The Natural History of Type-specific Human Papillomavirus Infections in Female University Students[1]

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Abstract

Little is known about the average duration of type-specific human papillomavirus (HPV) infections and their patterns of persistence. The objectives of this study were to evaluate the rate of acquisition and clearance of specific HPV types in young women. Female university students ($n = 621$) in Montreal were followed for 24 months at 6-month intervals. At each visit, a cervical specimen was collected. HPV DNA was detected using the MY09/MY11 PCR protocol followed by typing for 27 HPV genotypes by a line blot assay. The Kaplan-Meier technique was used to estimate the cumulative probability of acquiring or clearing a HPV infection considering types individually or in high-risk (HR) or low-risk (LR) groups defined by oncogenic potential. Incidence rates were 14.0 cases/1000 women-months (95% confidence interval, 11.4–16.3) and 12.4 cases/1000 women-months (95% confidence interval, 10.4–14.8) for acquiring HR and LR HPV infections, respectively. The 24-month cumulative rates of acquisition were highest for HPV-16 (12%), HPV-51, and HPV-84 (8%). Of the incident infections, HPV-16 was the most persistent (mean duration, 18.3 months), followed by HPV-31 and HPV-53 (14.6 and 14.8 months, respectively). HPV-6 and HPV-84 had the shortest mean duration time (<10 months) The mean durations of incident, same-type LR or HR HPV infections were 13.4 months and 16.3 months, respectively. Whereas the majority of episodes with a type-specific HPV infection cleared within 2 years, there were many women who were either reinfected with a new HPV genotype or presumably experienced reactivation of their initial infection.

Introduction

Whereas there is conclusive evidence that cervical HPV[3] infections are a necessary cause of cervical cancer (1, 2), the discrepancy between the high frequency of HPV infections in young, sexually active women and the relatively low occurrence of cervical lesions in the same population suggests that HPV is not a sufficient cause for cervical neoplasia (3). There is evidence that most HPV infections are transient, and only women who harbor a persistent HPV infection are likely to develop a cervical lesion (4, 5). However, there have been few studies designed to investigate the dynamics of HPV clearance or persistence. Describing the average duration of infection will be of great importance in establishing a clinically relevant definition of a persistent HPV infection that could be used for cervical screening and HPV vaccination studies (6).

In 1996, we began a prospective cohort study of the natural history of HPV infection and cervical neoplasia in a population of young university students in Montreal, Canada to study the rate of acquisition and clearance of specific HPV types in this population and to investigate risk factors for persistent HPV infections. This study presents the descriptive epidemiological results on the dynamics of acquisition, loss, and persistence of type-specific HPV infections.

Materials and Methods

Subjects. Female students attending either the McGill or the Concordia University Health Clinic were invited to participate if they intended to be in Montreal for the next 2 years and had not required treatment for cervical disease in the last 12 months. Recruitment was initiated in November 1996, and accrual was completed in January 1999. All eligible women were asked to return to the clinic every 6 months over a period of 2 years, for a total of five visits. The study protocol was approved by the Research Ethics Boards of McGill University and Concordia University. At each visit, a questionnaire was completed, and endo- and ectocervical cells from the uterine cervix were collected with two Accelon cervical biosamplers (Medscand Inc., Hollywood, FL). A Pap smear was prepared with the first sampler.

HPV DNA Detection. Preparation of the cell suspensions for HPV DNA testing has been described in detail elsewhere, with the use of QIAamp columns (Qiagen) for DNA purification (7). Five μl of DNA were first amplified for β-globin DNA with PC04 and GH20 primers to demonstrate the absence of inhibitors and the integrity of processed DNA (8, 9). β-Globin-

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[3] The abbreviations used are: HPV, human papillomavirus; LR, low-risk; HR, high-risk; CI, confidence interval.

