Viral Determinants of Human Papillomavirus Persistence following Loop Electrical Excision Procedure Treatment for Cervical Intraepithelial Neoplasia Grade 2 or 3

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Abstract

Background: **Persistent infection with carcinogenic human papillomavirus (HPV) causes cervical precancer (cervical intraepithelial neoplasia grade 2+) which, in the United States, is commonly treated using the loop electrical excision procedure (LEEP). Data from Atypical Squamous Cells of Undetermined Significance–Low-Grade Squamous Intraepithelial Lesion Triage Study were used to evaluate HPV persistence and reappearance after LEEP.**

Methods: **Cervical specimens, collected before LEEP and at 6-month study visits, were tested by L1-PCR for detection of ≥27 HPV types. HPV persistence, defined as the same HPV type being present before and 6 months after LEEP, was evaluated by: (*a*) genotype, (*b*) carcinogenicity, and (*c*) phylogenetic species. HPV infections that cleared post-LEEP (the complement of persistence) were followed for reappearance of the same type.**

Results: **HPV infections (n = 1,130) were detected among 481 women who underwent LEEP. Overall, 20.4% [95% confidence interval (95% CI), 18.2-22.9%] of infections persisted. Assessment of heterogeneity within the three categorizations of HPV showed that phylogenetic species best fit the data. Persistence was significantly greater by HPV types in the α3 species [all are noncarcinogenic; 40.9% (95% CI, 31.8-50.4%)] compared with HPV types in the α9 (HPV16 and related types) and α7 species (HPV18 and related types; 17.6% and 17.9%, respectively; $P < 0.001$ for both). HPV reappeared in 7.8% (95% CI, 6.1-9.9%) of infections that cleared after LEEP. Infections in the α3 species (22.6%) were the most likely to reappear compared with HPV types in the α9 (7.5%) and α7 (6.8%) species.**

Conclusions: **Patterns of HPV persistence and reappearance following LEEP were better explained by phylogenetic rather than standard classifications. HPV types likely to persist after LEEP as well as those likely to repopulate the cervical/vaginal epithelium were those in the α3 species, which are in effect not treated, but are not associated with cervical cancer and are unlikely to cause disease.** (Cancer Epidemiol Biomarkers Prev 2007;16(1):11–6)

Introduction

Persistent infection with carcinogenic human papillomavirus (HPV) is the cause of cervical cancer and its precursor lesion, cervical intraepithelial neoplasia grade 3 (CIN3). In the United States, women with CIN3 and the less serious cervical CIN2 (which together we will refer to as CIN2+) are commonly treated by the loop electrical excision procedure (LEEP). Women treated by LEEP have a residual risk (~10%) for posttreatment CIN2+, presumably due to persistent carcinogenic HPV infection (1, 2).

Using data from the Atypical Squamous Cells of Undetermined Significance–Low-Grade Squamous Intraepithelial Lesion Triage Study (ALTS), we recently reported (3), as have others (4, 5), that posttreatment CIN2+ could be best predicted by the presence or absence of HPV in cervical specimens collected 6 months after the LEEP. More specifically, women still infected with at least one carcinogenic type of HPV had a 15% risk of posttreatment CIN2+ over 2 years, whereas women who were either infected with non–cancer-associated HPV types or were completely HPV negative had a risk of 1.5% and 0%, respectively. Women who were HPV16 positive 6 months after LEEP had ~40% risk of developing posttreatment CIN2+ over 2 years, which was significantly greater than the risk associated with other carcinogenic types (11%; ref. 3).

Although different HPV genotypes clearly confer different risks for posttreatment CIN2+, recent data support the idea that genetically related HPV infections behave similarly and that patterns of viral natural history and carcinogenicity are strongly concordant with HPV phylogenetic analysis (6, 7). For example, HPV16 and related types in the α9 species often cause persistent infection and confer high risk for cervical precancer and cancer (in the absence of screening); the majority of HPV types in this species are considered carcinogenic. In contrast, many HPVs in the α3 and α15 species are also likely to cause persistent infection yet virtually never cause high-grade cervical disease (6).

To complement our study of risk of posttreatment CIN2+ by HPV type, we characterized type-specific and species-specific HPV persistence following LEEP.

