

# Chlamydia trachomatis, Herpes Simplex Virus 2, and Human T-Cell Lymphotropic Virus Type 1 Are Not Associated With Grade of Cervical Neoplasia in Jamaican Colposcopy Patients

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**Background:** A few recent studies have suggested that other sexually transmitted infections may increase the likelihood of a human papillomavirus (HPV) infection progressing to high-grade cervical neoplasia and cancer.

**Goal:** The goal was to assess whether exposures to *Chlamydia trachomatis*, human T-cell lymphotropic virus type 1 (HTLV-I), and/or human simplex virus type 2 (HSV-2) are greater in colposcopy patients with cervical intraepithelial neoplasia grade 3 or cancer (CIN3+) than in patients with low-grade cervical neoplasia (CIN1).

**Study Design:** Sequential patients (n = 447) attending a colposcopy clinic in Kingston, Jamaica, a country with high cervical cancer rates and high HTLV-I prevalence, were tested for (1) HPV DNA by L1 consensus primer (MY09/11) polymerase chain reaction assays, (2) *C trachomatis* DNA by ligase chain reaction, (3) *C trachomatis* antibodies by both microimmunofluorescence and a peptide (VS4) enzyme linked immunosorbent assay (ELISA), (4) HTLV-I antibodies by ELISA confirmed by western blotting, and (5) HSV-2 antibodies by a recombinant HSV-2-specific ELISA. Odds ratios and 95% confidence intervals were estimated with use of multinomial logistic regression models.

**Results:** HPV DNA detection was associated with grade of cervical neoplasia but other evaluated sexually transmitted infections were not.

**Conclusions:** HTLV-I, *C trachomatis*, and/or HSV-2 were not associated with severity of cervical neoplasia in Jamaican women.

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HUMAN PAPILLOMAVIRUS (HPV), a sexually transmitted DNA virus, is widely accepted as the central cause of cervical cancer.<sup>1,2</sup> However, infection by HPV is common and rarely results in a malignant outcome. It is important, therefore, to identify factors that may operate in the presence of HPV infection (HPV cofactors) to increase the likelihood of progression from CIN1 (mild morphologic changes associated with HPV infection) to cancer or cervical intraepithelial neoplasia grade 3 (CIN3), a well-established cancer precursor lesion.<sup>3</sup>

A few recent studies have provided support for a long-held idea that sexually transmitted infections (STIs) other than those due to HPV, such as *Chlamydia trachomatis*<sup>4,5</sup> and herpes simplex virus type 2 (HSV-2) infections,<sup>6</sup> may be such HPV cofactors. Mechanistically, coinfection of these STIs with HPV could increase risk of CIN progression by interfering with local immune responses, by inducing other (e.g., paracrine and autocrine) changes in the local cellular milieu, or by simply causing direct tissue damage and thus increasing the likelihood that HPV infection and associated lesions persist and progress.

Human T-cell lymphotropic virus type 1 (HTLV-I), like HIV, is a sexually transmitted retrovirus that may act as an HPV cofactor by interfering with general immune function.<sup>7</sup> A study in Japan found a significant association of HTLV-I with cervical cancer,<sup>8</sup> and a pilot study we conducted of sequential colposcopy patients (n = 198) in Jamaica, a country with high rates of cervical cancer and HTLV-I, showed elevated rates of HTLV-I infection among subjects with CIN3 or cancer (CIN3+) (OR = 3.82; 95% CI = 1.03–14.2) in comparison with subjects who had CIN1 or more benign pathologic conditions.<sup>9</sup> Although we controlled for detection of HPV DNA in the pilot study, the study lacked data regarding other STIs or additional cervical cancer risk factors.

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To address these issues in the current investigation, we enrolled a much greater number of patients from the same Jamaican colposcopy clinic as the pilot study, obtained detailed questionnaire data, and assessed exposure to multiple different STIs.

