Trichomonas vaginalis Is Associated with Pelvic Inflammatory Disease in Women Infected with Human Immunodeficiency Virus

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We assessed the association between the causative agents of vaginal discharge and pelvic inflammatory disease (PID) among women attending a rural sexually transmitted disease clinic in South Africa; the role played by coinfection with human immunodeficiency virus type 1 (HIV-1) was studied. Vaginal and cervical specimens were obtained to detect *Neisseria gonorrhoeae, Chlamydia trachomatis, Trichomonas vaginalis,* and bacterial vaginosis. HIV-1 infection was established by use of serum antibody tests. A total of 696 women with vaginal discharge were recruited, 119 of whom had clinical PID. Patients with trichomoniasis had a significantly higher risk of PID than did women without trichomoniasis ($P = .03$). PID was not associated with any of the other pathogens. When the patients were stratified according to HIV-1 status, the risk of PID in HIV-1–infected patients with *T. vaginalis* increased significantly ($P = .002$); no association was found in patients without HIV-1. *T. vaginalis* infection of the lower genital tract is associated with a clinical diagnosis of PID in HIV-1–infected women.

Pelvic inflammatory disease (PID) is a condition in which there is infection of the reproductive tract of women above the internal os of the cervix. This has classically been associated with an ascending cervical infection caused by the vaginal discharge pathogens of *Neisseria gonorrhoeae* and *Chlamydia trachomatis* and by the anaerobic bacteria associated with bacterial vaginosis (BV). Although *Trichomonas vaginalis* is a common cause of vaginal discharge that infects ~120 million women per year [1], less attention has been paid to its role as a possible cause of upper genital tract infection. This organism has previously been isolated from peritoneal fluid samples [2], and it has been implicated in potentiating bacterial upper genital tract infection [3–5].

Paisarntantiwong et al. [6] reported an association between vaginal trichomoniasis and PID among women colonized with *C. trachomatis*. In addition, trichomoniasis has been independently associated with adverse pregnancy outcomes, such as premature rupture of membranes, preterm delivery, and low birth weight [7–9]. The evidence linking the discharge-causing pathogens, including *T. vaginalis*, with HIV-1 transmission and acquisition is substantial and clear [10, 11]. However, literature addressing the influence of the interaction of HIV-1 infection and PID in terms of clinical presentation, microbiologic etiology, and response to therapy is inconclusive [12–16].

We hypothesized that *T. vaginalis* causes PID in HIV-1–infected women independent of the other established discharge-causing pathogens and BV. Therefore, we compared the prevalence of these pathogens in patients

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with and without HIV-1 who presented with vaginal discharge with or without clinical PID.

METHODS

Setting and patients. Women who attended the Africa Centre for Population Studies and Reproductive Health Sexually Transmitted Diseases Clinic in Kwamsane from March 1999 through November 2000 and who had symptoms of vaginal discharge only or of vaginal discharge with lower abdominal pain were invited to participate in the study after informed consent was obtained. A diagnosis of PID was made if, in addition to the presenting symptoms of genital discharge and lower abdominal pain, lower abdominal tenderness and cervical motion tenderness were elicited on examination [17]. The clinician who determined the subject's PID status was blinded to all results.

Specimen collection and processing. A calcium alginate swab and a Dacron swab were sequentially inserted 2–3 cm into the endocervix under direct vision; they were withdrawn while rotating. The swabs were used to inoculate New York City plates (Oxoid) for the detection of *N. gonorrhoeae* and to make a smear for chlamydia direct immunofluorescence testing (MicroTrak; Trinity Biotech), respectively.

A Dacron swab was used to collect vaginal specimens. A smear was made onto a glass slide, which was then stained with Gram stain and scored for BV by use of Nugent's criteria [18]. The swab was placed into Diamond's media for the isolation of *T. vaginalis.*

Blood samples we collected in EDTA tubes and screened with the Determine-HIV test for HIV testing. Those samples that tested positive had their results confirmed by 2 different ELISA tests (Murex HIV-1.2.0, manufactured by Abbott; Vironosticka HIV Uni-Form *plus* 0, manufactured by Organon Teknika). A random sample of 10% of the negative specimens was also subjected to ELISA confirmation.

