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Factors that influence the rate of epithelial maturation in the cervix of healthy young women

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Abstract

Purpose—To examine the longitudinal changes in the epithelial topography of the cervix in healthy young women; and to determine the sociodemographic, behavioral, and biological factors associated with the rate of cervical epithelial maturation.

Methods—Healthy young women were enrolled (10/00-9/02) as part of a larger study of human papillomavirus (HPV). At interval visits, interviews, infection testing, and colposcopy (3% acetic acid; 10X,16X magnifications) were performed. Areas of total cervical face and cervical immaturity, defined as columnar and early squamous metaplasia, were quantitatively measured using computerized planimetry. Cervical immaturity was expressed as percentage of total cervical face. This analysis includes the first consecutive 145 women with greater than 10% immaturity at baseline. The rate of cervical maturation was defined as change in percent-immaturity. Predictors included sociodemographics, sexual behaviors, and infections. Data analyses included multivariate generalized linear models with repeated measures.

Results—The baseline mean age was 17.8 years. Colposcopic photographs were available from 815 total visits, representing 2.7 years mean follow-up per woman and 5.9 month mean intervals. Women began the study with a median of 39% immaturity and ended with 8% immaturity. After adjusting for time and baseline percent-immaturity, an increased rate of cervical maturation was associated with oral contraceptive pill use (parameter estimate -0.023 , $p=0.04$) and smoking (-0.039 , $p=0.01$).

Conclusions—Cervical maturation was documented during relatively short time periods for the vast majority of these women. Oral contraceptive pills and smoking may accelerate the maturational process, representing increased cell proliferation and thus a possible greater vulnerability to HPV.

Keywords

Cervix; cervical epithelium; ectopy; cervical maturation; squamous metaplasia; transformation zone; colposcopy; adolescent women

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Introduction

Understanding the physiologic process of cervical maturation during adolescence and young adulthood may give us insight into the epidemiologic observation that adolescents and young adults appear vulnerable to certain pathogens. Women ages 15–24 years have the highest age-specific rates of reportable sexually transmitted infections (STIs) including *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and human papillomavirus (HPV).^(1, 2) This vulnerability is often attributed to risky sexual behaviors, but biologic factors including the epithelial topography of the cervix during this time period may also be contributory. During fetal development, the cervical epithelium is composed of a single layer of Mullerian columnar cells that are partially replaced by urogenital stratified squamous epithelium.⁽³⁾ An abrupt squamo-columnar junction is seen on the neonatal ectocervix. The epithelium remains relatively quiescent until puberty, when uncommitted basal cells of the columnar epithelium transform into squamous cells in a physiologic process termed “squamous metaplasia”.⁽⁴⁾ This epithelial area of transformation is referred to as the “transformation zone”. Over time, a new squamo-columnar junction is typically formed more proximally at the endocervix as the topography of the ectocervix transforms from a predominant columnar epithelium (considered to be more immature) to a predominant squamous epithelium (more mature). The cervical epithelium is the primary site of infection for common STIs. Columnar epithelial cells are known targets for *C. trachomatis* and *N. gonorrhoeae* infection,^(5–7) whereas the active transformation zone is an ideal host for HPV as host cell replication and differentiation is required for HPV growth. Understanding the natural history of cervical maturation may assist us in identifying factors that exacerbate or minimize the vulnerability of the epithelium to infection.