## Methods

### Subjects

Between 1993 and 1997, we enrolled sequential patients ( $n = 447$ ) presenting to the colposcopy clinic at the University of West Indies (UWI), Kingston, Jamaica, as part of a UWI and NIH institutional review board–approved study of cervical neoplasia. Eligibility was restricted to women 20–49 years of age, an age group in which both CIN3 and CIN1 are common, so that cases (CIN3+) and controls (CIN1) would be broadly representative of women with these conditions in the general population.<sup>3,10</sup> CIN1 was believed to be the most appropriate for comparison, since women with CIN1 are similar to cases except that they have not developed neoplasia of a high grade. Left untreated, only a small fraction of CIN1 patients would eventually be diagnosed with CIN3+, a circumstance suggesting that the comparison of CIN1 and CIN3+ does not, however, represent “overmatching.”<sup>11</sup>

The UWI colposcopy clinic is a referral center for follow-up of abnormal Papanicolaou smears in the Kingston, Jamaica, vicinity, representing one of just a few facilities where patients without a private gynecologist can receive colposcopic evaluation, although many women with a private physician are also frequently referred. Consequently, the clinic serves a broad range of women of poor, lower middle, and middle socioeconomic classes.

### Questionnaire Data and Specimen Collections

Each subject completed a detailed (30-minute) questionnaire regarding demographic, general health, obstetrical, and gynecological history and other risk-factor information administered by a trained interviewer. Just before colposcopy, cervical specimens for cytology and for DNA testing were obtained as follows. First, a standard Papanicolaou smear was prepared with use of an endocervical brush and Ayre’s spatula. Both implements were then placed in PreservCyt medium (Cytoc Corporation, Boxborough, MA) and agitated to collect residual exfoliated cervical cells for preparation of a ThinPrep monolayer Papanicolaou smear (Cytoc Corporation), in accordance with the manufacturer’s recommendations.

A second sample of exfoliated cervical cells was obtained for DNA testing with a polyester swab rubbed gently across the ectocervix and cervical os, placed in 1 ml of standard transport medium (STM; Digene Corporation, Gaithersburg, MD), and then stored at  $-70^{\circ}\text{C}$  until used for testing. Colposcopy was conducted, and biopsy specimens of any aceto-white cervical lesions and tissues suggestive of neoplasia were obtained for histologic evaluation. Blood was also collected by venipuncture and permitted to clot, and the resulting serum was stored at  $-70^{\circ}\text{C}$  until used for serologic testing.

### Pathology

A single expert pathologist (C. E.) examined all standard Papanicolaou smears, and a single cytopathologist (M. L. H.) specifically expert in the evaluation of thin-layer cytologic specimens examined all ThinPrep Papanicolaou smears. Both cytologists were asked to distinguish between high-grade squamous intraepithelial lesions (HSILs) of the moderate dysplasia subtype, consistent with a histologic diagnosis of CIN2, and HSILs of the severe dysplasia subtype, consistent with a histologic diagnosis of CIN3.

Histologic specimens were examined for cervical pathology (C. E.), masked to the original cytology interpretations.

The final diagnosis used in our analyses was the highest grade of disease detected by either cytology or histology. This reflects the growing awareness that both cytology and histology have limited intrarater and interrater agreement<sup>12</sup> (i.e., Kappa values in the range of 0.3–0.6, considered just fair to good, and awareness that histology is dependent on the colposcopist obtaining a biopsy specimen from the most severely neoplastic region.) To further address variability in diagnosis, we classified the “certainty” of each result as good (all cytologic and histologic results agreed exactly with regard to the presence and exact grade of lesion), intermediate (all agreed within one grade of disease), or poor. The exclusion of CIN2 from either the primary case definition (CIN3+) or control group (CIN1) also created a buffer, thereby reducing the likelihood of misclassification due to overlap between mild and moderate lesions and between moderate and severe lesions that would otherwise have occurred.

### HPV DNA Testing

HPV DNA was detected with use of L1 consensus primer MY09/MY11/HMB01 polymerase chain reaction (PCR) assays.<sup>13</sup> Control primer set PC04/GH20, amplifying a 268-bp cellular  $\beta$ -globin DNA fragment, was included in each assay to serve as an internal amplification control. Following proteinase K digestion of a specimen aliquot, 2–10  $\mu\text{L}$  of each cell digest was used in reactions containing 10 mmol/l Tris-HCL, 50 mmol/l KCL, 4 mmol/l  $\text{MgCl}_2$ , 200  $\mu\text{M}$  of each deoxyribonucleotide triphosphate, 2.5 U Taq DNA polymerase, and 0.5  $\mu\text{M}$  of each primer. There were 40 amplification cycles (95  $^{\circ}\text{C}$  for 20 seconds, 55  $^{\circ}\text{C}$  for 30 seconds, and 72  $^{\circ}\text{C}$  for 30 seconds), with a 5-minute extension period at 72  $^{\circ}\text{C}$  in the last cycle. Amplified material was then detected on filters individually hybridized with biotinylated type-specific oligonucleotide probes for multiple HPV types, including HPV 6, 11, 13, 16, 18, 26, 31–35, 39, 40, 42, 45, 51–59, 61, 62, 64, 66–70, 71 (AE8), 72, 73 (PAP238A), 81 (AE7), 83 (PAP291), 82(W13B and AE2), 84 (PAP155), 85 (AE5), 89 (AE6), AE9, and AE10.

