RESEARCH COMMUNICATION

Tuberculosis and Oncogenic HPV: Potential Co-infections in Women at High-risk of Cervical Cancer in Rural China

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Abstract

The study was embedded in Shanxi Province Cervical Cancer Screening Study II with the aim of examining the association between history of diagnosed tuberculosis or cervical inflammation and oncogenic human papillomavirus (HPV) infection, persistent oncogenic HPV infection, cervical intraepithelial neoplasia grade 3 or cervical cancer (CIN3+) in an isolated rural population of China. A total of 8,798 women were recruited for cervical cancer screening and an interviewer-administered questionnaire. Of the women in the study, 2.7% and 34% reported a diagnosis of tuberculosis and cervical inflammation, respectively. In the model for HPV infection, HPV persistence and CIN3+, we show an increasing magnitude of effect of tuberculosis with increasing severity of disease, as demonstrated by the increasing odds ratios from 1.68 for HPV positivity, to 1.75 for persistent HPV and then 2.08 for CIN3+. Women reporting a diagnosis of tuberculosis, cervical inflammation or both tuberculosis and cervical inflammation were at 75%, 22% and 113% higher odds of persistent HPV infection, respectively. One percent of the study population was diagnosed with tuberculosis and cervical inflammation, placing them at 90% and 113% higher odds of infection with HPV and persistent HPV, respectively. Tuberculosis and oncogenic HPV are identified for the first time as co-infections in rural unscreened women in Shanxi Province, China, highlighting the importance of infection history in assessing an individual's risk for HPV infection, persistence and CIN3+.

Keywords: Cervical cancer - CIN - inflammation - HPV - vaginal trichomoniasis - tuberculosis

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Introduction

Cervical cancer (CC) is a tremendous health burden in many areas of developing countries where screening is often limited. CC remains the second most commonly diagnosed cancer in women worldwide, with 85% of cases occurring in disadvantaged countries (Ferlay et al., 2008). Human papilloma virus (HPV) has been established as a necessary, but not sufficient, cause of CC (Walboomers et al., 1999; Schiffman et al., 2007). Less than 10% of all new HPV infections result in persistence or cervical disease, however those infections that persist for 6 months or more are at higher risk for continued persistence and disease progression (Syrjänen et al., 2009). This has led to a concerted effort to develop a better understanding of the role of the immune system and other cofactors in modulating HPV persistence (Einstein et al., 2009; Wang et al., 2009).

Recent studies have linked tobacco smoking, multiple sexual partners, early sexual onset, parity greater than 1, use of oral contraceptives and co-infection with HIV to HPV persistence and progression to cervical disease (Jamieson et al., 2002; Castellsague et al., 2003; Collins et al., 2010; Ferreira et al., 2010). It has been hypothesized that these recognized co-factors may enhance viral presence through either immune suppression or direct tissue damage (Castle et al., 2003). The strength of the cell-mediated immune response has also been implicated in HPV viral clearance (Man et al., 1998). Immunosuppressed individuals such as transplant recipients and HIV-infected persons are at increased risk for HPV-associated cervical lesions (Halpert et al., 1986; Petry et al., 1994; Man et al., 1998; Jamieson et al., 2002).

In addition to HIV, other sexually transmitted infections such as Chlamydiatrachomatis and herpes simplex virus type-2 have been cited as co-factors for HPV infection (Smith et al., 2002a; 2002b; Castle et al., 2003; Paba et al., 2008). Certain non-sexually

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transmitted infections, such as malaria, have been linked to a higher risk of high-grade cervical cancer (Odida et al., 2002). Recently, we reported that tuberculosis (TB) and cervical inflammation were associated with 67% and 15% higher odds of oncogenic HPV infection, respectively, in unscreened rural women from the Shanxi Province Cervical Cancer Study (SPOCCS) II (Belinson et al., 2003; Zhao et al., 2006). This observation is of particular relevance given the re-emergence of TB in the 1980s as a major public health threat (Bloom et al., 1992a; 1992b; Lienhardt et al., WHO et al., 2009).

