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Risk of human immunodeficiency virus infection and genital ulcer disease among persons attending a sexually transmitted disease clinic in Italy

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SUMMARY

To assess the relative importance of ulcerative and non-ulcerative sexually transmitted disease in the transmission of HIV, a seroprevalence study was conducted on 2210 patients at the sexually transmitted diseases (STD) clinic of the S. Maria e S. Gallicano Hospital in Rome, between 1989 and 1994. Among male patients, by univariate analysis, strong predictors of HIV infection were homosexuality, sexual exposure to a HIV-positive partner, hepatitis B virus infection, and positive syphilis serology. An increased risk was estimated for patients with past genital herpes (odds ratio (OR) 3·86, 95% confidence intervals (CI) 0·40–18·2), and primary syphilis (OR 5·79, 95% CI 0·59–28·6). By multivariate analysis, a positive association was found with homosexuality (OR 6·9, 95% CI 2·9–16·5), and positive syphilis serology (OR 3·5, 95% CI 1·3–9·2). An adjusted OR of 2·41 was calculated for current and/or past genital herpes. These results, although not conclusive, suggest a role of ulcerative diseases as risk factors for prevalent HIV infection, and indicate that positive syphilis serology is an unbiased criterion for identifying individuals at increased risk of HIV infection.

INTRODUCTION

The association between human immunodeficiency virus (HIV) infection and other sexually transmitted diseases (STD) is well documented [1–3]. Increased attention has been given in recent years to STDs causing genital ulceration, whose role in the heterosexual transmission of HIV has been established in several studies performed in Africa and other developing countries [4–8]. In the United States, studies conducted among homosexual men and heterosexual men and women attending STD clinics have shown a relationship between genital ulcer disease, mainly due to syphilis and genital herpes, and risk of HIV infection [9–14].

In this study an assessment of the relative importance of diseases causing genital ulceration and other non-ulcerative STDs in the transmission of HIV was attempted among patients attending a large clinic for STDs in Italy.

METHODS

Study population

A study of HIV seroprevalence was conducted at the STD outpatient clinic of the S. Maria e S. Gallicano

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Table 1. *Characteristics of the study population, Rome, Italy, 1986–94*

	Males		Females		
Risk factor	No.	%	No.	%	*P*
Year					
1989	1025	64·1	366	59·9	0·067
1993–4	574	35·9	245	40·1	
Age					
⩽ 30 years	689	43·1	333	54·5	0·000
> 30 years	910	56·9	278	45·5	
Education level					
⩽ 8 years	769	48·4	300	49·6	0·609
> 8 years	821	51·6	305	50·4	
Sexual preference					
Heterosexual	1278	81·0	590	99·7	0·000
Homosexual/bisexual	300	19·0	2	0·3	
Injecting drug use					
Yes	61	3·9	12	2·0	0·028
No	1511	96·1	593	98·0	
Sex partner IDU					
Yes	46	3·6	35	6·2	0·014
No	1221	96·4	531	93·8	
Sex partner HIV positive					
Yes	27	2·1	15	2·7	0·478
No	1229	97·9	542	97·3	
Blood transfusion					
Yes	45	2·9	21	3·6	0·443
No	1493	97·1	567	96·4	
No. of sex partners in the previous year					
0–2	887	55·6	537	88·3	0·000
⩾ 3	707	44·4	71	11·7	
Condom use in the previous year					
Always/often	482	31·5	78	13·2	0·000
Rarely/never	1050	68·5	512	86·8	
Diagnosis					
Normal physical examination	706	44·9	318	52·1	0·000
Non-ulcerative STD*	381	24·2	195	32·0	
Genital herpes (current)	155	9·9	19	3·1	
Genital herpes (previous)	25	1·6	12	2·0	
Primary syphilis	16	1·0	0	0·0	
Secondary syphilis	9	0·6	4	0·7	
Positive syphilis serology	281	17·9	62	10·2	
HIV					
Positive	80	5·0	10	1·6	0·000
Negative	1519	95·0	610	98·4	

* Non-ulcerative STDs included gonorrhoea, genital warts, nongonococcal urethritis (NGU) due to *Chlamydia trachomatis* or *Ureaplasma urealyticum*, vaginal or cervical infection due to *Trichomonas vaginalis*, *Candida albicans* or chlamydia, scabies, *Phthirus pubis* infestation.

Hospital in Rome, from February to December 1989 and from January 1993 to April 1994.