### HTLV-I Serology

Sera were screened for antibodies to HTLV-I/HTLV-II with both a recombinant p21e enzyme-linked immunosorbent assay (ELISA; Cambridge Biotech, Worcester, MA) and a whole-virus ELISA (Dupont, Wilmington, DE, or Genetic Systems, Redmond, WA). Reactivity of sera in either ELISA was confirmed by western blotting (Biotech Research Laboratories, Rockville, MD). Reactivity to both p24 and rp21e were defined as HTLV-positive. HTLV-I and HTLV-II infections were differentiated by synthetic peptide ELISA (United Biomedical/Olympus, Hauppauge, NY), recombinant protein-enhanced western blotting (Diagnostic Biotechnology, Singapore), or an algorithm comparing western blotting p24 and p19 band strength.<sup>14,15</sup>

### C trachomatis Ligase Chain Reaction (LCR) Assay

LCR assays (LCx, Abbott Laboratories, Abbott Park, IL) targeting the cryptic plasmid of *C trachomatis* were performed as previously described.<sup>16</sup> In brief, 100  $\mu\text{L}$  of each cervical STM specimen was placed in an LCR transport tube containing 0.5 ml of buffer and  $\text{Mg}_2\text{Cl}$  and then heated to 100  $^{\circ}\text{C}$  for 15 minutes to denature DNA. LCR amplification reactions were conducted by adding 100  $\mu\text{L}$  of each denatured test sample or a control into a microfuge tube containing a predispensed LCx reaction mixture of probes, polymerase, and ligase. The tubes were placed into a

TABLE 1. Comparison of Sociodemographic Factors, Behavior, and Prevalence of STIs Among Jamaican Colposcopy Patients, by Grade of Cervical Neoplasia

Variable	CIN1 (n = 201)	CIN2 (n = 117)	CIN3 <sup>+</sup> (n = 92)	P Value*
Mean age (y)	31.2	30.7	33.5	0.013
Mean age at first sexual intercourse (y)	17.1	17.1	16.6	0.23
Ever pregnant (%)	81.6	85.5	94.6	0.013
Mean number of pregnancies	2.8	2.7	3.0	0.41
Mean number of live births	2.1	2.1	2.5	0.19
Number of sex partners	3.5	3.9	4.1	0.16
Currently smoking cigarettes (%)	6.5	7.7	8.7	0.78
Currently using oral contraceptives or depo provera (%)	79.1	82.1	82.6	0.71
Current using IUD (%)	15.4	8.6	18.5	0.10
Ever had Pap test (%)	85.0	82.9	75.8	0.16
Some high school education or more (%)	54.2	54.7	44.6	0.30
HPV DNA-positive (HPV <sup>+</sup> ) (%)	54.3	87.4	95.4	<0.001
HSV-2-seropositive (%)	60.9	61.6	73.5	0.12
C trachomatis testing (%)				
MIF titer ≥8	90.1	89.7	92.9	0.71
Serovar D-K-specific MIF titer ≥8	20.3	16.8	22.4	0.61
Peptide ELISA OD ≥0.090	66.5	64.5	70.6	0.67
LCR-positive	9.2	10.2	9.4	0.96
HTLV-1-seropositive	4.0	1.7	7.8	0.10

\*The P value determined by analysis of variance (continuous variable) or Pearson  $\chi^2$  (categorical).

thermocycler (Perkin Elmer) programmed for 40 cycles. A micro-particle enzyme immunoassay was then run to detect *C trachomatis* amplicons.