The present study expands the previous report by focusing on risk factors for oncogenic HPV infection, HPV persistence, CIN3 and CC (CIN3+), with emphasis on TB and cervical inflammation as co-infections. We also analyze the associations of other risk factors including cigarette smoking, sexual history and menopausal state with HPV infection, cervical inflammation and disease, and TB. We hypothesize that history of TB may be an indicator for an immunological profile that is associated with increased susceptibility to oncogenic HPV infection, HPV persistence and CIN3+, potentially pointing to novel intervention strategies.

Materials and Methods

Participants

In May 2001, all women residing in 6 communes of Yangcheng County and 9 communes of Xiangyuan County, Shanxi Province, the People's Republic of China were invited to join SPOCCS II [Zhao et al., 2006]. The study design is described in detail elsewhere by Zhao and colleagues [Zhao et al., 2006]. Briefly, women aged 35-50 years were eligible if they: had an intact uterine cervix, were not currently pregnant, had no previous hysterectomy or pelvic irradiation and had not been screened for cervical neoplasia within the past 5 years. Enrollment in the study ended in June 2002. Of 9034 women screened, 8798 women met the eligibility criteria and were included in the final cohort. The study was approved by the Human Subjects Review Board at both the Cleveland Clinic Foundation, Cleveland, Ohio, the National Cancer Institute, Bethesda, Maryland, and the Cancer Institute/ Hospital of the Chinese Academy of Medical Sciences in Beijing, China.

Procedures

Cervical samples were collected from subjects at the local commune clinic by both self-test (self-sampling) and at the subsequent hospital, outpatient clinic visit by direct test (clinician sampling). Both cervical samples were tested for HPV DNA using the Digene Corporation Hybrid Capture II (HC II) assay, which detects thirteen oncogenic HPV sub-types including 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68. A specimen with ≥ 1.0 pg HPV DNA was considered positive. Details regarding specimen collection and diagnosis have been described previously (Zhao et al., 2006). The rate of persistent oncogenic HPV infection in SPOCCS II was defined based on previous reports of HPV persistence [Syrjänen et al., 2009; Schlecht et al., 2001; Smith et al., 2004]. Women who tested positive for oncogenic HPV infection by self-test and by direct test within a 10-month period were classified as a patient with persistent HPV infection. Questionnaires were administered by interviewers to subjects. TB, hepatitis, cervical inflammation, urinary tract infection (UTI) and vaginal trichomoniasis had sufficient numbers of observations for inclusion in the statistical models.

Statistical Analysis

The analyses were carried out in several phases taking into consideration the stratified cluster sample where the counties are the strata and the study communes within each county are the sampled clusters. In phase I, the proportion of the following reported diagnosed infections (TB, cervical inflammation, vaginal trichomoniasis), and diagnosed oncogenic HPV positive, oncogenic HPV persistence positive, and CIN3+ were calculated by commune. In phase II, we computed the Pearson chi-square test for independence to detect significant differences in recognized and potential risk factors among TB, cervical inflammation or vaginal trichomoniasis cases versus women who did not report a diagnosis of the aforementioned infections. This phase provided the covariates to be included in our models of infection. In phase III, statistically significant variables associated with TB, cervical inflammation or vaginal trichomoniasis were included in multiple logistic regression models to adjust for other covariates using the PC-SAS version 8.0 (SAS Institute I, 1999) callable, SUDAAN, Release 8.0 (Institute RT, 2002) software package, which took into account cluster sampling design effects. The outcomes for these models were: reported diagnosis of TB, cervical inflammation, and vaginal trichomoniasis. In phase IV, univariate analysis of risk factors for oncogenic HPV persistence was performed in 8286 women who received both the self-test and direct test for HPV. Women who were HPV positive at both time points were compared to women who were HPV negative at both time points. In phase V, oncogenic HPV positive, oncogenic HPV persistent positive and CIN3+ were considered as outcomes in multiple logistic regression models that included: 1) variables significantly associated with the odds for HPV persistence based on univariate analysis in this report; 2) the results of univariate analysis for HPV positive and CIN3+ as described earlier (Zhao et al., 2006); 3) variables those were significantly associated with the odds for TB, cervical inflammation, and vaginal trichomoniasis in this report; and 4) finally a variable for the interaction of TB and cervical inflammation that was significantly associated with the odds for HPV persistence based on univariate analysis . Effect modification and potential confounding were further examined by stratifying the analyses by categories of individual covariates and by examination of the results where cross products of relevant covariates were included in the logistic regression models. All findings were considered statistically significant based on a two-tailed P-value of ≤ 0.05 using SAS, version 8.0 (Institute RT, 2002).