Statistical analysis. χ^2 Tests were used to identify significant factors at baseline for PID. Relative risks and 95% CIs are reported. Breslow-Day tests were used to test for an interaction effect. Significant risk factors were examined in a logistic regression model to identify independent factors affecting PID.

RESULTS

A total of 577 subjects with vaginal discharge only and 119 subjects with clinical PID were recruited into the study after informed consent was obtained. The prevalence of HIV-1 infection in all women studied was 56%. There was no association between HIV-1 infection and a clinical diagnosis of PID: 76 (64%) of 119 women with PID were infected with HIV-1, as compared with 312 (54%) of 577 women with discharge and no signs of PID ($P = .6$).

The overall prevalence of sexually transmitted infections in this population was high; 525 (75%) of 696 subjects had at least 1 of the following: *N. gonorrhoeae* infection, *C. trachomatis* infection, *T. vaginalis* infection, or BV. BV was the most frequently diagnosed cause of vaginal discharge (in 481 subjects [69%]), followed by *T. vaginalis* (in 205 [29%]), *N. gonorrhoeae* (in 86 [12%]), and *C. trachomatis* (in 74 [11%]; table 1).

There was no difference in the overall prevalence of identifiable sexually transmitted infection etiology in women with PID (80%), as compared with women who had a discharge only (76%; $P = .8$). A significant association was seen between *T. vaginalis* and PID (45 of 119 subjects were coinfected), as opposed to those with discharge only (160 of 577; $P = .03$). A similar trend was seen with *N. gonorrhoeae,* although this did not reach statistical significance ($P = .06$; table 1).

When the prevalence of the various sexually transmitted infections and of BV was stratified according to the patient's HIV-1 serostatus, the associated risk of PID (as opposed to discharge only) in subjects infected with *T. vaginalis* disappeared among HIV-1–seronegative patients (RR, 0.8; 95% CI, 0.4–1.5; $P = .4$). However, this association with PID increased significantly among HIV-1–infected women who had *T. vaginalis* (RR, 1.9; 95% CI, 1.3–2.8; $P = .002$). No such association was seen with any of the other infections (tables 2 and 3). The association of PID and *T. vaginalis* in HIV-1–infected women remained, re-

Table 1. Prevalence of sexually transmitted infections and bacterial vaginosis among women with vaginal discharge only and among those with vaginal discharge and clinical pelvic inflammatory disease (PID).

Infectious agent or condition	No. (%) of patients				
	All ($n = 696$)	Had discharge only ($n = 577$)	Had discharge with PID ($n = 119$)	P	RR of PID (95% CI)
Bacterial vaginosis	481 (69)	397 (69)	84 (71)	.7	1.1 (0.7–1.5)
Trichomonas vaginalis	205 (29)	160 (28)	45 (38)	.03	1.5 (1.1–2.1)
Neisseria gonorrhoeae	86 (12)	64 (11)	22 (18)	.06	1.5 (1.0–2.3)
Chlamydia trachomatis	74 (11)	61 (10)	13 (11)	.8	1.0 (0.6–1.8)

Table 2. Prevalence of sexually transmitted infections and bacterial vaginosis among HIV type 1–infected women with vaginal discharge only and among those with vaginal discharge and clinical pelvic inflammatory disease (PID).

Infectious agent or condition	Percentage of patients: All (n = 388)	Had discharge only (n = 312)	Had discharge with PID (n = 76)	P	RR of PID (95% CI)
Bacterial vaginosis	78	79	78	.8	1.1 (0.65–1.8)
Trichomonas vaginalis	32	29	47	.002	1.9 (1.3–2.8)
Neisseria gonorrhoeae	16	14	21	.1	1.4 (0.9–2.3)
Chlamydia trachomatis	15	14	17	.5	1.2 (0.7–2.0)

gardless of whether trichomoniasis occurred as a single infection (RR, 3.4; 95% CI, 1.1–11.1) or in combination with ⩾1 of the other 3 conditions (RR, 2.1 95%, CI 1.2–3.6).