#### C trachomatis Serology

A modified microimmunofluorescence (MIF) technique utilizing yolk sac-grown elementary bodies specific for the 15 serovars of *C trachomatis* (A–L3) was used to measure antichlamydial antibodies in sera.<sup>17</sup> Antibody titers were determined with serial twofold dilutions. MIF titers  $\geq 1:8$  were considered positive, and positive results were subcategorized as low (1:8–1:128) or high titers (1:256 and higher). To reduce the effects of cross-reactive antibodies against either strains that cause trachoma or broadly reactive antibody presumably due to *C pneumoniae* infection, we further limited our data to serovars D–K, which are predominantly associated with gynecologic infections.<sup>4</sup> Thus, in keeping with an earlier positive report,<sup>4</sup> only MIF titers  $\geq 1:8$  against a D–K serovar were considered positive, and a titer  $\geq 1:256$  was categorized as a high titer.

A previously described in-house experimental ELISA was modified to use the major outer membrane protein of the VS4 peptide of *C trachomatis* (SS1-E).<sup>18</sup> In brief, 96-well microtiter plates were coated with the synthetic VS4 peptide. Diluted sera (1:100) were placed into the precoated plates and incubated for 1 hour at 36 °C. Plates were washed and incubated with goat antihuman IgG-HRP conjugate (Zymed Laboratories Inc., So. San Francisco, CA). After incubation, o-phenylenediamine substrate (Dako Corp., Carpinteria, CA) was added. The reaction was stopped with 8N H<sub>2</sub>SO<sub>4</sub>. Plates were read on a spectrophotometer at 492 nm. These ELISA optical density (OD) results were categorized into tertiles.

#### HSV-2 Serology

A recombinant gG-2-specific HSV-2 ELISA developed by Focus Technology (formerly MRL; Cypress, CA) was utilized for the detection of antibody to HSV-2.<sup>19</sup> OD values for each test specimen were compared to a standard (“cutoff calibrator”), and index values above 1.1 were considered positive. Test results classified

as equivocal (0.90–1.1; n = 2) were conservatively called negative for these analyses.

#### Analysis

Standard contingency table methods, with Pearson chi-square tests or, when appropriate, the Mantel extension test for trend, were used to assess possible univariate associations of categorical variables with grade of neoplasia. The associations of continuous variables with grade of neoplasia were assessed with analysis of variance. Odds ratios (ORs) and 95% confidence intervals (95% CIs) adjusted for relevant parameters (e.g., identified as part of preliminary data analysis) were determined with multinomial logistic regression. Our a priori main comparison was between patients with CIN3+ and those with CIN1, but for completeness, results comparing subjects with CIN2 to those with CIN1 are also shown. Dose–response relationships ( $p_{Trend}$ ) were assessed in multivariate logistic regression by treating ordinal variables as continuous (which assumes a linear trend).

Although limiting controls to those with CIN1, in part controlled for HPV and other risk factors for the development of cervical lesions, HPV infection was further controlled for by including detection of HPV DNA as a variable in multivariate statistical models and, in one set of models, by entirely restricting analysis to just those who were HPV DNA-positive for any type (HPV-restricted analysis). Thus, we attempted to minimize potential confounding by HPV when evaluating other STIs as cofactors.

Control for HPV type (e.g., oncogenic versus not oncogenic) was believed to be unnecessary in a cross-sectional study such as this, since the cross-sectional detection of HPV is only a proxy for lifetime level of exposure to all HPV (an exposure that is both common and often repeated in sexually active women<sup>20</sup>). In any event, secondary analyses controlling for detection of oncogenic HPV types through statistical adjustment in multivariate models did not meaningfully alter the estimated associations (data not shown).

TABLE 2. Results of multinomial logistic regression models examining the association of HTLV-I, *C. trachomatis*, and HSV-2 with CIN2 and CIN3+, compared with women diagnosed with CIN1 (as the referent group). One model statistically adjusted for the presence of any HPV type (HPV-adjusted) and the other restricted to HPV DNA positive women