Table 1 Prevalence and incidence[a] of infection with the most frequently detected HPV types and for groups according to oncogenicity

HPV type	Baseline prevalence (%)	No. of incident cases	Women-months of follow-up	Incidence rate (per 1000 woman-months) (95% CI)
HPV-6	2.7	29	12709	2.3 (1.5–3.3)
HPV-16	7.0	62	11928	5.2 (4.0–6.7)
HPV-18	3.1	24	12735	1.9 (1.2–2.8)
HPV-31	2.6	21	12854	1.6 (1.0–2.5)
HPV-39	1.0	247	13476	1.8 (1.1–2.5)
HPV-51	2.9	43	12588	3.4 (2.5–4.6)
HPV-53	4.3	31	12468	2.5 (1.7–3.5)
HPV-54	2.7	32	12783	2.5 (1.7–3.5)
HPV-56	2.6	19	12842	1.5 (0.9–2.3)
HPV-84	3.8	46	12475	3.7 (2.7–4.9)
Any HPV	29.0	155	8151	19.0 (16.1–22.3)
HR HPV	21.8	131	9344	14.0 (11.4–16.3)
LR HPV	14.8	128	10299	12.4 (10.4–14.8)

[a] The 10 types with the highest incidence rates are shown. Not shown are HPV-11, -26, -33, -35, -40, -42, -45, -52, -55, -57, -58, -59, -66, -68, -82, -83, and MM9.

positive specimens were further tested with the L1 consensus HPV primers MY09/MY11 and HMB01 and the line blot assay (Roche Molecular Systems) for the detection of 27 genital HPV genotypes (8, 10). HPV types were analyzed individually or in groups according to their oncogenic classification. HR HPV types included those genotypes that are most frequently found in cervical tumors: HPV-16; -18; -31; -33; -35; -39; -45; -51; -52; -56; -58; -59; and -68. All other individual types that were identified with the line blot assay were classified as LR HPV types (1): HPV-6; -11; -26; -40; -42; -53; -54; -55; -57; -66; -73; -82; -83; and 84 and 73 (10).

Statistical Methods. The estimates of the incidence rate for a given genotype only included women at risk of acquiring that given genotype, so that women with a prevalent infection (at enrollment) for a specific HPV type were excluded from that specific risk set. Patterns of type-specific HPV positivity were described by comparing the overall number of visits positive for a specific HPV type, allowing for intermittent negative results, with the number of consecutive visits with the same HPV type.

The Kaplan-Meier technique (11) was used to estimate the cumulative probability of acquiring a specific HPV type or grouped-type infection (HR HPV or LR HPV) as a function of the length of follow-up for each HPV type or grouped-type infection among women who were negative for the specific genotype or HPV group at baseline. The Kaplan-Meier method was also used to estimate the proportion of women who remained positive for a specific (incident) HPV type or grouped-type-specific infection by considering their index infection, when first detected, as time 0. Type-specific prevalent infections were not included in the analysis for clearance. Thus, for a woman with a prevalent infection, her index infection was the longest enduring type-specific infection that was newly acquired after enrollment. Time to an event was defined as the time until the first visit when a subject was no longer HPV positive (for a given type). Subjects with both HR and LR types in the index visit were assigned to either the HR group or the LR group, based on the type with the longest duration. If there was a tie, the index visit was assigned to the HR group. In case of a tie within the HR group or LR group, all longest persisting infections had to have cleared to be given "clearance" status. Subjects were censored at their last visit. The median and mean duration of infection were estimated directly with the actuarial (Kaplan-Meier) method. The Kaplan-Meier technique was also used to evaluate clearance of an overall HR or an overall LR HPV infection. In this second strategy (which simulated an analysis that would have been conducted with results from using a Hybrid Capture system), a HR or LR HPV infection was only considered to have cleared when a woman was no longer positive for any HR HPVs or any LR HPVs, respectively.

Results

A total of 635 women were initially recruited into the study. However, 13 subjects (2.0%) withdrew before completing their first questionnaire, along with 1 woman who had a β-globin-negative sample at visit 1 and did not return for visit 2. There were a total of 2650 completed visits at the time of this analysis (mean of 4.3 visits/subject), and 2570 (97.6%) of the cervical specimens were suitable for HPV DNA testing. Women with a β-globin-negative result were not excluded from the analyses; instead, the next visit with an informative HPV result was used. Loss to follow-up was approximately 10% per visit with approximately 90% of the participants returning for visit 3 (12 months) and 67.5% of the cohort returning for visit 5 (24 months), thus contributing a total of 13,353 woman-months of follow-up (mean, 21.5 months of follow-up/subject). The average time interval between visits was normally distributed, with the majority of women returning within 5–7 months of their previous visit. The mean age was 23 years (median age, 21 years; age range, 17–42 years), and 45% of the women had $\geq$5 lifetime sexual partners. The majority of women (81%) described themselves as Caucasian, 60% of the participants had never smoked, and 24% were current smokers.