HIV-1–infected women who had *N. gonorrhoeae* alone (OR, 2.2; 95% CI, 0.5–9.1; $P = .3$), BV alone (OR, 0.8; 95% CI, 0.4–2.6; $P = .5$), or a combination of the 2 (OR, 0.8; 95% CI, 0.2–2.7; $P = .7$) were not associated with PID. The number of HIV-1–infected women infected with *Chlamydia* species was too small to model.

DISCUSSION

PID is associated with significant morbidity and mortality. Its management consists of the immediate initiation of antimicrobial therapy to cover the possible causes of infection. *T. vaginalis* is the only vaginal-discharge–causing pathogen that is not part of the microbial differential diagnosis of PID. However, if present, this organism is inadvertently treated with metronidazole, which is used for possible infection with anaerobes. In this study, we show that, in HIV-1–infected women, lower genital tract infection with this organism is associated with an increased risk of PID ($P = .002$).

The association we describe does not necessarily imply a causal relationship. However, infection with *T. vaginalis* is increasingly recognized to be associated with reproductive tract complications including sepsis that occurs after abortion and after cesarean section [19, 20], as well as adverse pregnancy outcome [7–9]. This association has always been reported in combination with an altered state of the reproductive tract (e.g., pregnancy), the puerperium [13, 21], and coinfection with other sexually transmitted infections [6]. Our data suggest that coinfection with HIV-1 may also alter the host-microbe relationship, resulting in an apparent increased risk of PID in the presence of *T. vaginalis*. There are, however, no data suggesting a mechanism for such an association. The pathogenesis of infection with this protozoon is poorly understood. Lower genital tract infection results in an aggressive inflammatory response with punctate hemorrhages, thus allowing effective transmission and acquisition of HIV-1 infection [21, 22]. Theoretically, the proteinases produced by *T. vaginalis* have the potential to break down the protective cervical mucus plug, facilitating access of microbes from the lower to the upper genital tract [23]. These microbes may include not only the vaginal anaerobes, which are already implicated as causes of PID, but also *T. vaginalis* itself.

Although our data also suggest an increased risk of PID among patients infected with *N. gonorrhoeae*, irrespective of HIV-1 serostatus, this did not reach statistical significance. A lack of association is also noted for *C. trachomatis* and PID. A possible explanation for this is that the comparison group is women with

Table 3. Prevalence of sexually transmitted infections and bacterial vaginosis among HIV type 1–uninfected women with vaginal discharge only and among those with vaginal discharge and clinical pelvic inflammatory disease (PID).

Infectious agent or condition	Percentage of patients: All (n = 308)	Had discharge only (n = 265)	Had discharge with PID (n = 43)	P	RR of PID (95% CI)
Bacterial vaginosis	58	59	56	.7	0.9 (0.52–1.6)
Trichomonas vaginalis	25	26	21	.4	0.8 (0.4–1.5)
Neisseria gonorrhoeae	8	7	12	.3	1.5 (0.7–3.6)
Chlamydia trachomatis	5	6	0	.1	0.2 (0.01–3.2)

vaginal discharge and not "healthy" women. Therefore, a large proportion of patients in both the PID and non-PID groups are infected with *N. gonorrhoeae* and *C. trachomatis.*

This study has obvious limitations. The clinical diagnostic criteria used are subjective, but a diagnosis based on sonography or surgery is not always possible or feasible, especially in resource-poor areas, such as ours. In addition, *T. vaginalis* is associated with punctate hemorrhages and inflammation of the cervix (strawberry cervix), which may mimic the cervical motion tenderness associated with PID. If the latter is true, then PID may have been overdiagnosed among our patients, in which case, our results imply a more severe presentation of lower genital tract trichomoniasis among HIV-1–infected women.

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