Variable	No. of Women			HPV-Adjusted*		HPV DNA-Positive (Restricted)*	
	CIN1	CIN2	CIN3+	CIN2 OR (95% CI)	CIN3+ OR (95% CI)	CIN2 OR (95% CI)	CIN3+ OR (95% CI)
HTLV-seropositive							
No (referent)	191	114	83	1	1	1	1
Yes	8	2	7	0.45 (0.085–2.4)	1.9 (0.51–6.7)	0.48 (0.083–2.7)	1.4 (0.36–5.3)
<i>C trachomatis</i> , MIF IgG (titer)							
<8 (referent)	18	11	6	1	1	1	1
≥8	164	96	79	0.91 (0.38–2.2)	1.8 (0.58–5.7)	0.78 (0.27–1.9)	2.0 (0.57–7.2)
<8 (referent)	18	11	6	1	1	1	1
≥8–128	74	47	41	1.0 (0.41–2.6)	2.2 (0.67–7.3)	0.91 (0.32–2.5)	2.6 (0.69–9.8)
≥256	90	49	38	0.81 (0.33–2.0)	1.5 (0.47–5.0)	0.60 (0.22–1.7)	1.6 (0.44–6.0)
				$P_{Trend} = 0.42$	$P_{Trend} = 0.89$	$P_{Trend} = 0.16$	$P_{Trend} = 0.73$
<i>C trachomatis</i> , serovar D-K-specific MIF IgG (titer)							
<8 (referent)	132	82	62	1	1	1	1
≥8	37	18	19	0.86 (0.47–1.6)	1.2 (0.60–2.3)	0.59 (0.28–1.2)	1.2 (0.57–2.3)
<8 (referent)	132	82	62	1	1	1	1
≥8–128	21	9	10	0.81 (0.39–1.7)	1.0 (0.46–2.3)	0.80 (0.35–1.8)	1.2 (0.49–2.7)
≥256	16	9	9	0.98 (0.37–2.6)	1.5 (0.53–4.1)	0.27 (0.070–1.1)	1.1 (0.40–3.2)
				$P_{Trend} = 0.52$	$P_{Trend} = 0.75$	$P_{Trend} = 0.071$	$P_{Trend} = 0.73$
<i>C trachomatis</i> , peptide ELISA (OD)							
0–0–0.89 (referent)	61	38	25	1	1	1	1
0.09–0.259	58	30	27	0.89 (0.46–1.7)	1.3 (0.60–2.8)	1.1 (0.50–2.3)	1.6 (0.70–3.7)
≥0.260	63	39	33	0.87 (0.46–1.6)	1.3 (0.61–2.6)	0.80 (0.40–1.6)	1.3 (0.61–2.9)
				$P_{Trend} = 0.66$	$P_{Trend} = 0.55$	$P_{Trend} = 0.51$	$P_{Trend} = 0.51$
<i>C trachomatis</i> DNA (LCR)							
No (referent)	168	97	77	1	1	1	1
Yes	17	11	8	0.90 (0.38–2.2)	0.82 (0.29–2.3)	0.64 (0.25–1.6)	0.73 (0.26–2.0)
HSV-2-seropositive							
No (referent)	74	43	22	1	1	1	1
Yes	115	69	61	0.87 (0.50–1.5)	1.2 (0.61–2.3)	0.84 (0.45–1.5)	1.2 (0.58–2.4)

\*Adjusted for age (<25, 25–29, 30–34, ≥35 years) and ever pregnant. The models do not mutually adjust for the other (non-HPV) sexually transmitted infections.

## Results

The study enrolled 447 sequential colposcopy patients, aged 20 to 49 years, consistent with the enrollment goals set in our investigational design. Table 1 shows selected sociodemographic, behavioral, and laboratory data from the subset of subjects found to have cervical lesions, according to grade of neoplasia. The only significant behavioral or demographic factors found to be associated with grade of cervical neoplasia were age ( $P = 0.013$ ), with the median age of CIN3+ cases being approximately 2 years older than patients with CIN1, and ever being pregnant ( $P = 0.013$ ). HPV DNA prevalence rates among the subjects with CIN1 ( $n = 201$ ), CIN2 ( $n = 117$ ), and CIN3+ ( $n = 92$ ) were 54.3%, 87.4%, and 95.4%, respectively.

It is interesting that the low HPV DNA prevalence among those with CIN1 was similar to the findings observed during the earlier pilot study<sup>9</sup> and was not meaningfully affected (data not shown) by limiting analysis to only those women for whom the diagnosis was made with high certainty (i.e., those cases in which both results of cytology and histology all agreed exactly on the presence and grade of disease). Of those women who were HPV DNA-positive, 33.7% of CIN1 ( $n = 34$ ), 73.2% of CIN2 ( $n = 71$ ), and 79.3% of CIN3+ ( $n = 65$ ) patients were infected with one or more oncogenic types. HPV type 16 was almost 10-fold more prevalent in CIN3+ patients (34.9%) than in CIN1 patients (3.8%).