Unfortunately, few studies have examined the maturation process of the cervical epithelium in adolescents.^(4, 8, 9) Some cross sectional studies have reported associations between greater cervical maturation and higher numbers of years sexually active and numbers of sexual partners.^(8, 10) These behavioral factors may represent biological factors such as an increased exposure to infections or cervical trauma. Cervicitis has been associated with a loss of cervical columnar cells, a typical feature of the maturational process.⁽¹¹⁾ Consequently, STIs have been hypothesized to promote maturation via the induction of inflammation and subsequent cellular repair. In contrast, hormonal contraception has been linked to increased cervical ectopy (or less cervical maturation).^(12–14) Some have suggested that the appearance of increased immaturity is due to hormonal contraception causing tissue edema and eversion of the endocervix. However, more recent studies showed no association.^(15–17) Squamous metaplasia itself is thought to be triggered by the shift of the vaginal pH from the pre-pubertal level of >4.7 to the more acidic pubertal level of <4.5. In theory, maturation could be slowed or reversed by alkaline disturbances, as in bacterial vaginosis or with frequent exposure to semen which is strongly alkaline. Cigarette smoking is another possible influence since nicotine and its metabolites are detectable in cervical mucous.^(18, 19) Interestingly, both hormonal contraception and smoking have long been associated with an increased risk for cervical cancer,^(20, 21) and HPV infection is common during adolescence and young adulthood.⁽²⁾ As the cervical epithelium changes during this age, the possible relationships between hormonal contraception, smoking, and maturational activity could influence one’s vulnerability to HPV and other STIs. Since prior studies of cervical maturation were retrospective, cross-sectional, or case-control, it is difficult if not impossible to identify causal dynamic relationships.

The aims of this study were to: 1) examine the changes in epithelial topography of the cervix in healthy young women in a longitudinal design, and 2) determine the sociodemographic, behavioral, and biological factors associated with the rate of cervical epithelial maturation.

Methods

Participants

Study participants were selected from a larger cohort of women participating in an on-going longitudinal study of HPV that has been described elsewhere.(22) Briefly, beginning in 2000, women were voluntarily enrolled from a college health center and a family planning clinic. The inclusion criteria to the larger cohort were: ages 13–21 years, history of less than 5 years of sexual activity, and intent to attend interval visits for at least 3 years. The exclusion criteria were: history of treatment for squamous intraepithelial lesions (SIL) of the cervix, any immunosuppressive condition, or pregnancy. The only exclusion during follow-up was histology-proven incident cervical intra-epithelial neoplasia (CIN) 2/3. The study was approved by the Committee on Human Subject Research at the University of California, San Francisco and the San Francisco State University Committee on Human Research.

Study Design

Each participant had a baseline visit and was asked to return for 4-month interval visits. Each visit included a face-to-face interview to collect information concerning socio-demographic characteristics, behaviors, and medical history. A physical examination was performed by research staff clinicians to collect cervical and vaginal samples as described previously.(23) Briefly, bacterial vaginosis, Candidal, and *T. vaginalis* infections were diagnosed on routine normal saline and KOH wet mounts.(24) HPV DNA was detected from cervical vaginal lavage (CVL) samples using the Roche Reverse Line Blot assay.(25) Commercial PCR assays were applied to cervical swabs to test for *C. trachomatis* and *N. gonorrhoeae*. The assays for *C. trachomatis* and *N. gonorrhoeae* were performed at annual and any symptomatic visits. If a positive result was found, the cervical samples stored from a minimum of two prior visits were also tested to more closely characterize the onset of infection. After these samples were obtained, colposcopy was performed using 3% acetic acid applied to the cervix to accentuate the epithelial appearance.(26) After centering the cervix as much as possible, colpophotographs were taken at 10X and 16X magnifications using a Nikon camera (N2020 or N90S) attached to the colposcope and 35 mm film. In addition to the study visits, participants who reported genitourinary symptoms or concerns at any other time were seen by research staff for diagnosis and treatment of any infections.

Colpophotographic measurements

The visible areas of interest on the epithelium were measured using computerized planimetry techniques that were published previously(10, 27) and modified for this study.

Colpophotographs were viewed as digital images (1024×1536 pixels) using Adobe Photoshop® CS2. Nondestructive adjustment layers, sharpening tools, and shadow brightening tools were custom applied to each colpophotograph to improve image clarity. Only those Photoshop® tools that adjust all pixels consistently and thus preserve the integrity of the original image data were applied. Areas of immature epithelium, defined using standard colposcopic features for columnar epithelium and early-mid squamous metaplasia,(26) were outlined with digital freehand markings. Discontinuous areas required multiple discrete markings. The area of the total cervical face, defined as the cervical portio visible on clinical examination, was outlined with a combination of automated curves and freehand markings. Pixel counts were calculated to represent each marked area. “Percent-immaturity” was calculated as the area of immature epithelium divided by the area of total cervical face.