Results

Commune-specific prevalence rates of diagnosed infection were examined to investigate regional variations (Table 1). Rates of TB across communes ranged from 2 to 4.5% and zero to 2.3 % in Xiangyuan County and in Yangcheng County, respectively. For cervical inflammation the respective rates were 31 to 45% and 23.5 to 33.5%, while the rates for vaginal trichomoniasis ranged from 18.1 to 26.1% and to 5.9 to 22.6% . The rates of HPV positive ranged from 21.6 to 24.8% compared to 19.6 to 26% while the rates of the persistent positive ranged from 12.8 to 16.8 % and from 11.8 to 18.3 %, respectively. Finally the range in CIN 3+ in communes in Xiangyuan county was from zero to 2.7% while the range in rates across communes in Yangcheng county was from zero to 5.6%. Overall rates of TB, vaginal trichomoniasis, and cervical inflammation, were 2.7, 21.0 and 34.0 %, respectively. The mean age at diagnosis of TB by X-ray was 25±8.4 years with a range of 1-46 years. The overall rate of UTI was 4.8%. Hepatitis was reported by 3.6% of the women at an average age of 26±8.4 years. Demographic information collected has been described elsewhere (Zhao et al., 2006).

Among the total, 8,286 women had both the HPV self-test and HPV direct test within 10 months. Of these, 15.2% tested positive in both, 10.5% in the self-test only, 8.6% in the direct test only, and 65.7% tested negative in both. The overall rate determined by the direct test was 23.6% and by the self-test was 25.7%, with moderate consistency between the two (kappa=0.489, P<0.0001). CIN 3+ were diagnosed in 2.4% of the women. Rates of CIN1 and CIN2 were 4.0% and 2.0%, respectively. CIN2 was not used as an endpoint in this study, since controversy exists regarding the potential for CIN2 to progress to a more invasive disease.

Multiple logistic regression models (Table 2) were

Table 1. Proportions (%) of Infections and CIN3+ by Commune in SPOCCS-II

Commune[1] (No.)	Infection TB[2]	CI[2]	VT[2]	HPV[3]	HPV-P[4]	CIN3+
11 (1342)	4.5	34.9	25.3	23.9	14.1	1.9
12 (688)	3.8	35.5	22.1	23.5	16.6	2.0
13 (747)	2.8	45.9	26.1	24.4	16.6	2.1
14 (705)	3.1	41.4	20.9	23.7	13.7	1.8
15 (432)	2.5	33.3	20.1	24.8	16.8	2.1
16 (414)	2.4	30.2	18.1	22.9	16.0	1.7
17 (837)	2.0	34.9	22.6	21.6	13.5	2.3
18 (410)	3.2	35.9	19.3	23.2	13.8	2.7
19 (86)	5.8	31.4	22.1	23.3	12.8	0.0
21 (1007)	1.9	24.2	22.6	26.0	16.0	2.7
22 (517)	2.3	31.5	15.1	23.6	15.4	2.5
23 (645)	1.7	33.5	16.1	25.6	18.3	4.5
24 (863)	1.4	29.8	16.6	20.3	13.7	2.9
25 (54)	0.0	33.3	13.0	24.1	17.0	5.6
26 (51)	0.0	23.5	5.9	19.6	11.8	0.0
Total (%)	2.7	34.0	21.0	23.6	15.2	2.4

[1]Numbers beginning with 1 indicate Xiangyuan County, numbers beginning with 2 indicate Yangcheng County; Second digit indicates commune number; [2]Proportion based on at least 1 diagnosis by a health care provider; [3]Includes women who tested positive for oncogenic HPV DNA by self-test, direct-test, or both; [4]Includes women who tested positive for oncogenic HPV DNA on both self-test and direct test within a 10-month period; CI, cervical inflammation; VT, vaginal trichomoniasis; HPV-P, HPV-persistent positive