During the first period all patients seen at the STD clinic were enrolled in the study. In 1993–4, all patients reporting genital ulcer or erosion and all patients attending the clinic on a random day a week were

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recruited. Informed consent was obtained from all study participants. After informed consent, the patients provided a blood sample and were interviewed using an anonymous questionnaire by a trained study clinician, to collect information on age, sex, education, sexual preference, number of sexual partners and use of condoms in the previous year, and any history of intravenous drug use, blood transfusions and STD.

Laboratory methods

The serum samples collected in both study periods were stored at −20 °C until tests were performed. All samples were tested for HIV antibodies by enzyme linked immunosorbent assay (ELISA). Reactive sera were confirmed by Western blot analysis Abbott HIV, Abbott Diagnostics, Wiesbaden, Germany). Antibodies against hepatitis B core antigen (anti-HBc) were determined using a commercially available ELISA (Abbott Laboratories, North Chicago, IL). Anti-HCV antibodies were determined with the third-generation Ortho HCV 3.0 ELISA (Ortho Diagnostic Systems, Raritan, New Jersey). Reactive sera were controlled with an additional, more specific, test (Chiron Riba HCV 3.0 SIA, Ortho Diagnostic Systems, Raritan, New Jersey) consisting of a qualitative immunoblot assay which utilizes hepatitis C virus (HCV) antigens, immobilized as individual bands onto test strips.

Syphilis serologic testing was performed with the rapid plasma reagin (RPR) screening test and the haemaglutination test for *Treponema pallidum* (TPHA) and an EIA test for IgM and IgG for confirmation. Dark field microscopy was performed on any lesion or rash compatible with primary or secondary syphilis.

The diagnosis of genital herpes was clinical. Serological testing for chlamydial infection was made by ELISA. Vaginal and urethral specimens were either examined by Gram stain or cultured for *Neisseria gonorrhoeae*.

Statistical analysis

Unadjusted odds ratios (OR) and 95% confidence intervals (CI) were computed separately by sex to assess univariate associations between behavioural, clinical and laboratory risk factors and prevalent HIV infection. A logistic regression model was used to identify variables independently associated with anti-HIV-1 positivity. For each variable the reference category was the most favourable level of exposure (youngest age, highest education level, lowest number of sexual partners, use of condoms, absence of a history of STDs).

Frequency distributions, univariate analysis, and logistic regression analyses were performed using BMDP statistical software [15].

RESULTS

Demographic, behavioural and clinical characteristics

A population group of 2595 patients (1581 in 1989 and 1014 in 1993–4) were enrolled into the study. A total of 1391 in the first period and 819 in the second period provided a blood sample, an acceptance rate of 88 and 81%, respectively. These 2210 patients comprise the subjects for this analysis. No significant differences were observed between subjects who underwent serologic testing and those who refused. The general characteristics of study patients by gender are shown in Table 1. More men (1599) than women (611) entered the study, which reflected the general demographic profile for the clinic's population. A higher proportion of men reported three or more sexual partners in the year before the clinic visit (44 *vs.* 12%), same-sex partner exposure (19 *vs.* 0·3%) and parenteral drug use (4 *vs.* 2%). In addition, prevalent HIV infection and serologic evidence of syphilis were more common in male than in female patients (5 *vs.* 1·6%, and 18 *vs.* 10%, respectively).

Predictors of HIV infection by univariate analysis

Among male patients, excluding intravenous drug users and subjects who had received blood transfusion after 1975, the relative odds of HIV infection were highest for homosexual or bisexual men (OR 13·4, 95% CI 7·2–24·3) and for patients, having an HIV positive sexual partner, with antibodies to core antigen of hepatitis B virus, and with reactive syphilis serology (Table 2). An increased risk of HIV infection was observed also for patients reporting a history of genital herpes infection (OR 3·86, 95% CI 0·40–18·2), for patients with primary syphilis (OR 5·79, 95% CI 0·59–28·6) and for patients who were anti-HCV

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Table 2. *Univariate analysis of risk factors for HIV infection among male patients attending a sexually transmitted disease clinic, Rome, Italy, 1989–94 (intravenous drug user and transfused subjects are excluded)*