Use of the percent-immaturity measure improved the ability to compare colpophotographs since absolute pixel counts do not account for variations in photographic conditions (angle of view) and inconsistent cervical size portrayed on the photographs taken at different visits for a single woman. Furthermore, we purposely viewed the colpophotographs from all study visits

on the screen simultaneously. This approach allowed a consistency in the definition of the total cervical face and the identification of any photographs that may have been incorrectly labeled. We performed a small analysis to compare our simultaneous approach to that of measuring single colpophotographs in random order. The variability of the calculated percent-immaturity ranged from 15–20% when colpophotographs were measured in random order by two reviewers. However, this variability was reduced to 5–10% using the simultaneous approach and appears consistent with previous work indicating an error rate of 5–10% upon repeat measurements of a single photo due to the inherent practical limitations of gross freehand outlining.^(10, 27) Additionally to minimize error, if the cervical epithelial topography was deemed to show no change compared to the previous colpophotograph, the identical value of percent-immaturity was entered into the dataset. Otherwise, complete measurements and pixel calculations were performed. Given the inherent coarseness of using colpophotographs, these approaches allowed us to focus on gross cervical changes rather than small subtle changes that would be difficult to reproduce and indistinguishable from measurement error. The reviewer (L.H.) was blinded to all participants' characteristics and study data. A subset (10%) of colpophotographs was measured by a second blinded reviewer (A-B.M.) and measurements were reconciled.

Other measures and statistical analyses

Since our specific aim was to examine maturational changes in the cervical epithelium, women from the larger cohort were selected for this analysis according to these additional inclusion criteria: a minimum of 10% immaturity on the first available colpophotograph, and minimum of two years of follow-up during which the colpophotographs were adequate for image analysis. Among the larger cohort of 616 women, approximately one-third of women were eligible for this analysis. The first consecutive 145 eligible women (enrolled October 2000–September 2002) were selected.

All of the variables in this analysis were time-dependent except for the static predictor variable, ethnicity. Each visit with an available colpophotograph was analyzed. "Visit (x)" refers to the visit currently under analysis; "visit (x-1)" is the nearest prior visit; and visit (x-2) is the second nearest prior visit. The outcome variable was the rate of cervical maturation, calculated from the percent-immaturity at visit (x) minus the percent-immaturity at visit (x-1). A negative value indicated a decrease in cervical immaturity and an assumption of interim squamous metaplasia. In contrast, a positive value indicated an increase in cervical immaturity and an assumption of interim reversal to more immaturity. The time-dependent predictors assessed at visit (x) were: chronological age, time since menarche, time since first intercourse, number of lifetime sexual partners, number of new sexual partners since the previous visit, frequency of sexual intercourse (number of episodes in the prior 2 months), condom use (in the prior 2 months: never, rarely, half the time, most of the time, or always), and the following dichotomous variables (currently yes or no): cigarette smoking, pregnancy, and any hormonal contraceptive use. The time-dependent variables for the genital infections were also dichotomous (yes or no). Bacterial vaginosis, candida, and HPV were each defined as any infection detected between 2–4 months prior to visit (x) and were inclusive of all diagnoses made at study and non-study visits. *C. trachomatis*, *N. gonorrhoeae*, and *T. vaginalis* infection were each defined as any infection detected between 4–8 months prior to visit (x) to allow more time for the treatment and resolution of acute inflammation and cellular repair. To address the possibility of other timeframes of longitudinal influence, we also examined alternative definitions that included infections up to 18 months prior to visit (x), or included infections from visit (x) to (x-1), or visit (x-1) to visit (x-2), but all of these results were unchanged and thus not presented here.