The results of STI serological assays were correlated with one another and with sexual behavior. MIF titers restricted to serovars D–K, for example, were strongly associated with lifetime number of sex partners ( $P = 0.005$ , Pearson chi-square test) as well as with *C trachomatis* MIF ELISA OD values ( $P = 0.03$ ). Similarly, HSV-2 seropositivity was strongly related to higher numbers of lifetime sex partners ( $P < 0.001$ , Pearson chi-square test) and with HTLV-I seropositivity ( $P = 0.01$ , Pearson chi-square test) and was nonsignificantly associated with *C trachomatis* MIF titers for serovars D–K ( $P = 0.1$ , Pearson chi-square test).

None of the STIs examined (except for HPV), however, had a clear association with CIN3+ (Tables 1 and 2). HTLV-I seroprevalence was 4.0% among subjects with CIN1, compared with 7.8% among those with CIN3+ ( $P = 0.10$ ). In a multivariate model adjusting for age (<25, 25–29, 30–34, and ≥35 years), ever being pregnant, and the presence of HPV DNA, HTLV-I seroprevalence was not significantly associated with CIN3+ (OR = 1.9; 95% CI = 0.51–6.7), and restricting this analysis to just those cases and controls who were HPV DNA-positive for any type did not alter the findings (OR = 1.4; 95% CI = 0.36–5.3).

Exposure to *C trachomatis* as measured by DNA positivity, an indicator of current infection, showed no association with CIN3+ (OR = 0.82; 95% CI = 0.29–2.3, HPV adjusted). Nor was *C trachomatis* seropositivity, an indicator of past infection, associated with CIN3+, regardless of whether *C trachomatis* seroreac-

tivity was measured by a positive MIF result (titers  $\geq 1:8$ ) for any *C trachomatis* serovar (OR = 1.2, 95% CI = 0.60–2.3, HPV adjusted), for serovars D–K (OR = 1.8, 95% CI = 0.58–5.7, HPV adjusted), for high MIF antibody titers ( $\geq 1:256$ ) (OR = 1.5; 95% CI = 0.47–5.0, HPV adjusted), or for high MIF antibody titers ( $\geq 1:256$ ) for serovars D–K (OR = 1.5; 95% CI = 0.53–4.1, HPV adjusted) or by using the VS4 peptide ELISA. Restricting the analysis to HPV DNA–positive (any type) cases and controls did not appreciably alter the results.

HSV-2 seroprevalence was high among all subjects. Among patients with CIN1, the HSV-2 seroprevalence was 60.8%, compared with 73.5% among those with CIN3+ ( $P = 0.12$ ), but neither the multivariate model with HPV adjustment (OR = 1.2; 95% CI = 0.61–2.3) nor the multivariate model restricted to HPV DNA–positive women (OR = 1.2, 95% CI = 0.58–2.4) suggested any association of HSV-2 seropositivity with CIN3+.

## Discussion

To assess the possibility that certain STIs may increase the risk of developing CIN-3 and cancer, we determined the prevalence of antibodies to HSV-2, *C trachomatis*, and HTLV-I among sequential colposcopy clinic patients in Kingston, Jamaica. Jamaica is of special interest because Jamaican women have high rates of cervical cancer<sup>21</sup> and high rates of infection with HTLV-I.<sup>22</sup> Two earlier studies,<sup>8,9</sup> including a pilot study conducted in Jamaica by our research group, had suggested an association of HTLV-I with CIN3+. In the current investigation, however, we failed to detect differences between patients with CIN3+ and those with CIN1 in their seroprevalence for HTLV-I or the other STIs evaluated.

Based on an a posteriori assessment, the current investigation had 80% power to detect an OR of 2.3 for the associations of both *C trachomatis* and HSV-2 seropositivity with CIN3+, an effect size in keeping with recent positive reports.<sup>4–6</sup> Given our negative results, it is also noteworthy that few previously published studies regarding the effects of STIs on cervical neoplasia appropriately controlled for HPV infection.<sup>23,24</sup> Several studies, for example, used statistical adjustment for HPV seropositivity to control for HPV exposure,<sup>5,24</sup> an approach that could have resulted in a positive bias due to the insensitivity of HPV serology and the mutual sexual association of HPV and other STIs.<sup>25</sup>

Among the few previous studies that controlled for HPV infection by detection of HPV DNA are two positive reports from a single research group that combined the data of several cervical cancer case-control studies it had conducted in different countries.<sup>4,6</sup> In these combined data sets, seropositivities for *C trachomatis* (OR = 2.1; 95% CI = 1.1–4.0)<sup>4</sup> and for HSV-2 (OR = 2.4; 95% CI = 1.1–5.2)<sup>6</sup> were found to be significantly greater in cervical cancer cases than in HPV DNA–positive normal controls. In the latter study, it is of note that there was significant heterogeneity in the association of HSV-2 seroprevalence with invasive cervical cancer by country.