Table 2. Adjusted Odds Ratios (95% CI) for Infections in SPOCCS-II

Tuberculosis[1]	Yes (239)	No (8,559)	OR (95% CI)
Current smoking			
No	223 (93.3)	8,272 (96.6)	1.00
Yes	16 (6.7)	287 (3.4)	1.93 (1.07-3.49)
Multiple sexual partners, husband			
No	184 (77.0)	7,317 (85.5)	1.00
Yes	55 (23.0)	1,242 (14.5)	1.48 (1.08-2.02)
Birth control method			
None, IUD	79 (33.1)	1,748 (20.4)	1.00
Tubal ligation	160 (66.9)	6,811 (79.6)	0.46 (0.34-0.62)
Cervical Inflammation	Yes (2,992)	No (5,806)	OR (95% CI)
Current smoking			
No	2,907 (97.2)	5,588 (96.3)	1.00
Yes	85 (2.8)	218 (3.7)	0.66(0.56-0.78)
Marital status			
Married	2,936 (98.1)	5,643 (97.2)	1.00
Divorced[#]	56 (1.9)	163 (2.8)	0.62 (0.45-0.85)
Miscarriage			
No	2,545 (85.1)	5,181 (89.2)	1.00
Yes	447 (14.9)	625 (10.8)	1.41 (1.19-1.68)
Induced abortion			
No	1,833 (61.3)	3,876 (66.8)	1.00
Yes	1,159 (38.7)	1,930 (33.2)	1.35 (1.22-1.50)
Multiple sexual partners, husband			
No	2,427 (81.1)	5,074 (87.4)	1.00
Yes	565 (18.9)	732 (12.6)	1.32 (1.08-1.62)
Multiple sexual partners, subject			
No	2,082 (69.6)	4,560 (78.5)	1.00
Yes	910 (30.4)	1,246 (21.5)	1.42 (1.21-1.68)
Birth control method			
IUD, others	580 (19.4)	1,247 (21.5)	1.00
Tubal ligation	2,412 (80.6)	4,559 (78.5)	1.18 (1.06-1.30)
Vaginal trichomoniasis[1]			
No	1,964 (65.6)	4,989 (85.9)	1.00
Yes	1,028 (34.4)	817 (14.1)	3.05 (2.69-3.46)
Vaginal trichomoniasis	Yes (1,845)	No (6,953)	OR (95% CI)
Stillbirth			
No	1,676 (90.8)	6,467 (93.0)	1.00
Yes[1]	69 (9.2)	486 (7.0)	1.30 (1.14-1.49)
Miscarriage			
No	1,568 (85.0)	6,158 (88.6)	1.00
Yes	277 (15.0)	795 (11.4)	1.22 (1.03-1.46)
Multiple sexual partners, subject			
No	1,300 (70.5)	5,342 (76.8)	1.00
Yes	545 (29.5)	1,611 (23.2)	1.18 (1.00-1.40)
Cervical inflammation[1]			
No	817 (44.3)	4,989 (71.8)	1.00
Yes	1,028 (55.7)	1,964 (28.2)	3.05 (2.69-3.46)
Urinary tract infection[1]			
No	1,680 (91.1)	6,696 (96.3)	1.00
Yes	165 (8.9)	257 (3.7)	2.29 (1.78-2.93)
Marital status			
Married	1,791 (97.1)	6,788 (97.6)	1.00
Divorced[#]	54 (2.9)	165 (2.4)	1.40 (1.03-1.90)
Hepatitis			
No	1,760 (95.4)	6,723 (96.7)	1.00
Yes	85 (4.6)	230 (3.3)	1.46 (1.02-2.10)

[1]Proportion based on at least 1 diagnosis by a health care provider
[#]or widowed