Data analyses included bivariate and multivariate generalized linear models with repeated measures. Once an individual woman's cervical epithelium appeared largely mature (<10%

immaturity) and stable, her subsequent visits were excluded to avoid misleading errors in the repeated measures models. Each model was adjusted for time that was entered a priori in 4-month increments, and for percent-immaturity measured at baseline since greater immaturity at baseline was significantly associated with an accelerated rate of maturation during follow-up (correlation coefficient=0.83, $p<0.0001$). This finding was expected since a woman who begins the study with a greater area of percent-immaturity would inherently have a greater likelihood to demonstrate cervical maturational activity during follow-up. A separate bivariate model was created for each predictor variable discussed above. Then an initial multivariate model included all predictor variables, and the least significant variable was removed in each subsequent iteration. Two-way interaction terms between time and the independent variables were examined. Goodness of fit was evaluated using the Akaike information criterion (AIC) or the Bayesian information criterion (BIC) when we performed model comparisons and selections. The final most parsimonious model retained the time variable, percent-immaturity measured at baseline, and all predictor variables found to be significant at $p<0.05$. Since we observed that some women experienced short time periods of increasing cervical immaturity, a small subanalysis was conducted to examine factors associated with this reversal towards more immaturity. These analyses compared the time intervals of reversal to those of no change as a dichotomous outcome variable. Bivariate and multivariate generalized estimating equation models were constructed for the predictor variables above.

Results

Sociodemographic characteristics and behaviors

Colpophotographs were adequate for measurement from 815 visits for the 145 women, yielding 465.1 woman-years of follow-up, and means of 5.6 visits (SD, 1.8) and 2.7 years (SD, 1.0) of follow up per woman. The mean time between these visits was 5.9 months (SD, 2.2 mo). The demographic characteristics at baseline, and the behaviors and infections over the entire study period are shown in Table 1. This young cohort with a mean baseline age of 17.8 years was ethnically diverse and had been sexually active for a mean of 1.8 years at baseline. Vaginal intercourse was common during the study, with a mean of 7 episodes per month. Consistent condom use was reported at only 17% of visits, and hormonal contraception use at 28%. The hormonal contraception consisted largely of oral contraceptive pills containing 30–35 micrograms of estrogen (74%) or 20 micrograms of estrogen (12%). No progestin-only pills were reported. The remaining hormonal methods were injections of medroxyprogesterone acetate (21%) and medroxyprogesterone acetate/estradiol cypionate (0.9%). *C. trachomatis* infections were diagnosed 27 times in 19 distinct women; and *N. gonorrhoeae* and *T. vaginalis* were rare. Within the study, women presented with current pregnancies at 2% of visits.

Cervical maturation

Our sample of 145 women began the study with a median area of cervical immaturity of 39% (interquartile range, 20%, 52%) and ended the study with a median of 8% (3%, 14%) immaturity. From the 815 visits, 670 short intervals were derived between each visit (x) and visit (x-1) as shown in Table 2. Among these short intervals, 388 (58%) demonstrated a decrease in percent-immaturity (median -8%, range -64 to -0.6%) suggesting maturational change; 234 (35%) had no change; and 48 (7%) had an increase in percent-immaturity (median +12%, range +0.1 to +62%) suggesting reversal towards more immaturity.

By study-end compared to baseline, 137 (95%) women had experienced a net maturational change of -25% immaturity (median) observed during a mean of 2.7 years of follow-up. In these 137 women, the incremental patterns of change varied. For the majority (n=91) of women, the net maturation was produced from a mixture of short intervals of maturation and no change,

as in Figure 1. A smaller number of women (n=46) exhibited a net maturation via a mixture of short intervals that included reversal as well as maturation and no change, as in Figure 2. Additionally in our sample, there were 2 (1%) women who experienced no net change during a mean 2.6 years of follow-up time; and 6 (4%) women who had a net reversal change of +8% immaturity (median) during a mean of 3.1 years of follow-up.