Table 1 shows the prevalence at baseline and incidence for the most common HPV genotypes and grouped-type infections. The prevalence of HR HPV infections was 21.8%, and the prevalence for LR HPV infections was 14.8%. There were 327 women who had HPV detected at one or more visits during the study, and of those women, 124 (38%) had coinfections with a HR and a LR HPV type at the same visit. The three most common HPV types at enrollment were HPV-16 (7%), HPV-53 (4.3%), and HPV-84 (3.8%). HPV-16 (5.2/1000), HPV-84 (3.7/1000), and HPV-51 (3.4/1000) were the most frequent newly acquired infections, with all incidence rates expressed per month. The incidence rates for HR HPV and LR HPV infections were very similar (14.0/1000 and 12.4/1000, respectively). The cumulative rate for new HPV infections was 18.0% (95% CI, 14.1–21.9) at 1 year and 36.4% (95% CI, 31.3–41.5)

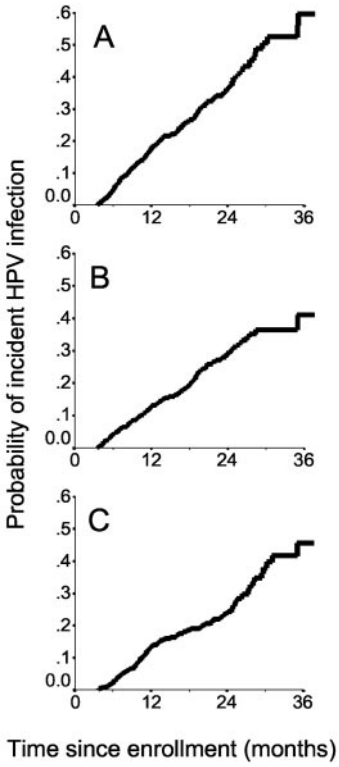

Fig. 1. Cumulative risk of incident HPV infections. *A*, acquisition of any HPV type among women who were HPV negative at enrollment ($n = 420$). *B*, acquisition of a HR HPV infection among women who were HR HPV negative at enrollment ($n = 460$). *C*, acquisition of a LR HPV infection among women who were LR HPV negative at enrollment ($n = 498$).

at 2 years. The equivalent rates for HR HPV were 12.7% (95% CI, 9.6–15.8) and 29.0% (95% CI, 24.4–33.4), and for LR HPV they were 13.4% (95% CI, 10.4–16.4) and 23.7% (95% CI, 19.7–27.7; Fig. 1).

Table 2 shows the pattern of persistence in the cohort. Of those women with 2 or more positive visits with the same HPV type, over 80% harbored these infections at consecutive visits, regardless of the number of positive visits and infection type group. This suggests that persistence tended to be above the levels of viral load that can be effectively detected by standardized cervical sampling coupled with a validated PCR assay.

The most persistent infections, with median retention times of >1 year, included those with types HPV-31 (20.0 months), HPV-16 (19.4 months), HPV-54 (16.8 months), and HPV-53 (13.9 months; Table 3). The median retention time for the types that cleared most rapidly (HPV-6 and HPV-84) was ~6.5 months. The median time for clearance of an incident (type-specific) HR HPV infection (13.2 months) was slightly longer than the median time to loss of an incident (type-specific) LR HPV infection (12.3 months). Approximately 53% or 56% of the women with an incident LR or HR HPV infection, respectively, still remained positive after 1 year of follow-up. The mean durations of most incident infections were very similar to the median estimates, although HPV-16 was the most persistent type (mean duration, 18.3 months), followed by HPV-53 (mean duration, 14.8 months) and HPV-31 (mean duration, 14.6 months). HPV-6 and HPV-84 had the shortest mean duration times. The mean time for clearance of an incident (type-specific) LR HPV or HR HPV infection was close to 13 or 16 months, respectively. The mean duration of an overall LR or HR HPV episode (not necessarily type-specific persistent) was between 16 and 17 months, respectively. After 1 year of follow-up, among women with an incident HPV infection, approximately 59% to 61% of the women remained positive for an overall LR or HR HPV episode, respectively.