Table 3. Adjusted Odds Ratios of HPV Persistence in SPOCCS-II

Risk factor	HPV+ve[1] (1,261)	HPV-ve[2] (5,447)	OR (95% CI)
Multiple sexual partners, subject			
No	829 (65.7)	4,306 (79.1)	1.00
Yes	432 (34.3)	1,141 (20.9)	1.60 (1.06-2.42)
Multiple sexual partners, husband			
No	978 (77.6)	4,785 (87.9)	1.00
Yes	283 (22.4)	662 (12.1)	1.67 (1.38-2.02)
Post-menopausal			
No	1,081 (91.5)	4,734 (93.7)	1.00
Yes	100 (8.5)	317 (6.3)	1.34 (1.10-1.63)
Bathing location			
Home	663 (52.6)	3,101 (56.9)	1.00
Public house	598 (47.4)	2,346 (43.1)	1.34 (1.19-1.51)
Abnormal vaginal discharge			
No	724 (57.4)	3,437 (63.1)	1.00
Yes	537 (42.6)	2,010 (36.9)	1.28 (1.09-1.50)
Tuberculosis[3]			
No	1,204 (95.5)	5,312 (97.5)	1.00
Yes	57 (4.5)	135 (2.5)	1.75 (1.27-2.40)
Cervical inflammation[3]			
No	776 (61.5)	3,643 (66.9)	1.00
Yes	485 (38.5)	1,804 (33.1)	1.22 (1.09-1.35)
TB and cervical inflammation[3]			
No	1,238 (98.2)	5,401 (99.2)	1.00
Yes	23 (1.8)	46 (0.8)	2.13 (1.53-2.95)
Vaginal trichomoniasis[3]			
No	1,035 (82.1)	4,278 (78.5)	1.00
Yes	226 (17.9)	1,169 (21.5)	0.66 (0.51-0.85)

HPV+ve/-ve, Oncogenic HPV persistent positive/negative; [1]Includes women who tested positive for oncogenic HPV DNA on both self-test and direct test within a 10-month period; [2]Includes women who tested positive by self-test or direct-test no more than once, and those who tested negative for oncogenic HPV DNA; [3]Proportion based on at least 1 diagnosis by a health care provider

computed to examine the associations between recognized and potential risk factors and the odds ratio of diagnosed TB, cervical inflammation and vaginal trichomoniasis, respectively. Women identifying themselves as current smokers, as opposed to never or former smokers, had 93% higher odds of TB and 34% lower odds of cervical inflammation. Women who reported their husbands had multiple sex partners had 48% and 32% higher odds of TB and cervical inflammation, while multiple sexual partners on the part of the subject placed women at 42% and 18% higher odds of cervical inflammation and vaginal trichomoniasis, respectively. Tubal ligation, in contrast with other forms of birth control or contraception, was associated with 54% lower odds of TB and 18% higher odds of cervical inflammation. Divorced or widowed women were at 38% lower odds of cervical inflammation compared to married women. Women who experienced either miscarriage or induced abortion were at 41% and 35% higher odds of cervical inflammation, respectively, when compared to women who did not report a miscarriage or an induced abortion. Women who reported a diagnosis of vaginal trichomoniasis as compared to those who did not report this diagnosis were at 205% higher odds of cervical inflammation. Women with at least one stillbirth or miscarriage compared to neither were at 30% and 22% higher odds of vaginal trichomoniasis,

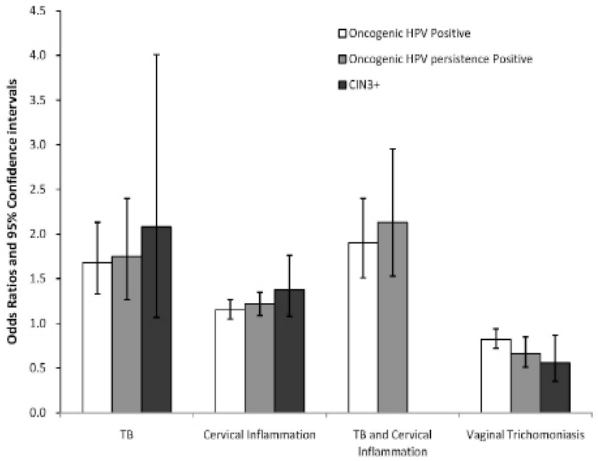

Figure 1. Trends in adjusted odds ratios (95% CI) for HPV Oncogenic Subtypes and CIN3+. Data was not reported for the combined effect of TB and cervical inflammation on CIN3+ due to small numbers.

respectively. Women who reported at least one diagnosis of cervical inflammation had 205% higher odds of vaginal trichomoniasis, and women who reported at least one diagnosis of UTI had 129% higher odds.