Factors that influence cervical change

The bivariate analyses to examine associations between each predictor variable and the rate of cervical maturation are shown in Table 3. Three factors were significantly associated with an increased rate of maturation: oral contraceptive pill use ($p=0.05$), smoking ($p=0.01$), and frequency of sexual activity ($p=0.03$). Table 4 demonstrates the final multivariate model in which two factors, oral contraceptive use and smoking, remained significant. Oral contraceptive use was associated with an additional 2.3% maturational change for each time increment of 4 months, compared to non-use of oral contraception ($p=0.04$). For example, an average acceleration of 14% maturational change over two years would be gained due to continuous use of oral contraception compared to non-use of oral contraception. Similarly, smoking was associated with an additional 3.9% maturational change for each time increment compared to non-smoking ($p=0.01$). This would translate to an acceleration of 23% maturational change during two years of smoking compared to non-smoking. Two-way interactions between time and each predictor variable, time-by-oral-contraception and time-by-smoking, were not significant (data not shown). Since the oral contraceptive use may be a proxy for new sexual partners, another multivariate model was created to adjust for the number of new partners, but the results were unchanged (data not shown).

For sub-analyses of the factors associated with reversal, intervals of reversal were selected for comparison to intervals of no change. We selected the 34 reversal intervals during which the percent-immaturity increased at least +5% and excluded the remaining 15 reversal intervals to avoid possible measurement error. Bivariate models indicated that a pregnant woman was more likely to experience reversal than a non-pregnant woman (OR 9.27, 95% CI 2.35–36.51, $p<0.01$), whereas an oral contraceptive user was less likely to experience reversal than a non-user (OR 0.45, CI 0.21–0.96, $p=0.04$). These results remained consistent in a multivariate model controlling for smoking and the number of recent sexual partners. Again, pregnancy was associated with an increased odds for reversal (6.77, 1.56–29.34, $p=0.01$). Oral contraceptive use was associated with a decreased odds for reversal at a marginal significance level (0.43, 0.18–1.01, $p=0.05$).

Discussion

This is the first prospective study to our knowledge that documents the dynamic maturational process in the cervix of healthy adolescent women. This study was unique in the recording of frequent cervical changes by using a quantitative measure of the epithelial topography. Most women had evidence of at least minor maturational changes during brief 4–8 month time periods. During the 2–3 year observation, the change was more dramatic with an overall 31% replacement of the columnar and metaplastic epithelium with mature squamous epithelium. Individual women appeared to have times of substantial acceleration as well as times of dormancy.

Oral contraceptive use and smoking were the two key factors found to accelerate maturation, and are also each known to be important risk factors for cervical neoplasia. (20–21) Estrogen and progesterone are known to promote cell proliferation, and intraepithelial estrogen and progesterone receptors are significantly more plentiful in the transformation zone compared to other cervical tissues.(28) Therefore, stimulation by supraphysiologic exogenous doses of estrogen and progesterone may conceivably accelerate squamous metaplasia in the

transformation zone. However, a traditional past belief was that oral contraceptive use results in increased cervical immaturity, as estrogen was thought to cause cervical stromal edema and a resultant eversion of the columnar epithelium of the endocervix out onto the ectocervix. Previous studies supporting this concept were cross sectional. In contrast, our prospective study found the opposite effect of enhanced maturation from oral contraceptive use. Current-day low-dose formulations may result in hormonal levels in a range capable of stimulating the receptors in the cervical epithelium without inducing stromal edema whereas the higher doses in older studies may have exerted both effects. To our knowledge, nicotine effects on the cervix have not previously been investigated with reference to epithelial maturation. In addition to nicotine and its metabolites being present in cervical secretions,(18, 19) cervical enzymes are known to metabolize tobacco-specific carcinogens, and tobacco-specific nitrosamines cause DNA damage in cervical tissues.(29, 30) Our data suggest that during the normal physiologic process of cervical maturation, tobacco accelerates squamous metaplasia. We hypothesize that the link between oral contraceptive use, smoking and cervical cancer may be this increase in cell proliferation that increases one's vulnerability to HPV, DNA damage, and subsequent neoplasia. Previous work has demonstrated that a more rapidly maturing epithelium rather than the absolute area of immature epithelium is a significant risk factor for incident low-grade squamous intraepithelial lesions, a clinical manifestation of HPV infection.(27) Smoking has also been associated with changes in cervical cytokine secretion,(22) which may alter the metaplastic process.