Discussion

We decided to present estimates of time to clearance for incident infections only, rather than pooling both prevalent and incident cases. Calculating the average duration times for both prevalent and incident infections could result in an overestimation of duration of an infection because prevalent cases could overrepresent persistent infections at any point in time. We considered two definitions for HPV clearance when HPV types were classified into HR or LR groups. There were many instances when a participant had an infection with more than one HR or LR HPV type at the same visit or at a later visit. Therefore, the first definition was based on mutually exclusive HR or LR groups, and only the most persistent HPV type was included in the analysis. If a woman had an equally persistent HR and LR HPV infection, then she was only included in the HR group, and a woman had to have cleared the longest persisting type before her HPV infection was considered cleared. This approach had the advantage of not mixing a preexisting type and a newly acquired type in the definition of persistence (or in this case, clearance). However, this strategy also has its limitations because we selectively chose the most persistent HR or LR type-specific infection from each subject (with an incident HPV infection) and then estimated a global average duration for any HR or any LR HPV infection. This method may lead to an overestimation or an underestimation of the average duration of specific HPV group infections. For example, if our cohort happened to have an overrepresentation of one specific HR HPV type that happened to persist, on average, for a longer duration than other HR types, then this analysis would tend to inflate the overall duration of HR HPV infections. Therefore, this approach will lead to altered estimates of duration based on the type distribution of HPV infections in a particular population. In our cohort, the median duration of the most (type-specific) persistent (incident) HR HPV infection (13.2 months) was slightly longer than the median duration of a LR HPV infection (12.3 months), but the CIs of the two estimates overlapped considerably.

In our second definition of HR or LR HPV clearance, a woman had to have been negative for any HR type or any LR type subsequent to any incident HR infection or LR infection at the previous visit, respectively. Thus, the concept of persistence could include a preexisting (incident) HR (or LR) type infection mixed with a newly acquired (different) HR (or LR) type infection, provided there was not an intermittent visit that was HR (or LR) HPV negative. Whereas this approach does not directly capture HR or LR HPV persistence with the same type, it does attempt to describe the dynamics of an infection that may contain more than one HR (or LR) infection that is only resolved when all HR (or LR) types are cleared. It also simulates the strategy used by the majority of other researchers who have published data on the median duration of HPV infections (12–16).

Given the large number of participants with more than one HR or LR HPV infection throughout the study, it was not surprising that the estimated median duration of an overall (incident) HR HPV (16.6 months) or an overall (incident) LR HPV (14.7 months) episode was longer than the estimated

Table 2 Persistence *versus* intermittency of HPV infections according to number of visits with the same type-specific infection

No. of visits with the same type-specific infection[a]	No. of subjects with same type HR HPV infections		No. of subjects with same type LR HPV infections	
	Any combination of positive visits	Positivity in consecutive visits only (%)	Any combination of positive visits	Positivity in consecutive visits only (%)
2	73	68 (93.2)	43	36 (83.7)
3	33	27 (81.8)	21	18 (85.7)
4	24	23 (95.8)	7	6 (85.7)
5	16	16 (100.0)	5	5 (100)

[a] These are mutually exclusive groups and refer to the exact number of women with two, three, four, or five visits with a type-specific HR or LR HPV infection.