	No. HIV-positive/ No. tested	HIV-positive (%)	Crude OR	95% CI
Age				
< 30 years	18/641	2·8	1·0	
⩾ 30 years	39/855	4·6	1·65	0·94–2·92
Education level				
> 8 years	28/789	3·5	1·0	
⩽ 8 years	29/697	4·2	1·18	0·69–2·00
Sexual preference				
Heterosexual	15/1193	1·3	1·0	
Homosexual/bisexual	41/284	14·4	13·4	7·22–24·3
Sex partner IDU				
No	30/1175	2·6	1·0	
Yes	1/21	4·8	1·91	0·25–14·7
Sex partner HIV positive				
No	29/1172	2·5	1·0	
Yes	4/122	18·2	8·76	2·79–27·5
No. of sex partners in the previous year				
0–2	23/832	2·8	1·0	
⩾ 3	34/660	5·2	1·91	1·11–3·28
Condom use in the previous year				
Always/often	30/449	6·7	1·0	
Rarely/never	16/990	1·6	0·23	0·12–0·43
Diagnosis				
Normal physical examination	16/665	2·4	1·0	
Non-ulcerative STD*	7/352	2·0	0·82	0·28–2·14
Genital herpes (current)	3/149	2·0	0·83	0·15–2·97
Genital herpes (previous)	2/23	8·7	3·86	0·40–18·2
Primary syphilis	2/16	12·5	5·79	0·59–28·6
Secondary syphilis	0/7	0·0	—	—
Positive syphilis serology	27/258	10·5	4·74	2·41–9·40
Anti-HBc				
Negative	17/1026	1·7	1·0	
Positive	40/468	8·5	5·55	3·01–10·3
Anti-HCV				
Negative	37/1231	3·0	1·0	
Positive	5/62	8·1	2·83	0·84–7·60

* Non-ulcerative STDs included gonorrhoea, genital warts, nongonococcal urethritis (NGU) due to *Chlamydia trachomatis* or *Ureaplasma urealyticum*, vaginal or cervical infection due to *Trichomonas vaginalis*, *Candida albicans* or chlamydia, scabies, *Phthirus pubis* infestation.

positive (OR 2·83, 95% CI 0·84–7·60). A significant positive association was also found between anti-HIV positivity and reporting three or more sexual partners in the previous year. Surprisingly, using condoms rarely or never was associated with a lower risk of HIV infection.

Among female patients, the risk of prevalent HIV infection was high if they reported having either a seropositive or intravenous drug user as a sexual partner (Table 3). A positive association was also found with a previous genital herpes infection.

Predictors of HIV infection by multivariate analysis

To evaluate the independent effect of each risk factor, after adjusting for the confounding effect of the other variables, a logistic regression model including all the

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Table 3. *Univariate analysis of risk factors for HIV infection among female patients attending a sexually transmitted disease clinic, Rome, Italy, 1989–94 (intravenous drug user and transfused subjects are excluded)*

Risk factor	No. HIV-positive/ No. tested	HIV-positive (%)	Crude OR	95% CI
Age				
< 30 years	4/321	1·2	1·0	
⩾ 30 years	2/258	0·8	0·62	0·11–3·42
Education level				
> 8 years	2/289	0·7	1·0	
⩽ 8 years	4/285	1·4	2·04	0·37–11·3
Sex partner IDU				
No	3/512	0·6	1·0	
Yes	2/25	8·0	14·8	2·34–93·0
Sex partner HIV positive				
No	4/523	0·8	1·0	
Yes	1/11	9·1	13·0	0·24–14·6
No. of sex partners in the previous year				
1–2	6/513	1·2	—	
⩾ 3	0/65	0·0	—	—
Condom use in the previous year				
Always/often	0/75	0·0	—	
Rarely/never	5/485	1·0	—	—
Diagnosis				
Normal physical examination	3/302	1·0	1·0	
Non-ulcerative STD	2/184	1·1	1·10	0·09–9·65
Genital herpes (current)	0/19	0·0	—	—
Genital herpes (previous)	1/11	9·1	9·97	0·17–135
Primary syphilis	—	—	—	—
Secondary syphilis	0/3	0·0	—	—
Positive syphilis serology	0/59	0·0	—	—
Anti-HBc				
Negative	6/490	1·2	—	
Positive	0/87	0·0	—	—
Anti-HCV				
Negative	1/446	0.2	—	
Positive	0/21	0·0	—	—

variables associated with prevalent HIV infection by univariate analysis was run for male patients, excluding subjects who reported intravenous drugs or blood transfusion after 1975 (Table 4). No significant association was found with age and number of sexual partners in the previous year. A strong positive association with HIV infection was found for homosexuality (OR 6·37, 95% CI 2·67–15·2) and a positive syphilis serology (OR 2·73, 95% CI 1·00–7·45). Adjusted OR of 2·70 and 2·41 were calculated for having a HIV positive sexual partner and current and/or past genital herpes, respectively, although there were not statistically significant, probably due to the small number of subjects in these categories. A negative association still remained between condom use and risk of HIV infection.