Materials and Methods

Overview. ALTS was a randomized controlled trial that compared three strategies for the initial management of equivocal and low-grade cytologic abnormalities. The methods have been described previously (8, 9). Consent was obtained from all participants in accordance with the guidelines of the

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Note: Dr. Kreimer was a Cancer Prevention Fellow in the Division of Cancer Prevention at the time of this work.

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U.S. Department of Health and Human Services; the study was approved by the institutional review board of the NIH and the participating centers. Women (n = 5,060) with a community-read cytologic interpretation of either atypical squamous cells of undetermined significance (n = 3,488) or low-grade squamous intraepithelial lesion (n = 1,572) were enrolled and followed for 2 years. Women were randomized into one of three management arms: immediate colposcopy, HPV triage, and conservative management. At enrollment, the arms differed in their referral criteria for colposcopy: in the immediate colposcopy arm, all women were referred; in the HPV triage arm, Hybrid Capture 2 (Digene Corp., Gaithersburg, MD)–positive women, women with a missing Hybrid Capture 2 result, or women with an enrollment cytologic interpretation of high-grade squamous intraepithelial lesion were referred; and in the conservative management arm, referral was based on a cytologic interpretation of high-grade squamous intraepithelial lesion. In the HPV triage arm, nearly all colposcopy referrals were related to positive Hybrid Capture 2 tests.

At study visits, which occurred every 6 months for 2 years regardless of study arm, nurse-clinicians conducted an interview, a pelvic examination, and collected two cervical specimens. The interview addressed demographic characteristics and potential cofactor information (e.g., oral contraceptive pill use, smoking, and parity). The first specimen was collected with a Papette broom (Wallach Surgical, Orange, CT) and used for cytologic assessment and HPV testing using Hybrid Capture 2. The second sample was collected with a Dacron swab in specimen transport medium (STM; Digene Corp., Gaithersburg, MD) and used for investigational HPV DNA typing that is presented in this analysis. Thin-layer liquid-based cytology was interpreted by the pathologists at the clinical centers. Women with a cytologic interpretation of high-grade squamous intraepithelial lesion were referred to colposcopy. Women who had a colposcopy-directed biopsy diagnosed as histologic CIN2+ were offered treatment by LEEP, which was done using a standard protocol. Detailed information about the procedure was recorded, including whether additional procedures were done (i.e., 'top-hat', ablation, and endocervical curettage).

HPV Testing. HPV DNA was assayed using L1 PGMY09/11 consensus primers with line blot hybridization done on cervical STM samples. Early in ALTS, the following 27 genotypes were detected: HPV 6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51, 52, 53, 54, 55, 56, 57, 58, 59, 66, 68, 73 (MM9), 82 (MM4), 83 (MM7), and 84 (MM8; ref. 10). For the remainder of the trial, these 11 HPVs were also genotyped (for a total of 38 HPV genotypes): HPV 61, 62, 64, 67, 69, 70, 71, 72, 81, 82V, and 89 (11). Consequently of this difference in testing protocol, the baseline prevalence of some noncarcinogenic HPV infections was likely underestimated. At baselines 38.0% of this study population were tested for 27 HPV types, whereas the remainder were tested for all 38 HPV types; analyses were conducted among both groups with similar results. For the main findings of this article, the analysis representing testing for 38 HPV types is presented.

Classification of HPV Types by Carcinogenicity and Phylogenetic Species. The following HPV types were considered carcinogenic: HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68 (7, 12); the remainder were considered noncarcinogenic. Based on previously derived classifications (6), HPV infections were categorized into the following species: α9 (included HPV types 16, 31, 33, 35, 52, 58, and 67), α11 (HPV 73), α7 (HPV 18, 39, 45, 59, 68, and 70), α3 (HPV 61, 62, 72, 81, 83, 84, and 89), α4 (HPV 57), α5 (HPV 26, 51, 69, and 82), α6 (HPV 53, 56, and 66), α1 (HPV 42), α8 (HPV 40), and α10 (HPV 6, 11, and 55), and α13 (HPV 54). Species that were closely genetically related based of HPV phylogenetic analysis were combined (6); specifically, the few infections in the α11 (n = 16) species were combined with infections in the α9 species (α9/α11); the α5 and α6 species were combined (α5/α6) as were infections from the α1, α8, α10, and α13 species (α1/α8/α10/α13).