In contrast to several other reports, our final model found a lack of influence of sexual activity and number of partners on cervical maturation. The most likely explanation is the prospective design of our study and the ability to examine other cofactors that may be related to risky sexual behavior, such as hormonal contraceptive use and smoking.(31) Alternatively, our study assessments began only after a women's sexual debut and we may have missed any factors that were influential around the time of sexual debut.

One study limitation was the coarseness of our methodology of colpophotography and computerized planimetry for the gross measurement of the epithelium. Fine cervical changes were not possible to study and other influences may not have been detected. Overall, our methodology appears to be relatively accurate since we were able to identify a previously documented factor, pregnancy,(32) as a cause of "reversal" of maturation. We also acknowledge our inability to assess genetic influences on the rate of maturation. We examined several behavioral and infectious factors to reflect the complexity of exposures at the cervix, but epigenetic factors are also likely important contributors to cervical development in young women. Finally, our sample of women was selectively comprised of sexually active women who volunteered to have frequent colposcopic examinations during an on-going study of HPV. However, the larger cohort study did not enroll women based on HPV status. Consequently, we feel that our sample represented a relatively unbiased sexually active population for this study.

In summary, our longitudinal data is the first to support that many sexually active healthy adolescents undergo active epithelial maturation in the cervix over relatively short periods of time. Oral contraception and smoking are identified as important accelerators of maturation. This increased cell proliferation may represent a dynamic response by the epithelium, but we postulate that it may enhance a woman's vulnerability to HPV infection and DNA damage. Understanding the complexities of cervical epithelial changes could reveal biological factors that contribute to the high rates of STIs in adolescent women.

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A. 21% immaturity
at Time 0

B. 8% immaturity
at 11 mo of follow-up

C. 4% immaturity
at 27 mo of follow-up

D. 4% immaturity
at 31 mo of follow-up

Figure 1.

Selected colpophotographs for one woman with short intervals of maturation change or no change

A. 44% immaturity
at time 0

B. 61% immaturity
at 3 months follow-up

C. 37% immaturity
at 11 months follow-up

D. 22% immaturity
at 14 months follow-up

E. 19% immaturity
at 29 months follow-up

Figure 2.

Selected colpophotographs for one woman with short intervals of reversal or maturation change

Table 1

Demographic characteristics, behaviors, and test results (N=145 women)

Demographic characteristic, behavior, or test result	mean (standard deviation) or n (%) ^a
Age at baseline, years	17.8 (2.1)
Ethnicity	
African-American	10 (7%)
Asian	24 (16%)
Latina/Hispanic	48 (33%)
White	43 (30%)
Mixed/Other	20 (14%)
Age of menarche, years	12.3 (1.3)
Age of first intercourse, years	16.0 (2.1)
Number of lifetime sexual partners at study entry	4.5 (6.3)
Number of new sexual partners since previous visit	0.4 (0.8)
Episodes of sexual activity in past 2 months	14 (16)
Condom use in past 2 months	
Never/rarely	479 (61%)
Half/most of the time	173 (22%)
Always	136 (17%)
Current pregnancy	20 (2%)
Current hormonal contraceptive use	221 (28%)
Current smoking	196 (26%)
<i>Chlamydia trachomatis</i> infection ^b	27 (3%)
<i>Neisseria gonorrhoeae</i> infection ^b	1 (0.1%)
<i>Trichomonas vaginalis</i> infection ^b	0 (0%)
Bacterial vaginosis ^c	12 (1%)
Candidal infection ^c	15 (2%)
HPV infection by any of 32 types ^c	234 (31%)
HPV infection by high risk types ^{c,d}	179 (23%)
HPV infection by low risk types ^{c,e}	46 (6%)

HPV, human papillomavirus

^a Behaviors that were repeatedly measured are reported for all 815 visits by the 145 women. Variations in the denominators are due to missing data.^b defined as any infection during the 4–8 months prior to each visit^c defined as any infection during the 2–4 months prior to each visit^d High-risk types: 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, 82.^e HPV Low-risk types: 2, 6, 11, 40, 42, 54, 55, 57, 67, 69, 70, 72, 83, 84.