In an analysis of the risk factors for persistent oncogenic HPV infection, we found that history of TB or cervical inflammation were associated with 75% and 22% higher odds of infection, respectively. Individuals reporting a diagnosis of both TB and cervical inflammation were at 113% higher odds (Table 3). Multiple sexual partners on the part of the subject or her husband was associated with 60% and 67% higher odds for persistent HPV infection, respectively. Women who were post-menopausal (v. premenopausal) were at 34% higher odds of infection with persistent HPV. Bathing location (public facility v. home) was associated with 34% higher odds of persistent HPV infection. History of ever having an abnormal vaginal discharge was associated with 28% higher odds of persistent HPV. Vaginal trichomoniasis, an indicator of treatment, was associated with 34% lower odds of persistent HPV infection.

Based on the results of univariate analysis, variables that were significantly associated with history of diagnosed infections, HPV positivity, HPV persistence and CIN3+ were then entered in multiple logistic regression models for oncogenic HPV infection, persistent oncogenic HPV infection (Table 3) and CIN3+. After adjusting for other covariates, women reporting (v. women not reporting) a diagnosis of TB had 68%, 75% and over 100% increased odds of HPV oncogenic infection, persistent HPV and CIN3+, respectively (Figure 1). Women reporting compared to women not reporting a diagnosis of cervical inflammation had 15%, 22% and 38% higher odds of HPV oncogenic positivity, persistent HPV and CIN3+, respectively. Conversely, a diagnosis of vaginal trichomoniasis (v. no diagnosis of vaginal trichomoniasis) was associated with 18%, 34% and 44% lower odds of HPV positivity, persistent HPV and CIN3+, respectively. TB and cervical inflammation were diagnosed in 1% of the women and associated with 90% higher odds of oncogenic HPV infection and 113% higher odds of persistent HPV

infection.

Discussion

Our results are consistent with the novel hypothesis that TB may provide an immunological profile that is associated with an increased susceptibility to HPV infection. In the model for HPV infection, HPV persistence and CIN3+, we show an increasing magnitude of effect of TB with increasing severity of disease, as demonstrated by the increasing odds ratios from 1.68 for HPV positivity, to 1.75 for persistent HPV and then 2.08 for CIN3+. Our results agree with Safaeian et al., where a 60% higher prevalence of oncogenic HPV infection was observed in women with a history of TB, herpes zoster or oral candidiasis (Safaeian et al., 2008). Cervical inflammation had an increasing odds ratio from HPV positivity to CIN3+ with the greatest magnitude of effect observed at the most advanced disease stage. Women ever diagnosed with cervical inflammation had 38% higher odds of CIN 3+, implying that diagnosis of cervical inflammation may predispose an individual to advanced stage disease perhaps from inflammation involved in carcinogenesis. In contrast, the odds ratio associated with vaginal trichomoniasis decreased with increasing disease severity. Women who presented with vaginal trichomoniasis were most likely treated for the infection, suggesting that diagnosis of vaginal trichonomiasis may serve as a proxy for access to health care (Zhao et al., 2006).

Multivariate models were carried out to identify risk factors associated with HPV, TB, cervical inflammation and vaginal trichomonasis. The rates of TB agree with reported rates in rural non-direct observed therapy, short-course (DOTS) areas in China, and are higher than rates in urban areas (Xianyi et al., 2002). In agreement with other studies, smoking was a risk factor for both TB and cervical inflammation, indicating that smoking and nicotine metabolites may play a role in local immune suppression and disease pathogenesis (Lin et al., 2007; Kundu et al., 200; Louvanto et al., 2010]. We did not observe that current smoking was significantly associated with oncogenic HPV persistence, although recent studies have shown that both early initiation of smoking and current smoking are significant predictors of oncogenic HPV persistence (Louvanto et al., 2010). Rates of smoking in our study participants may be lower than those in other studies (Louvanto et al., 2010) that observe the association.

Persistent HPV infections are important events in the development of cervical cancer and are the focus of recent HPV vaccine development (Bornsteinn et al., 2007). While a persistent HPV infection is widely acknowledged as a necessary precursor to cervical cancer, there is no general consensus regarding the duration of infection necessary to classify an infection as persistent or transient (Woodman et al., 2007). A persistent infection is generally classified as a woman who tests positive for HPV on two or more occasions, the interval between them ranging between 2 months to 7 or more years (Woodman et al., 2007). The rate of persistent oncogenic HPV infection in SPOCCS II was estimated by assessment of oncogenic HPV infection at two time points within a 10-month period. Given this relatively short interval, it is possible that a fraction of women classified with persistent infections may instead have transient HPV infections. Additionally, because the HC II assay does not distinguish sub-types of HPV, the women who tested positive for oncogenic HPV at both the initial and follow-up clinical screening could have been infected with either the same or different oncogenic subtypes of HPV and therefore may be transiently infected.