Statistical Analysis. Women (n = 686) underwent LEEP at study enrollment or during the follow-up period (68% of LEEPs were done following the enrollment study visit). Women were excluded if (a) CIN2+ was not diagnosed on either the colposcopy-directed biopsy or LEEP (n = 20), (b) the pre-LEEP HPV (n = 28) or post-LEEP HPV (n = 72) results were missing, (c) the woman was HPV negative at the visit corresponding to the LEEP (n = 28), or (d) the interval between biopsy and LEEP was >6 months (n = 56) due to the concern that the covariates measured at the time of biopsy would no longer be relevant. The remaining 482 women had 1,136 HPV infections (median no. of infections per woman, 2; range, 1-10) detected in the cervical specimens collected at the study visit corresponding to the LEEP. HPV types present in less than five women before LEEP were excluded [HPV26 (n = 2 infections), HPV57 (n = 1), and HPV69 (n = 3)]; one woman who was singly infected with HPV26 was excluded as a consequence. Multiple infections were included as separate units for each of the constituent types to create an analytic data set with 1,130 units per infections among 481 women. Because women can have multiple infections, generalized estimating equations (GEE) (13) were used to account for potential exchangeable correlation of infections within a woman (see Measures of Association section below).

Definition and Categorizations of Outcomes. HPV test results were evaluated before the LEEP (from either the visit just before the LEEP or immediately preceding the LEEP at the same visit) and after the LEEP (from the study visit immediately following LEEP). HPV persistence was defined as the same HPV type being present before and after LEEP. The complement of persistence, HPV clearance, was said to have occurred when a HPV infection that was present before LEEP was subsequently absent (or negative) after LEEP (at the 6-month follow-up visit). Infections that cleared after LEEP were followed for the remainder of the study to evaluate HPV reappearance (of the same type). HPV persistence and reappearance were evaluated in three different categorizations: (a) singly by genotype (27 levels), (b) by grouping individual HPV infections by carcinogenicity (two levels: carcinogenic and noncarcinogenic), and (c) by grouping individual HPV infections into phylogenetic species (five levels, α9/α11, α7, α3, α5/α6, and α1/α8/α10/α13).

Goodness of Fit by Each Categorization. Likelihood ratio testing was used to compare logistic regression models with test for differences in the proportions of HPV persistence against the null hypothesis of overall HPV persistence. Persistence was evaluated in each of the three categorizations; this analysis was repeated for reappearance. χ^2 tests were used to determine if significant differences existed in the proportion of persistence or reappearance within categorizations; exact methods were used when fewer than five infections were present in a cell.

Measures of Association. The odds of HPV persistence at the study visit after LEEP was modeled as a binary variable (persistence versus clearance) using GEE to examine the effects of treatment, HPV-related variables, age (as a continuous variable), and potential cofactors, including smoking (categorized as never/former/current), oral contraceptive pill use (never/ever), and parity (0/1/2 or more live births). The associations of each categorization of HPV (genotype, carcinogenicity, or species) with both persistence and with reappearance (given initial clearance) were investigated simultaneously with a single continuation ratio logistic regression model (14).

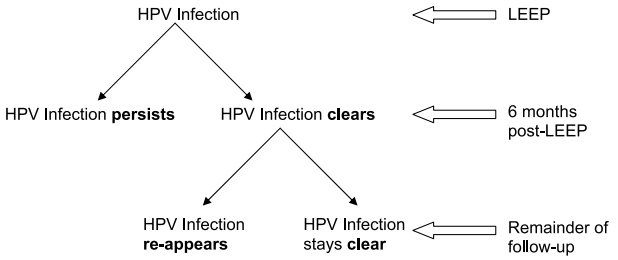

Figure 1. Schema of the continuation ratio model used to simultaneously investigate HPV persistence and reappearance after LEEP.

This model enabled us to estimate the odds ratios (OR) for each categorization with persistence as well as ORs for the same categorization with reappearance and then test the null hypothesis that the pairs of ORs for persistence and reappearance were equal (Fig. 1); for P values ≥ 0.05, we would reject the null hypothesis in favor of the alternative that the ORs differ significantly. For the continuation ratio logistic regression models, standard errors (SEs) was not used because the SEs using GEE were virtually identical to SEs ignoring this correlation (correlation of multiple infections within a woman was 0.07).