Table 2

Changes in percent-immaturity of the cervical epithelium during entire study and during discrete intervals

Direction of net change over entire study (n=145 women)	No of women (%)	Amount of change in percent-immaturity, median	Total follow-up time, mean years (SD)
Maturation	137 (95%)	-25%	2.7 (1.0)
No change	2 (1%)	NA	2.6 (0.8)
Reversal	6 (4%)	+8%	3.1 (1.1)
Direction of change during each short interval (n=670 intervals)	No of intervals (%)	Amount of change in percent-immaturity, median	Duration of interval, mean months (SD)
Maturation	388 (58%)	-8%	7.5(5.6)
No change	234 (35%)	NA	6.5(4.7)
Reversal	48 (7%)	+12%	9.1(7.2)

Table 3

Bivariate analyses of the associations between demographic, behavioral, and infectious factors and the rate of cervical epithelial maturation^a

Demographic, behavioral, or infectious factor	Parameter estimate	Standard error	p value
Age	0.003	0.003	0.37
Ethnicity ^b			
African-American	−0.011	0.035	0.76
Asian	0.003	0.025	0.91
Latina/Hispanic	−0.014	0.020	0.48
Mixed	−0.025	0.027	0.35
Age of menarche	−0.004	0.006	0.53
Age of first intercourse	0.002	0.004	0.65
Number of lifetime sexual partners at study entry	<0.001	0.001	0.98
Number of new sexual partners since the previous visit	−0.006	0.006	0.31
Frequency of sexual activity	−0.001	<0.001	0.03
Condom use ^c			
Always	−0.009	0.014	0.50
Most of the time	−0.015	0.015	0.33
Half the time	−0.011	0.018	0.53
Rarely	−0.026	0.016	0.10
Current pregnancy	0.035	0.031	0.27
Current oral contraceptive pill use	−0.022	0.011	0.05
Current medroxyprogesterone injection use	0.001	0.023	0.96
Current smoking	−0.038	0.015	0.01
<i>Chlamydia trachomatis</i> infection ^d	<0.001	0.028	0.99
<i>Neisseria gonorrhoeae</i> infection ^d	0.023	0.132	0.86
<i>Trichomonas vaginalis</i> infection ^{d,e}	--	--	--
Bacterial vaginosis ^f	−0.047	0.039	0.23
Candidal infection ^f	−0.019	0.035	0.59
HPV infection by any of 32 types ^f	0.002	0.006	0.78
HPV infection by high risk types ^{f,g}	−0.008	0.012	0.55
HPV infection by low risk types ^{f,h}	0.018	0.021	0.40

HPV, human papillomavirus

^aRate of cervical epithelial maturation was defined as the change in percent-immaturity between two sequential visits. Each model was adjusted for percent-immaturity at study entry, and the time variable was entered in 4-month increments.

^bReferent group was Caucasian.

^cReferent group was non-use of condoms.

^ddefined as any infection during the 4–8 months prior to each visit

^eInsufficient number of positive infections to generate a parameter estimate

^fdefined as any infection during the 2–4 months prior to each visit

^gHigh-risk types: 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, 82.

^hHPV Low-risk types: 2, 6, 11, 40, 42, 54, 55, 57, 67, 69, 70, 72, 83, 84.

Table 4Multivariate analysis of the associations between behaviors and the rate of cervical epithelial maturation ^a

Behavior	Parameter estimate	Standard Error	p value
Percent-immaturity at baseline	0.617	0.035	<0.01
Current oral contraceptive pill use	-0.023	0.011	0.04
Current smoking	-0.039	0.015	0.01

^aRate of cervical epithelial maturation was defined as the change in percent-immaturity between two sequential visits. The time variable was entered in 4-month increments.

Akaike information criterion (AIC) = -775.2

Bayesian information criterion (BIC) = -769.3