Table 3 Different measures of the time to loss of an incident infection with specific HPV types and for grouped-type specific infections

HPV type	No. of cases (*n*)	Median retention time (95% CI) (mo)[a]	Mean retention time (95% CI) (mo)[a]	Proportion (%) remaining positive at 1 year (95% CI)[a]
HPV-6	26	6.4 (4.9–7.8)	8.7 (6.8–10.6)	42 (19–65)
HPV-16	62	19.4 (11.4–27.5)	18.3 (12.9–23.7)	62 (46–78)
HPV-18	25	9.4 (4.8–14.0)	11.6 (8.8–14.4)	40 (15–65)
HPV-31	21	20.0 (13.4–26.6)	14.6 (11.0–18.1)	62 (35–89)
HPV-39	24	8.0 (5.8–10.1)	11.0 (7.0–14.9)	32 (3–61.9)
HPV-51	45	9.0 (7.7–10.4)	10.5 (8.4–12.7)	35 (14–56)
HPV-53	31	13.9 (11.1–16.8)	14.8 (11.4–18.3)	62 (41–83)
HPV-54	34	16.8 (8.0–25.7)	13.2 (10.2–16.1)	58 (34–82)
HPV-56	19	8.4 (3.2–13.6)	10.6 (7.9–13.2)	40 (13–67)
HPV-84	47	6.6 (6.0–7.2)	9.9 (7.0–12.8)	23 (7–41)
HR-HPV[b]	124	13.2 (10.2–16.2)	16.3 (13.7–18.9)	56 (44–68)
LR-HPV[c]	73	12.3 (11.4–13.5)	13.4 (11.4–15.4)	53 (41–65)
Any HPV episode[d]	155	17.3 (12.8–21.7)	17.0 (15.1–18.8)	62 (52–72)
HR-HPV episode[e]	131	16.6 (14.5–18.7)	17.4 (14.7–20.1)	61 (51–71)
LR-HPV episode[f]	128	14.7 (10.9–18.4)	15.8 (13.3–18.3)	59 (49–69)

[a] Estimates from actuarial analysis using the Kaplan-Meier technique.
[b] HR HPV infections were grouped according to the longest persisting (incident) HR type-specific infection.
[c] LR HPV infections were grouped according to the longest persisting (incident) LR type-specific infection.
[d] Episode refers to consecutive visits with any type.
[e] Episode refers to consecutive visits with HR (not necessarily type-specific) infection.
[f] Episode refers to consecutive visits with LR group infection (not necessarily type-specific).

duration of the longest HR or LR HPV (type-specific) infection (13.2 months and 12.3 months, respectively). Most other studies have observed that the median duration of LR infections is <5 months, whereas the median duration of HR HPV infections is usually twice as long (8–10 months; Refs. 14 and 15). Whereas our second definition of HPV clearance highlights the high frequency of infection and reinfection with the same or different types (within the same oncogenic group), contrary to other reports (14, 15), our results suggest that the average duration of newly acquired HR or LR types does not differ substantially. However, most of the studies that have been able to estimate the duration of HPV infections have generally evaluated clearance of prevalent or mixed prevalent and incident infections (12–15), and only a few had assays for detecting a substantial number (>10) of LR types (14, 16). These different design issues could affect the estimates of duration of grouped HR and LR HPV infections.

Another explanation for our findings of a similar average duration for incident HR HPV and LR HPV infections is that there may not have been enough follow-up time to reveal the real average length of time to clearance. Only half of the women had a detected cleared incident HPV infection, *versus* 70% of those with LR HPV (data not shown). The remaining women with an incident HPV infection were censored, *i.e.*, possibly persistent. When we looked at clearance of prevalent HPV infections only, the majority of those prevalent HPV episodes cleared before the last follow-up visit (data not shown). Among the latter, HR HPV infections persisted for an average of 19.5 months (95% CI, 16.9–22.1 months), whereas LR HPV infections were cleared, on average, within 16 months (95% CI, 13.7–18.0 months).