Results

Before LEEP, 1,130 HPV infections were detected among the 481 women who had histologic CIN2+ and underwent at least one LEEP; overall, 20.4% [95% confidence interval (95% CI), 18.2-22.9%] of these infections persisted to the subsequent study visit (Fig. 2). When categorized by carcinogenicity, noncarcinogenic HPV infections (28.2%; 95% CI, 23.3-33.6%) were significantly more likely to persist after LEEP compared with carcinogenic HPV infections (17.8%; 95% CI, 15.3-20.5%; $P = 0.0001$; Fig. 2). When categorized by phylogenetic relatedness, HPV types from the α3 species, all of which are noncarcinogenic, were the most likely to persist: 40.9% (95% CI, 32.3-50.0%) of 115 initial infections were still present after LEEP. By comparison, HPV types in the α9/α11 (17.6% of 567; $P < 0.001$) and α7 species (17.9% of 195; $P < 0.001$), the majority of which are carcinogenic, were significantly less likely to persist post-LEEP. HPV types in the α5/α6 species (16.0% of 169; $P < 0.001$), some of which are carcinogenic and others that may be carcinogenic, were also less likely to persist than the average of types in α3. In addition, HPV types in the α1/α8/α10/α13 species (26.2% of 84; $P = 0.03$), which are relatively uncommon in cervical specimens and are noncarcinogenic, were significantly less likely to persist than types in the α3 species.

HPV Persistence among Women. A sensitivity analysis was conducted to evaluate HPV persistence by species among women who were coinfected with at least one HPV infection from both α3 and α9/α11species. This was done as a form of biological 'matching' to evaluate HPV persistence among infections that share the same environment (i.e., the same genital tract of the same woman). There were 83 women positive for at least one infection from both α3 and α9/α11 species at the time of LEEP; of these, 13.3% remained positive for both species following LEEP, 12.0% remained positive for only HPV types in the α9/α11species, 41.0% stayed positive for only HPV types in the α3 species, and 33.7% became HPV negative for both (Mc Nemas's χ^2 $p = 0.0003$).

Evaluation of the Categorization of HPV Persistence. Assessment of heterogeneity within categorizations of HPV persistence by carcinogenicity (2 levels; $P = 0.002$), species (5 levels; $P < 0.001$), or HPV type (27 levels; $P = 0.002$) showed that some strata within each categorization have significantly different HPV persistence (Table 1). Compared with overall persistence, using carcinogenicity as the covariate did not fit the data well ($P = 0.2$); further categorization using species as the covariate significantly improved the model ($P < 0.05$) because using the binary classification of carcinogenic versus noncarcinogenic masked the heterogeneity in HPV persistence specifically seen in the two noncarcinogenic species groups, α3 and α1/α8/α10/α13. Although there was no additional heterogeneity by type within species (all P values >0.05), many types had sparse data that limit the power to detect heterogeneity (Table 1).

Predictors of HPV Persistence. Factors that could potentially confound the analysis of overall HPV persistence versus clearance at the visit after LEEP were investigated (Table 2). Phylogenetic species remained the strongest predictor of HPV persistence: compared with the α9/α11species, infections in the α3 species were thrice as likely to persist following LEEP (OR, 3.3; 95% CI, 2.1-5.0). More extensive disease present prior to LEEP, approximated by the number of pathology blocks from the LEEP specimen that contained CIN2+, significantly increased the odds of HPV persistence 1.7-fold (95% CI, 1.2-2.3). No association between HPV persistence and age, oral contraceptive pill use, smoking, or parity was noted (data not shown).

HPV Clearance and Subsequent Reappearance. HPV infections that were present before LEEP and did not persist at the visit after LEEP (defined at cleared infections, $n = 899$) were followed for reappearance during the follow-up period (median no. of study visits after clearance was 3, representing ~18 months). HPV infections in women with fewer than two visits following the LEEP were excluded because reappearance could not be assessed ($n = 79$; Table 1). Overall, HPV was subsequently detected in cervical specimens in 7.8% (95% CI, 6.1-9.9%) of the remaining 820 infections that cleared after LEEP (defined as reappearing). On average, noncarcinogenic HPV infections (11.1%; 95% CI, 7.3-16.3%) were nearly significantly more likely to reappear after LEEP compared with carcinogenic HPV infections (6.8%; 95% CI, 5.1-9.1%; $P = 0.06$). Again, using carcinogenicity alone as the covariate was not the best fit for the data. Using species to categorize data significantly improved the fit of the model

