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RESEARCH NOTE

Detection of *Chlamydia trachomatis* and herpes simplex virus type 1 or 2 in cervical samples in human papilloma virus (HPV)-positive and HPV-negative women

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ABSTRACT

This study investigated whether the prevalence of human papilloma virus (HPV) in association with *Chlamydia trachomatis*, herpes simplex virus (HSV)-1 and/or HSV-2 was greater in high-grade than in low-grade or control cervical biopsy specimens. HPV-positive (n = 86) and HPV-negative (n = 213) women were screened for HPV, HSV and *C. trachomatis* by PCR. The most common HPV genotypes were HPV-16, HPV-6 and HPV-33; mixed HPV infection (n = 12) was also seen. A higher prevalence of *C. trachomatis*, HSV-1 and HSV-2 was found in HPV-positive samples. High-risk HPV genotypes and combined HPV + *C. trachomatis* or HPV + HSV-1, but not HSV-2, infections were associated with a greater risk of developing cervical carcinoma.

Keywords Cervical carcinoma, *Chlamydia trachomatis*, herpes simplex virus, human papilloma virus, risk-factors

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Human papilloma virus (HPV) is the main aetiological factor in cervical neoplasia [1]. However, the high incidence and long latency of HPV suggest that additional factors are involved in inducing cervical intra-epithelial neoplasia (CIN) [1,2], including herpes simplex virus (HSV) [3] and *Chlamydia trachomatis* [4,5]. These factors act by increasing the HPV-associated CIN risk through several mechanisms, including modulation of host immunity. Previous infection has been reported to be the most significant factor for HPV persistence [5], and a relationship between HPV and other sexually transmitted infections has been suggested. The presence of HSV-2 in HPV-positive cases suggests that it could initiate mutations and carcinogenesis [6,7], and may act with HPV in increasing the risk of CIN [7,8], although contradictory results have also been obtained [9]. *C. trachomatis* has also been associated with an increased risk of cervical neoplasia in HPV-positive patients [8], with the risk imparted by *C. trachomatis* being proportional to its titre, which is elevated in older women [4]. The present study investigated the association between HPV and HSV-1, HSV-2 and/or *C. trachomatis*, and correlated the results with other CIN risk-factors.

In total, 299 women (86 HPV-positive and 213 HPV-negative, based on Pap smear tests and confirmed by PCR [10]) attending obstetrics and gynaecology outpatient clinics were recruited to the study (Table 1), and were asked to complete and sign a standard questionnaire/consent form after the purpose of the study was explained and after all institutional ethical requirements were met. Endocervical scrapings were collected using a speculum-assisted Ayre's spatula. Where endocervical sampling could not be performed, vaginal specimens (n = 14) were collected using a sterile Ayre's spatula after separation of the labia minora and intra-vaginal 180° rotation of the spatula.

Table 1. Characteristics of study participants

Variable	HPV-positive	HPV-negative	p	OR	95% CI
Number	86	213			
Age (years)					
Mean ± SD	33.6 ± 7.1	33.6 ± 7.4	0.961		
Range	20–50	20–59			
Smokers	36 (41.9%)	54 (25.4%)	0.002	2.36	1.38–4.03
Pregnancies	65 (75.6%)	151 (70.9%)	0.477	1.27	0.72–2.26
No. partners[a]					
1	35 (49.3%)	137 (80.1%)			
2–5	29 (40.8%)	17 (9.9%)	0.031	2.30	1.13–4.64
> 5	7 (9.9%)	17 (9.9%)	0.424	1.61	0.62–4.19
Pap smear					
Negative	22 (25.6%)	192 (90.1%)	< 0.001	26.60	13.73–51.54
Positive	64 (74.4%)	21 (9.9%)			
CIN grade					
0	22 (25.6%)	192 (90.1%)			
1	42 (48.8%)	21 (9.9%)	< 0.001	17.46	8.80–34.62
2	6 (7.0%)	0	< 0.001	0.103	0.07–0.15
3	16 (18.6%)	0	< 0.001	0.103	0.07–0.15

CIN, cervical intra-epithelial neoplasia; HPV, human papilloma virus.
[a]Based on 71 HPV-positive and 171 HPV-negative women for whom reliable information was collected.

HPV was amplified with the L1 consensus primers MY09 and MY11 [10]. HPV genotypes (HPV-6/11/16/18/33) were determined by hybridisation with genotype-specific biotinylated DNA probes, followed by visualisation with a DNA enzyme immunoassay (DiaSorin, Salluggia, Italy). *C. trachomatis* DNA was detected using the Amplicor STD Amplification and CT Detection systems (Roche Diagnostics, Mannheim, Germany), with detection by hybridisation with *C. trachomatis*-specific biotinylated probes. HSV-1 and HSV-2 were detected by nested PCR [11]. Statistical analysis was performed using SPSS v.13 software (SPSS Inc., Chicago, IL, USA), with Pearson's chi-square test used to assess intergroup significance, and Student's t-test used to determine differences in means.

While HPV-positive women were comparable to HPV-negative women in terms of age and previous pregnancies, a higher prevalence of smokers, women with multiple male sexual partners and women with positive Pap smears was seen among HPV-positive women (Table 1). Of the 64 HPV-positive women with abnormal cytology, 42 were graded CIN I, six were graded CIN II, and 16 were graded CIN III; all abnormal cytology results in HPV-negative women were graded CIN I (Table 1). The most prevalent genotypes were HPV-16 (n = 48), HPV-6 (n = 10) and HPV-33 (n = 6). Mixed HPV infections (n = 12) included eight patients with high-risk/high-risk (HR/HR) genotypes and four with high-risk/low-risk (HR/LR) genotypes. The mean age of *C. trachomatis*-positive (n = 29), HSV-1-positive (n = 40) and HSV-2-positive (n = 33) women was comparable to that of HPV-positive cases. Abnormal cytology was significantly greater in women with HPV-16 and mixed HR/HR infections, as well as in those with HSV-1 and *C. trachomatis* infections.