Interestingly, in those countries (The Philippines and Spain) with the lowest HSV-2 seroprevalence among controls (9.2% and 9.4%, respectively), the association of HSV-2 seroprevalence with cancer was the strongest. By contrast, in those countries (Brazil and Columbia) with the highest HSV-2 seroprevalence among controls (42.0% and 56.9%, respectively), the association was attenuate or null, consistent with the null results in our Jamaican population with high seroprevalence ( $\geq 0\%$ ).

The current negative results and the reduced HSV-2 effect previously observed in countries with high HSV-2 seroprevalence raises the possibility that, in general, the epidemiologic relation of STIs with CIN3+ is weaker in populations with high rates of STIs.

This would be expected if these relationships were not biologic in nature. That is, in populations with low rates of STIs, the few seroprevalent individuals are atypical and are likely to be unusual for their high-risk sexual behavior, making STI seropositivity a potentially strong surrogate marker for exposure to oncogenic HPV.

In contrast, in populations that have high rates of STIs and presumably more universal exposure to oncogenic HPV, STI seropositivity may not be as strong a surrogate marker of oncogenic HPV infection. Were there a true biologic relationship between STIs and high-grade cervical neoplasia, though, the effect would still be observed even in populations with high rates of STIs, assuming adequate statistical power.

Alternatively, these conflicting findings could also be explained by several relevant differences between our study design and that of earlier studies. In particular, we used patients with CIN1 as the comparison group, whereas other investigations used normal individuals or normal individuals with HPV infection.<sup>4–6,24</sup> Null results in our study but positive results in these prior investigations would have been expected if the STIs studied had an effect on the probability of low-grade cervical neoplasia but did not affect the likelihood of its progression.

Analogously, inclusion of women with CIN3 in the case group (78 CIN3 and 14 cancer cases) in the current study but not in the aforementioned studies could explain our null results if the STIs assessed mainly promote progression from carcinoma in situ to frank carcinoma. We also cannot entirely exclude the possibility that in examining a population with universally high rates of STIs, as reflected in the high prevalence of HSV-2 antibodies detected in this study and the high rates of HPV seropositivity in Jamaica as a whole,<sup>26</sup> we may have inadvertently matched on the risk factors we had hoped to study.

Our current results are also inconsistent with the findings of our own pilot study that suggested a strong association between HTLV-I and grade of neoplasia.<sup>9</sup> Because recent data indicate that HTLV-I-associated immune suppression may increase with age or duration of infection,<sup>27</sup> we believed it was important to explore whether the differences in our two sets of results could be explained by the younger age of the cases in the current investigation (mean = 33.5 years) in comparison with the pilot study (mean = 39 years). However, secondary analysis of the pilot study data demonstrated that the greatest effect was observed among those 30–49 years of age (data not shown) versus those >49 years of age and therefore suggests that age differences between the participants in the two studies do not explain the discordant HTLV-I results.

We note that only women attending this colposcopy clinic were enrolled in this study. Women who never attended a screening clinic and were not referred for colposcopy would not have participated in this study. Likewise, women who visited private physicians would not have been included in this study.

Thus, we did not have a fully representative population. We also selected controls with microscopic indications of HPV infection, which represents a subset of HPV-positive women who may be less able to control infection and/or more likely to have persistent infection. Selection of these controls may lead to a bias toward null association if these women are on average more likely to remain coinfecting and therefore more likely to seroconvert than all HPV-infected women.

In conclusion, we infer from our data that coinfections by HTLV-I, *C trachomatis*, and HSV-2 did not contribute significantly to the risk of high-grade cervical neoplasia, after controlling for HPV. Given the conflicting findings to date, we suggest that the next step in understanding these relationships needs to be retrospective cohort investigations that permit accurate assessment of

the temporality of STI infections in relation to disease, involving direct DNA measurements of HPV and relevant STIs in the cervix.

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