between HCMV and CIN grade were not ($P > 0.001$) (Fisher's exact two-tailed test).

[b] For pregnant women, the associations between HPV and CIN grade, between AAV 2 and CIN grade, and between HCMV and CIN grade were not statistically significant ($P > 0.001$) (Fisher's exact two-tailed test).

[c] There was no significant difference between HPV prevalence among pregnant women and that among nonpregnant women ($P > 0.001$ [Fisher's exact two-tailed test]; odds ratio = 0.5839 [95% confidence interval, 0.2894 to 1.178]). There was a statistically significant ($P < 0.001$) increase in the prevalence of AAV 2 among pregnant women compared to that among nonpregnant women (odds ratio = 0.1215 [95% confidence interval, 0.0357 to 0.4131]). HCMV infection was found only in nonpregnant women, but no significant difference was established ($P > 0.001$).

between HCMV or AAV 2 infection and a higher CIN grade for either nonpregnant or pregnant women.

There was no significant difference between HPV prevalence among pregnant women (75.5%) and that among nonpregnant women (64.2%). However, there was a statistically significant ($P < 0.001$) increase in the presence of AAV 2 in pregnant women (17%) compared to that in nonpregnant women (2.4%). HCMV infection was found only in nonpregnant women (5.5%), but no significant difference was established.

There was no statistical correlation between HPV and AAV 2 or HCMV infections among either nonpregnant women or pregnant women. All but one of the AAV 2-positive samples (12 of 13 samples) were also HPV positive (odds ratio = 6.358). HCMV was present in 5.9% (4 of 68) of the HPV-negative samples and in 3.5% (5 of 141) of the HPV-positive samples.

There was a significant increase ($P < 0.001$) of all HPV types except low-risk HPV 6 and 11 with increasing CIN grade among nonpregnant women (Fig. 1A). The frequencies of different HPV types among pregnant women were incoherent (Fig. 1B).

The distribution of different HPV types among nonpregnant Croatian women found in this study and in a previous epidemiological report (10) is consistent with the general trend found in most of the developed countries. The exception was the incoherent frequencies of different HPV types according to CIN grade among pregnant women, probably due to the low number of samples ($n = 53$) in our study group.

A contradictory role of AAVs in cervical cancer has been

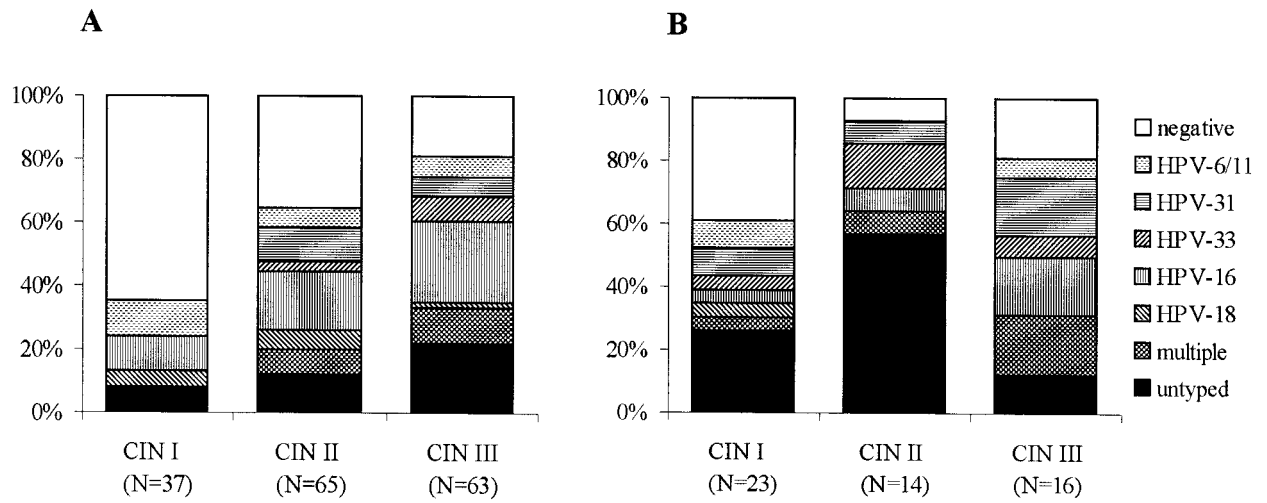

FIG. 1. Distributions of different HPV genotypes among nonpregnant (A) and pregnant women (B) according to CIN grade.