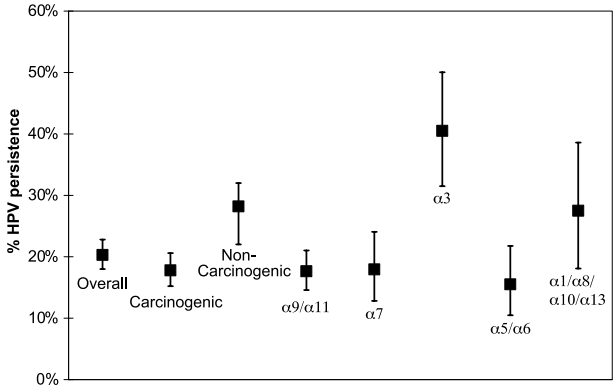

Figure 2. Overall HPV persistence at the visit following LEEP, stratified by HPV carcinogenicity and phylogenetic species. Carcinogenic HPV types include HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68. All other HPV types were considered not associated with cancer (noncarcinogenic). HPV types were grouped into the following species: α9/α11(HPV 16, 31, 33, 35, 52, 58, 67, and 73), α7 (HPV18, 39, 45, 59, 68, and 70), α3 (HPV 61, 62, 72, 81, 83, 84, and 89), α5/α6 (HPV51, 53, 56, 66, and 82), and α1/α8/α10/α13 (HPV 6, 11, 40, 42, 54, and 55).

(p=0.05) because the two noncarcinogenic species, α3 and α1/α8/α10/α13, have significantly different reappearance (22.6% versus 7.4%; P = 0.03) and should therefore not be combined. Infections in the α3 species were the most likely to reappear (22.6% of 62 cleared infections), whereas HPV types in the α9/α11 (7.5% of 428 cleared infections) and α7 (6.8% of 146 cleared infections) species reappeared less commonly. Within each species, the proportion of each of the HPV types that reappeared was similar (all P values >0.3; Table 3); infections within a species seemed to behave similarly with regard to reappearance, although small sample size may have limited the power to detect heterogeneity.

A sensitivity analysis was conducted to evaluate the potential role of HPV tests being considered 'cleared' after LEEP when they may have actually been false negative. Infections that 'cleared' at the post-LEEP visit were required to remain 'cleared' for one additional visit. Similar results were obtained for overall, carcinogenic versus noncarcinogenic and species-specific reappearance (data not shown).

Simultaneous Investigation of Persistence and Reappearance. Using the continuation ratio model, the separate ORs for carcinogenicity with persistence and carcinogenicity with reappearance were not significantly different (P = 0.9; Table 3). Overall, reappearance was significantly less common than persistence (OR, 0.6; 95% CI, 0.4-0.7; data not shown). Again, this carcinogenicity-only model does not fit as well as the species model (P = 0.03), implying that the lumping of species into carcinogenic or not masks heterogeneity in HPV persistence and reappearance by phylogenetic relatedness. Instead, fitting the continuation ratio model with species as the covariate fits the data well (P = 0.6) and shows that each association of species with reappearance does not significantly change from that for persistence (P = 0.6), implying that the species that most commonly persisted after LEEP were also the species that most commonly reappeared. For each species, the ORs for persistence were not significantly different compared with the ORs for reappearance (all P values >0.2; Table 3).

Table 1. HPV infections present before LEEP, persistent after LEEP, cleared after LEEP, and reappeared during the follow-up period, by HPV genotype, carcinogenicity, and phylogenetic species