Whereas some recent studies have shown the median duration of new or prevalent HPV infections to be less than 10 months in young and middle-aged women (14–16), distinguishing between an infection that has truly resolved and a false negative test result due to poor sampling, low levels of virus, or insensitive measurement tests is very difficult (12). As a result, the clearance rate may be somewhat overestimated, whereas the frequency of persistent infections may be underestimated. At least one study (12) observed that the median duration for a HPV infection (not necessarily type specific) was greater than 1 year (13.8 months), and when Moscicki *et al.* (13) considered various definitions of clearance by modeling different number of consecutive HPV negative tests since the last HPV positive test, the median duration of the infection increased as the definition of clearance became more conservative. The authors concluded that it took approximately 15 months for 50% of the women in their study to clear a prevalent HPV infection, conditional on three consecutive negative HPV tests (but not necessarily a type-specific HPV infection). The median duration for any incident HPV episode in our cohort was 17.3 months (95% CI, 12.8–21.7 months). Whereas misclassification of HPV status may have occurred in our study, our results suggest that there were few false negative results because very

few women with a persistent type-specific infection had an intervening visit with a negative test result, and more than 80% of same type persistent infections occurred during consecutive visits.

The time interval between visits can also influence the assessment of persistence. With a shorter interval between visits, the clearance time for HPV episodes would appear earlier, as would time of acquisition of a new HPV infection. The shorter interval may lead to improved precision of clearance time but would not necessarily change the estimate of mean duration. For practical purposes, we opted for 6-month intervals between testing opportunities because the interval between HPV tests should be consistent with existing clinical guidelines for monitoring cervical cytological abnormalities and is currently defined by most practice standards at 6-month intervals. In addition, between-test intervals of 6 months are more coherent with the biological rationale for using persistent HPV infection as an outcome in trials of HPV vaccine efficacy because it allows for the onset of induced immunity in clearing immediate postvaccination transient infections. With short testing intervals, such infections could mistakenly be interpreted as persistent and be counted as vaccine failure events, a scenario that would lead to a biased estimate of the vaccine efficacy.

Prevalence of cervical HPV infections has been investigated in numerous studies (17). Nonetheless, the geographical variation in type distribution has not been extensively documented, except for HPV-16, which appears to be the most frequently occurring type in most countries (4, 12, 14, 16, 18, 19). Our results are in agreement with these previous studies, with a point prevalence of HPV-16 at enrollment of 7% in the whole population or 24% of all HPV-positive samples at baseline. HPV-53 appears to be very prevalent in different populations, including ours, but the high prevalence of HPV-84 (previously MM8) in our group differs from other recent studies presenting type-specific prevalence from cohorts with a broader age range, which show HPV-84 to be rare (14, 19, 20). Among those women who were HPV negative at baseline in our cohort, approximately 36% were infected with HPV at some time during the 2-year period of the study. Recent longitudinal cohort studies have shown the 36-month cumulative incidence rates for acquiring any new HPV infection to range from 43% to 51% (3, 12, 16) among women in their early 20s and younger. The high incidence rates of HPV-16, -51, and -84 that were seen in our group have also been observed in a cohort of university students in New York (16) and in young women attending gynecology clinics for routine screening in Arizona (15). As an important caveat, we generally considered the natural history of LR or HR HPV infections independent of a coinfection with a different HPV group. Coinfections at the same visit made up 38% of the overall infections in our cohort, of which the majority were a LR type accompanied by one or more HR types (data not shown).

Another limitation of the study was that only 27 HPV types could be detected with our system. Although the most frequent and important (in terms of oncogenic potential) HPV types have been included in our line blot PCR assay, our results are not directly comparable with those of studies that could detect more than 35 types (14, 16). It is conceivable that one or more LR HPV types not included in our probe set could have been present in our population and thus would have constituted false negative results, although the extent of the bias is probably small because of the rarity of such types.

In conclusion, the natural history of cervical HPV infections in this cohort of university students is anything but static. There is frequent acquisition of both HR and LR HPV types. The median duration of the longest persisting, newly acquired LR and HR HPV infections is a little over 12 months, with great variation in the average duration of type-specific infections (range, 6.3–20 months among the 10 most common types). Whereas the majority of the incident type-specific HPV infections cleared within 2 years, there were also many women who were either reinfected with a different HPV type or presumably experienced reactivation of their initial infection. Results from ongoing variant analyses of HPV-16 and HPV-18 in our cohort will help us determine how many of those type-specific reinfections actually represent the same infection. Finally, whether coinfections influence the natural history of type-specific infections still needs to be further explored, although preliminary investigations suggest that whereas risk of acquisition may be higher among women with coinfections, persistence is not affected (21–23).

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