HSV-1, HSV-2 and *C. trachomatis* were more prevalent among HPV-positive women (Table 2). Combined HSV-1/HSV-2 and HSV-1/*C. trachoma-*

Table 2. Frequency of isolation of herpes simplex virus (HSV)-1, HSV-2 and *Chlamydia trachomatis* from women positive or negative for human papilloma virus (HPV)

	HPV-negative		HPV-positive				
	n	%	*n*	%	p	OR	95% CI
HSV-1	11	5.6	29	43.3	< 0.001	12.84	5.9–27.9
HSV-2	19	9.7	14	20.9	0.031	2.45	1.2–5.2
HSV-1 + HSV-2	1	0.5	10	11.6	< 0.001	27.90	3.5–221.6
C. trachomatis	13	6.7	16	25.8	< 0.001	4.84	2.2–10.8
C. trachomatis + HSV-2	1	0.5	2	2.3	0.200	5.05	0.5–56.4
C. trachomatis + HSV-1	1	0.5	7	8.1	0.001	18.79	2.3–155.1

tis, but not HSV-2/*C. trachomatis* infection, was significantly more prevalent among HPV-positive cases (Table 2). Predictors of abnormal cytology were determined by logistic regression analysis, with the dependent variable being a Pap smear and the independent variables being age, number of sexual partners, abortions, smoking, other infections and HPV, *C. trachomatis*, HSV-1 and HSV-2 infections. The only variables that were selected were HPV ($p < 0.001$; OR 22.340) and *C. trachomatis* (p 0.006; OR 5.910); HSV-1 or HSV-2 were not associated significantly with abnormal cytology.

It has been suggested by some studies [3,5,6], but not by others [9], that *C. trachomatis* and/or HSV are predisposing factors for HPV infection. The present study demonstrated an increased prevalence of *C. trachomatis* and HSV-1 among HPV-positive patients, as was expected because of the similarity in their mode of transmission [5,12]. HPV infection was more common among women with multiple sexual partners, and those who were smokers or had abnormal cytology [1]. Abnormal cytology was associated with a lower risk for CIN in HPV-negative women, as all the abnormal cytology seen in HPV-negative women was graded CIN 1, compared with 65.6% of HPV-positive women who were graded CIN 1 and 34.3% who were graded CIN 2/3. As demonstrated previously [13], including in Lebanon [14], HPV-16 was the most prevalent genotype among HPV-positive women with abnormal cytology.

The association of HSV-2 with abnormal cytology was modest compared with that of HPV [15]. While HSV-2 may cooperate with HPV, its effects appear to be indirect, indicating that HSV-2 is not a significant risk-factor for cervical carcinoma [6,9], in contrast with the association between HSV-1 and CIN in HPV-positive women [16]. Abnormal cytology was more prominent in women with HPV-16 and mixed HR/HR HPV, *C. trachomatis* and HSV-1 infection. While HSV-1 and HSV-2 are associated commonly with labial and genital lesions [17], concomitant HSV-1 infection may exert a protective role in HSV-2-infected individuals [18]. In contrast, it has also been suggested that HSV-2 induces CIN, which becomes more pronounced with HPV infection [16].

HPV-positive women harbouring HSV-1, or both HSV-1 and HSV-2, were more likely to have a positive Pap smear than HSV-2-positive patients, suggesting that HSV-2 acts by cross-immunising against HSV-1 [19], and/or by inhibiting HPV expression. Abnormal cytology was also significantly higher in patients infected with HR/HR than in those infected with HR/LR genotypes, suggesting that the timing of these infections was crucial in precipitating abnormal cytology, and that infection with an LR genotype may facilitate development of cell-mediated immunity. *C. trachomatis* was a risk-factor for HPV persistence [5], and may act as a co-factor with HPV in precipitating abnormal cytology by inducing expression of pro-inflammatory mediators [20], altering cell-to-cell adhesion, and affecting cellular differentiation [20]. The results of the present study are in agreement with studies that linked *C. trachomatis* with cervical neoplasia after controlling for HPV, but contradict others that found no association between *C. trachomatis* and low-grade CIN in HPV-adjusted analyses. Accurate determination of HPV and other co-infections in relation to cervical cancer is of relevance in assessing the role of these sexually transmitted infections in cervical diseases.

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RESEARCH NOTE

Treatment of acute post-surgical infection of joint arthroplasty

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ABSTRACT

The best antibiotic regimen for acute prosthetic joint infection, treated without removal of the implant, has not been well-defined. This study describes the use of a protocol based on oral rifampicin combinations to treat 47 cases that were followed prospectively for a 2-year period. The regimen used most commonly was levofloxacin 500 mg/24 h plus rifampicin 600 mg/24 h for a mean duration of 2.7 ± 1 months. The cure rate was 76.9%, and the only independent risk-factor associated with treatment failure was infection caused by methicillin-resistant *Staphylococcus aureus* or *Enterococcus* spp. (OR 17.6, p 0.003). Overall, the results suggested that use of oral antibiotics, including rifampicin, for 2–3 months was a good treatment option.

Keywords Acute prosthetic joint infection, antibiotic regimen, levofloxacin, rifampicin, treatment

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Acute post-surgical prosthetic infection can be treated successfully by open debridément and prolonged intravenous antimicrobial therapy. However, it has not yet been established which

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