	Present before LEEP, *n*	Persistent after LEEP, *n* (%)	Cleared* after LEEP, *n*	Reappeared during follow-up, *n* (%)
Overall HPV infection	1,130	231 (20.4)	820	64 (7.8)
Carcinogenic HPV	839	149 (17.8)	630	43 (6.8)
Noncarcinogenic HPV	291	82 (28.2)	190	21 (11.1)
α9/α11†	567	100 (17.6)	428	32 (7.5)
16	263	58 (22.1)	195	11 (5.6)
31	74	9 (12.2)	56	3 (5.4)
33	45	3 (6.7)	38	2 (5.3)
35	38	3 (7.9)	31	4 (12.9)
52	75	12 (16.0)	57	8 (14.0)
58	44	11 (25.0)	28	3 (10.7)
67	12	1 (8.3)	10	0 (0)
73	16	3 (18.8)	13	1 (7.7)
P		0.052		0.4
α7	195	35 (17.9)	146	10 (6.8)
18	57	7 (12.3)	46	3 (6.5)
39	40	4 (10.0)	33	1 (3.0)
45	30	8 (26.7)	19	2 (10.5)
59	29	7 (24.1)	20	0 (0)
68	24	5 (20.8)	17	3 (17.6)
70	15	4 (26.7)	11	1 (9.1)
P		0.2		0.3
α3	115	47 (40.9)	62	14 (22.6)
61	18	7 (38.9)	10	5 (50.0)
62	21	12 (57.1)	7	1 (14.3)
72	8	3 (37.5)	5	2 (40.0)
81	8	4 (50.0)	4	0 (0)
83	22	9 (40.9)	13	2 (15.4)
84	18	6 (33.3)	11	2 (18.2)
89	20	6 (30.0)	12	2 (16.7)
P		0.7		0.4
α5/α6	169	27 (16.0)	130	4 (3.1)
51	63	11 (17.5)	47	1 (2.1)
53	29	5 (17.2)	22	1 (4.5)
56	33	7 (21.2)	24	0 (0)
66	24	4 (16.7)	19	2 (10.5)
82	20	0 (0)	18	0 (0)
P		0.2		0.4
α1/α8/α10/α13	84	22 (26.2)	54	4 (7.4)
6	12	1 (8.3)	8	0 (0)
11	6	0 (0)	5	0 (0)
40	6	0 (0)	6	0 (0)
42	16	6 (37.5)	8	1 (12.5)
54	30	9 (30.0)	19	3 (15.8)
55	14	6 (42.9)	8	0 (0)
P		0.1		0.8

NOTE: HPV types considered carcinogenic are in bold. P represents P value for the χ^2 test for heterogeneity.
*Restricted to HPV infections in women with at least two study visits following the LEEP to assess reappearance (n = 79 infections excluded).
†Only HPV73 is in the α11 species; the majority of this category represents HPV types in the α9 species.

Table 2. Predictors of post-LEEP HPV persistence

	Persisted after LEEP, n = 231	Cleared after LEEP, n = 899	OR* (95% CI)
HPV species			
α9/α11	100 (17.6)	467 (82.4)	1.0
α7	35 (17.9)	160 (82.1)	1.0 (0.7-1.6)
α3	47 (40.9)	68 (59.1)	3.3 (2.1-5.0)
α5/α6	27 (16.0)	142 (84.0)	0.8 (0.5-1.3)
α1/α8/α10/α13	22 (26.2)	62 (73.8)	1.6 (0.9-2.7)
Infection status at LEEP			
Single	24 (14.3)	144 (85.7)	1.0
Coinfected with other types	207 (21.5)	755 (78.5)	1.6 (1.0-2.6)
Adjusted for species †			1.4 (0.9-2.3)
No. CIN2+ blocks cut from the LEEP specimen ‡			
0-1	89 (16.4)	453 (83.6)	1.0
2 or more	142 (24.2)	446 (75.6)	1.7 (1.2-2.3)
Adjusted for species †			1.7 (1.2-2.3)
Diagnosis of the LEEP specimen			
<CIN2	51 (17.0)	249 (83.0)	1.0
CIN2+	180 (21.7)	650 (78.3)	1.4 (1.0-2.0)
Adjusted for species †			1.5 (1.0-2.3)

*The odds of persistence versus clearance are based on generalized estimating equations, as described in Materials and Methods.
†ORs were adjusted for species because that was the strongest predictor of HPV persistence after LEEP.
‡ Used to approximate extent of disease at LEEP.