DISCUSSION

The relationship between HIV infection and sexually transmitted diseases has been reported world-wide [2, 6, 7, 14, 16, 17] and has led to the hypothesis that STDs, particularly those causing genital ulceration, might be biological cofactors of HIV transmission. Genital ulceration may increase the susceptibility to HIV by disruption of the genital epithelium, local recruitment of HIV target CD4 lymphocytes or by inducing immunosuppression and increased susceptibility to viral infections.

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Table 4. *Risk factors for HIV infection among male patients attending a sexually transmitted disease clinic* (*multiple logistic regression analysis*) *Rome*, *Italy*, *1989–94* (*intravenous drug user and transfused subjects are excluded*)

Risk factor	Adjusted OR	95% CI
Age		
< 30 years	1·0	
⩾ 30 years	0·73	0·31–1·72
Sexual preference		
Heterosexual	1·0	
Homosexual/bisexual	6·37	2·67–15·2
Sex partner HIV positive		
No	1·0	
Yes	2·70	0·74–9·94
No. of sex partners in the previous year		
0–2	1·0	
⩾ 3	0·70	0·31–1·58
Condom use in the previous year		
Always/often	1·0	
Rarely/never	0·35	0·15–0·83
Diagnosis		
Normal physical examination	1·0	
Non-ulcerative STD	1·24	0·39–3·94
Genital herpes (current or previous)	2·54	0·72–8·92
Positive syphilis energy	2·73	1·00–7·45
Anti-HBc		
Negative	1·0	
Positive	2·17	0·95–4·99

This study suggests that recurrent genital herpes infection and primary syphilis might increase the risk of HIV infection among male patients attending the Rome STD clinic, although the estimated OR of 3·86 and 5·79, respectively, did not reach the statistical significance, due to the small number of subjects reporting these exposures. We did not use serology for diagnosis of genital herpes because of the weak correlation between a positive test and clinical disease. In addition, the residual cross-reactivity between herpes simplex viruses HSV-1 and HSV-2 excludes its use for diagnosis in absence of clinical symptoms.

Positive syphilis serology was strongly associated with prevalent HIV infection in these patients (OR 2·73, 95% CI 1·00–7·45), and, interestingly, this association remained also after adjusting for possible confounders by multivariate analysis. This finding is consistent with those reported by other authors [11, 14], but whether a reactive syphilis serology indicates a pre-existing genital ulcer or is a marker of high-risk sexual behaviour is still unclear. Syphilis is likely to be a reliable indicator for certain high risk behaviours that are difficult to assess with accuracy with history alone.

A recent review of the literature [18] shows that, although most of the epidemiological evidence suggests there is truly an association between genital ulcer disease and HIV, only a few studies have accurately examined the temporal relationship between the disease and subsequent HIV seroconversion. Moreover, the high variability in definition of genital ulceration, or the use of laboratory tests as a proxy of previous genital ulcer might account for different strengths of association found in different studies [19]. An example is the lack of consistency between studies investigating the association between genital herpes infection, which in Western countries is a common cause of genital ulceration, and HIV, with a stronger association reported in those studies which used HSV-2 serology for the diagnosis [10, 20, 21].

Many conditions and behaviours associated with

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an increased risk of genital ulcerative diseases are likewise associated with increased risk of HIV infection. The control of potential confounders is *per se* difficult, because the assignment of subjects to specific risk groups is largely based on self-reported assessments of risk behaviour, which may be subject to recall bias or the bias of social desirability. The apparent paradox in our study of an increased risk of HIV infection among subjects more frequently using condoms may be due to these types of bias, and is consistent with an observation reported in a Thai study [22]. Subjects may be reporting their recent condom use, after previously engaging in high risk sex or, alternatively, they may misreport their actual condom use for reasons of social desirability, especially in settings such as STD clinics, where reporting condom use is perceived as the correct response.

In conclusion, despite the many problems in measuring the true risk of HIV infection associated with previous genital ulcer disease, the evidence provided by the published studies is in favour of a true association. The results of this study, although not conclusive, suggested a role of ulcerative diseases such as genital herpes and syphilis, in the study population, as risk factors for prevalent HIV infection. The consistent association between syphilis serology and HIV infection indicates that positive syphilis serology is an unbiased criterion for identifying individuals at increased risk of HIV infection.