reported in the last few decades (7, 16). Although several epidemiological studies showed a negative association of AAVs with HPV-associated cervical cancer, some studies did not find AAVs in the cervix at all (16).

Han et al. (11) detected AAV in 50% of cervical samples of healthy patients by means of PCR, indicating that AAV is commonly carried in the genital tract and can be sexually transmitted. Walz et al. (19) showed that HPV could act as an AAV helper virus. In our study, 92.3% (12 of 13) of the AAV 2-positive samples were also HPV positive, pointing to a role for HPV as AAV 2 helper virus.

Several collective studies suggest that AAV 2 could be directly related to the suppression of the oncogenic role of HPV infection in cervical oncogenesis (9, 13). In the present study, we found AAV 2 in only 6% (13 of 218) of cervical specimens from women with CIN, while Walz et al. (20) detected AAV in 63% of biopsy samples from subjects with high-grade CIN. On the other hand, Strickler et al. (16) did not detect any AAV 2 in cervical specimens and also found no relationship between AAV antibodies and the presence or grade of neoplasia. In a study by Lanham et al. (14), AAV was found in similar proportions of high- and low-grade CIN samples. These findings provide no evidence that AAV infection plays a role in cervical tumorigenesis.

Ahn et al. (1) found no significant difference between AAV 2 prevalences in microdissections with CIN I to III and those in adjacent normal tissues, supporting the notion that AAV 2 is not associated with cervical tumorigenesis. Our findings are in concordance with that study, since no association between AAV 2-positive women and CIN grade was found.

A study by Erles et al. (9) showed that pregnant women are significantly more frequently seropositive for AAV than nonpregnant controls are, suggesting a reactivation of latent AAV in specific hormonal conditions and slight immunosuppression. Similar results were found in the present study; AAV 2 was more frequent in pregnant women than in nonpregnant women (20.5 versus 2.4%). Thus, AAV could be considered to be protective against CIN progression only during pregnancy.

Chan et al. (5) detected HCMV in 9.5% of cervical samples. However, there was no association between HCMV detection and cervical lesions or between HPV status and HCMV positivity, indicating that HCMV is most probably a bystander rather than a cofactor in cervical cancerogenesis. This finding could also explain why there was a low HCMV positivity rate for cervical samples (4.1%) in our study.

Thompson et al. (17) studied the role of HCMV in the development of cervical carcinoma. As expected, 86.4% of cervical cancer biopsy specimens were HPV positive by PCR, in comparison to only 3.9% of the samples being HCMV positive. No unusual histological diagnosis or more aggressive clinical behavior was observed in HCMV-positive samples, which led to the conclusion that HCMV is not associated with cervical cancer. Our results are in accordance with that study, since HCMV infection was detected in only 4.1% of the women in our study group and no statistical correlation between HCMV infection and increasing CIN grade was found.

Our findings indicate that HCMV and AAV 2 infections did not contribute to an increase or decrease in the grade of CIN. However, there was a marked increase in the AAV 2-positive rate among pregnant women compared to that among nonpregnant women in all CIN groups. A similar outcome was found for HPV, but only among women with CIN II; no such outcome was observed for HCMV.

Our results confirm the already established role of HPV in CIN development. Furthermore, these results provide no evidence that AAV 2 and HCMV infections have any impact on CIN development. HCMV is probably mostly a transient sexually transmitted infection, whereas HPV infection is usually persistent. Our data point out that HPV could indeed be an AAV helper virus and that AAV as such can be considered sexually transmissible.

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