Discussion

The majority (79.6%) of HPV infections present prior to LEEP were subsequently absent at follow-up. The behavior of HPV infections after treatment could be predicted based on established phylogenetic classifications. HPV infections in the α9/α11 and α7 species, most of which are considered carcinogenic, did not commonly persist or reappear following treatment. Instead, HPV infections in the α3 species, none of which are associated with increased risk of cervical cancer, were the most likely to induce a persistent infection: >40% of infections present before LEEP persisted at the visit after LEEP. Further, infections from the α3 species were also most likely to reappear after LEEP (23%). Even when other potential factors were investigated, including treatment-related variables and cofactors that increase HPV persistence, the strongest predictor of persistence and reappearance remained infection with HPV types in the α3 species. Therefore, we believe that the epidemiology of HPV infection following treatment for CIN2+ by LEEP can be predicted by phylogenetic relatedness, as genetically related HPV types share patterns of behavior with regard to persistence and reappearance.

These data support the current belief that phylogenetic analysis can offer clues about the behavior of certain HPV types (7, 15). Previous analyses have established that genotypes within phylogenetic species tend to be similar with respect to the (*a*) anatomic site of likely infection, (*b*) chance that the infection persists, and (*c*) level of risk conferred for disease. Some of the data to support these conclusions came from a screening study in Guanacaste, Costa Rica that showed that non–cancer associated types in the α3 species were reported to have a predilection for vaginal epithelium compared with HPVs in other species (16). From the same cohort, it was shown that HPV types in the α9 species caused persistent infection and conferred high risk for cervical precancer, whereas types in the α3 and α15 species were also likely to cause persistent infection yet almost never caused high-grade cervical disease (6). Finally, women with infections from the α9/α11 species were more likely to have cytologic abnormalities.[4] In this U.S. population of treated women, additional clues were revealed. Specifically, HPV types likely to persist after LEEP, as well as those likely to repopulate the cervix, were those in the α3 species, which are considered noncarcinogenic.

Although α3 infections present no risk to the cancer infected patients, it is important to understand the underlying mechanism for persistence. Given the vaginal tissue tropism of HPV infections in the α3 species, it is possible that these infections are not treated by excision of the transformation zone during LEEP, in which case, persistence could be considerable. It is also possible that persistence is actually reinfection from an intrinsic source, such as the cervix outside of the excised area (LEEP treats a relatively small area of the genital mucosa) or an extrinsic source, such as a male partner. Incomplete or inadequate treatment may also explain some persistent HPV infection. These virologic margins become the responsibility of the immune response of the host. Although each of these is important, it is beyond the scope of the data to investigate these hypotheses.

Although the evidence suggests that a biological explanation for these findings is merited, there were methodologic limitations. Although PCR is considered the 'gold standard' for HPV detection, the sensitivity of detection differs by individual HPV types (17), which could result in some misclassification of persistence or clearance. Additionally, HPV data were missing for 11 HPV types from the enrollment visit for some women, before the extended line blot assay was used for the remainder of ALTS. Although this likely resulted in an underestimate of the baseline prevalence of some noncarcinogenic HPV types specifically in the α3 species, it would probably not modify the conclusions because we were most interested in the proportions of HPV infections prior to LEEP that persisted. In addition, although specimens were supposed to be collected only from the cervix, it is possible that in some instances the specimen collection device could have contacted the vagina. As HPV types in different species have been reported to infect different anatomic areas of the female genital tract, unintentionally sampling the vagina could confuse the findings of HPV status on the cervix (16).

This study adds to the understanding of HPV behavior following treatment. We determined that noncarcinogenic types in the α3 species are most likely to persist and reappear after treatment for high-grade cervical disease. It seems, from the analysis restricted to women coinfected with HPVs from the α3 and α9/α11 species, that the removal of the transformation zone, commonly accomplished during LEEP, can in fact treat some infections and not others. Importantly, the α3 HPV types are in effect not treated, but they are not associated with cervical cancer and are unlikely to cause cervical disease. Their detection, by design or unplanned cross-reactivity of HPV test methods, should be avoided in posttreatment monitoring of patients.

Table 3. Simultaneous evaluation of HPV persistence and reappearance using continuation ratio models

	Persistence OR	Reappearance OR	P*
HPV species			
α9/α11		Referent category	
α7	1.6	1.0	0.4
α3	3.2	3.5	0.8
α5/α6	1.0	0.4	0.2
α1/α8/α10/α13	1.0	0.9	0.8

*P value tests the hypothesis that each of the persistence ORs is not significantly different compared with the reappearance OR. Because all P values are >0.05, it seems that the odds for persistence and reappearance are similar within each of the categories of phylogenetic species.

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