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REFERENCES

1. Quinn TC, Glasser D, Cannon RO, et al. Human immunodeficiency virus infection among patients attending clinics for sexually transmitted diseases. N Engl J Med 1988; **318**: 197–203.
2. Cannon RO, Schmid GP, Moore PS, Pappaioanu M. Human immunodeficiency virus (HIV) seroprevalence in persons attending STD clinics in the United States, 1985–1987. Sex Transm Dis 1989; **16**: 184–9.
3. Chiasson MA, Stoneburner RL, Lifson AR, et al. Risk factors for human immunodeficiency virus type 1 (HIV-1) infection in patients at a sexually transmitted disease clinic in New York City. Am J Epidemiol 1990; **131**: 208–20.
4. Greenblatt RM, Lukehart SA, Plummer FA, et al. Genital ulceration as a risk factor for human immunodeficiency virus infection. AIDS 1988; **2**: 47–50.
5. Simonsen JN, Cameron DW, Gakinya MN, et al. Human immunodeficiency virus infection among men with sexually transmitted diseases. Experience from a center in Africa. N Engl J Med 1988; **319**: 274–8.
6. Cameron DW, D'Costa LJ, Maitha GM, et al. Female to male transmission of human immunodeficiency virus type 1: risk factors for seroconversion in men. Lancet 1989; ii: 403–7.
7. Plourde PJ, Pepin J, Agoki E, et al. Human immunodeficiency virus type 1 seroconversion in women with genital ulcers. J Infect Dis 1994; **170**: 313–17.
8. Rodrigues JJ, Mehendale SM, Shepherd ME, et al. Risk factors for HIV infection in people attacking clinics for sexually transmitted diseases in India. BMJ 1995; **311**: 283–6.
9. Holmberg SD, Stewart JA, Gerber AR, et al. Prior herpes simplex virus type 2 infection as a risk factor for HIV infection. JAMA 1988; **260**: 1429–33.
10. Stamm WE, Handsfield HH, Rompalo AM, Ashley RL, Roberts PL, Corey L. The association between genital ulcer disease and acquisition of HIV infection in homosexual men. JAMA 1988; **260**: 1429–33.
11. Quinn TC, Cannon RO, Glasser D, et al. The association of syphilis with risk of human immunodeficiency virus infection in patients attending sexually transmitted disease clinics. Arch Intern Med 1990; **150**: 1297–302.
12. Chirgwin K, De Hovitz JA, Dillon S, McCormack WM. HIV infection, genital ulcer disease, and crack cocaine use among patients attending a clinic for sexually transmitted diseases. Am J Public Health 1991; **81**: 1576–9.
13. Telzack EE, Chiasson MA, Bevier PJ, Stoneburner RI, Castro KJ, Jaffe HW. HIV-1 seroconversion in patients with and without genital ulcer disease. A prospective study. Ann Intern Med 1993; **119**: 1181–6.
14. Torian LV, Weisfuse, Makki HA, Benson DA, Di Camillo LM, Toribio FE. Increasing HIV-1 seroprevalence associated with genital ulcer disease, New York City, 1990–1992. AIDS 1995; **9**: 177–181.
15. BMDP Statistical Software Manual, vol. 2. University of California Press, 1990: 1047–76.
16. Mehendale SM, Rodrigues JJ, Brookmeyer RS, et al. Incidence and predictor of human immunodeficiency virus type 1 seroconversion in patients attending sexually transmitted disease clinics in India. J Infect Dis 1995; **172**: 1486–91.
17. Kassler WJ, Zenilman JM, Erickson B, Fox R, Peterman TA, Hook EW. Seroconversion in patients attending sexually transmitted disease clinics. AIDS 1994; **8**: 351–5.
18. Dickerson MC, Johnston J, Delea TE, White A, Andrews E. The causal role for genital ulcer disease as a risk factor for transmission of human immunodeficiency virus. Sex Transm Dis 1996; **23**: 429–40.

19. Rompalo AM, Shepherd M, Lawlor JP, et al. Definitions of genital ulcer disease and variation in risk for prevalent human immunodeficiency virus infection. Sex Transm Dis 1997; **24**: 436–42.
20. Hook EW, Cannon RO, Nahmias AJ, et al. Herpes simplex virus infection as a risk factor for human immunodeficiency virus infection in heterosexuals. J Infect Dis 1992; **165**: 251–5.
21. Stamm WE, Handsfield HH, Rompalo AM, Ashley RL, Roberts PL, Corey L. The association between genital ulcer disease and acquisition of HIV infection in homosexual men. JAMA 1988; **260**: 1429–33.
22. Nelson DI, Celentano DD, Eiumtrakol S, et al. Changes in sexual behavior and a decline in HIV infection among young men in Thailand. New Engl J Med 1996; **335**: 297–303.