The initial prevalence rate of HPV infection reflects either incident or pre-existing infection (Schlecht et al., 2001; Smith et al., 2004). The persistence rate of oncogenic HPV infection in SPOCCS II was 15.2% and was comparable to the rate found in another study over a 7-year period (Smith et al., 2004). The rate of persistence in this study may have been underestimated, because the initial HPV diagnosis was based on the self-test, and the second HPV diagnosis was based on the direct test, with the sensitivity of the former test lower than that of the latter test (Belinson et al., 2003). Factors influencing the odds of persistent HPV positivity included sexual behaviors, hygienic practices, and history of infections including TB (Table 3). Being post-menopausal rather than pre-menopausal was another risk factor for HPV persistence and perhaps a surrogate for age, which has been directly associated with persistent HPV infection in earlier reports (Smith et al., 2004; Ferreira et al.,2010;).

Although it is difficult to determine the exact chronology of events using data from reported diagnosis of infections, it is possible to draw preliminary conclusions regarding the relationship between co-infections and HPV infection, HPV persistence and development of CIN3+. Host mechanisms for defense against TB and HPV both involve cell-mediated immunity. Infection with HPV and the tubercle bacilli result in chronic immune activation, followed by sustained production of inflammatory cytokines and prostaglandins (Dinarello et al., 2006; Kundu et al., 2008; Einstein et al., 2009), and subsequent suppression of cell-mediated immune responses and promotion of angiogenesis. Chronic inflammation associated with HPV infection, TB and other diseases may increase the production of reactive oxygen species and other potential damaging metabolites that aid carcinogenesis (Dinarello et al., 2006; Kundu et al., 2008).

Nutritional status may also play a role in the immunological defense against HPV infection. Many populations in Asia suffer from marginal to deficient serum levels of vitamin A, vitamin C and selenium, which fluctuate with seasonal availability of dietary-rich sources of these micronutrients (Yang et al., 2007; Ratnasinghe et al., 2000a; 2000b). The variation in season-specific serum levels of nutritional status has been associated with increased risk of respiratory infections, including tuberculosis (Rios et al., 2000; Thorpe et al., 2004) in children and increased risk of lung cancer in Chinese tin miners (Forman et al.,1990; 1994). Recent evidence from SPOCCS II implicates a role for season in HPV infection (Zhao et al., 2006). Individuals with chronic infections may have depleted nutritional stores , that may increase risk of progression from HPV infection to persistence and invasive cancer (Dinarello et al., 2006; Kundu et al., 2008).

The conclusions that can be drawn from this study are limited by the study design and collection of self-reported medical histories. Due to the cross-sectional nature of the study, it is difficult to determine cause and effect between proposed co-infections and HPV infection, HPV persistence and cervical disease. Furthermore, the reliability of infection rates is uncertain given the data were self reported. Indeed report of any infection may depend on access to care or access to information and therefore be a biased prevalence rate of infections (Zhao et al., 2006). For instance, rates of tubal ligation are high when rates of diagnosed infections are high. However, rates of infection did not correlate with distance or travel time to the regional hospital, and the rates observed in Shanxi Province are consistent with prevalence rates in a recent report of non-DOTS areas in China (Zhao et al., 2003). We conclude that rates of self-reported infection are more likely to be underestimated than over estimated given the correlation between reported diagnoses and access to care observed with infections such as vaginal trichomoniasis (Zhao et al., 2006).

These findings highlight the importance of the contributions of individual and combined history of infections, illustrated by TB, in an individual's risk for HPV infection, HPV persistence and CIN3+. Future research investigating immunologic host defense for TB, gynecologic infections and HPV individually and in combination in the same patient may lead to novel prevention and intervention strategies for HPV infection, HPV persistence and subsequent invasive cervical disease in rural populations. Improving the control and management of infections in general may help protect women against invasive disease and may be the key to implementing prevention and intervention strategies, including HPV vaccines, in resource-poor settings.

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