The effects of urethritis on seminal plasma HIV-1 RNA loads in homosexual men not receiving antiretroviral therapy


Methods: Prospective case-control study. HIV-1 infected homosexual men, not receiving ART for at least 3 months, with (cases) and without (controls) symptomatic urethritis, were recruited. Blood and semen samples were collected for HIV-1 RNA quantification at presentation, before antibiotic therapy, and at 1 and 2 weeks.

Results: 20 cases (13 gonococcal urethritis and/or chlamydial urethritis (GU/CU) and seven non-specific urethritis (NSU)) and 35 controls were recruited. Baseline characteristics and blood plasma viral load were similar in cases and controls. Mean log semen plasma viral loads were higher among those with GU/CU compared with controls (4.27 log versus 3.55 log respectively; p = 0.01) but not in those with NSU (3.48 log; p = 0.82). Following antibiotics, semen plasma viral loads fell by a mean of 0.25 log (95% CI: 0.03 to 0.47) in those with GU/CU. Semen plasma viral loads did not fall in those with NSU.

Conclusions: In this study of 55 homosexual men not on ART, semen plasma viral loads were approximately fivefold higher in those with GU/CU, but not NSU, compared with controls. Treatment of GU/CU resulted in reduction in semen plasma viral loads. Although absolute effects were considerably lower when compared to patients from a similar study from sub-Saharan Africa, our data demonstrate the potential for sexually transmitted infections to enhance HIV infectivity of men not receiving ART in the developed world.

Abbreviations: ART, antiretroviral therapy; BPVL, blood plasma viral loads; GU, gonococcal urethritis; NGU, non-gonococcal urethritis; NSU, non-specific urethritis; PCR, polymerase chain reaction; p/hpf, polymorphs per high power field; SPVL, seminal plasma viral loads

Most HIV-1 infections in adults worldwide occur sexually, and there is biological and epidemiological evidence that the quantity of virus in genital secretions is an important determinant of transmission. We have recently demonstrated no effect of sexually transmitted infections (STIs) on seminal plasma viral loads (SPVL) in homosexual men on fully suppressive antiretroviral therapy (ART) living in the United Kingdom. Among those not on ART, urethritis has been associated with increased genital shedding of HIV-1 in studies from sub-Saharan Africa, with differences of over 100,000 copies/ml in median SPVL observed in cases of gonococcal urethritis (GU) compared to those without STIs in one study. In the developed world, a limited number of small studies have suggested that STIs may increase SPVL in those not taking ART.

It is possible that effects of STIs on SPVLs, observed in Africa, may be greater than those observed in the developed world. HIV-1 infected individuals in Africa have, on average, higher blood plasma viral loads (BPVL) and states of immune activation than those in the developed world and the effects of inflammatory cytokines on viral replication may be greater on prevalent subtypes in Africa compared to subtype B, found more commonly in Europe and North America. Furthermore, delayed health seeking behaviour of those with STIs in the developing world may allow STIs to have greater impact before treatment is given. We studied the effects of urethritis on SPVL in homosexual men with HIV infection who were not on ART.

Methods: HIV-1 infected homosexual men not receiving ART for at least 3 months attending two UK sexual health clinics either with urethritis (cases) or for a sexual health check up but with no STI (controls) were recruited prospectively between November 2000 and October 2002. Patients were excluded if they had an episode of urethritis or systemic illness in the previous month. Participants had routine urethral swabs for gonorrhoea (by microscopy and culture), chlamydia (using ligase chain reaction; Abbott Diagnostics, Abbot Park, IL, USA), and non-gonococcal urethritis (NGU) by microscopy. For the study, in those who were negative for gonorrhoea on microscopy, NGU was defined as those patients symptomatic for urethritis with five or more polymorphs per high power field (p/hpf) on microscopy or those asymptomatic patients with 10 p/hpf or more on microscopy. This definition was used because of our observation that consistency of microscopy alone for predicting NGU is poor when polymorph counts are low. The initial diagnosis of NGU was changed to non-specific urethritis (NSU) if subsequent chlamydia and gonococcal culture tests remained negative. In addition to serological tests for syphilis taken on their first visit, blood was also collected for HIV-1 RNA quantification and patients then provided a semen sample by masturbation into a sterile container. All patients provided semen samples before voiding urine and were advised against using lubricants during masturbation.

Patients diagnosed with urethritis, whether GU or NGU, were treated with appropriate antibiotics. Cases and controls were asked to attend the following week (visit 2) and 2 weeks later (visit 3) for repeat smears, gonococcal culture, and blood and semen samples. At all visits, clinical and demographic data were collected including sexual histories.
A sample size of 20 cases and controls was required to give approximately 80% power to detect as significant a difference in mean log-SPVL at first visit of 0.7 (that is, a fivefold difference in SPVL), as observed previously in Africa, relative to a standard deviation of measurements in each group of 0.8, and taking the standard 5% significance level. It was decided to try to recruit more controls to increase this power.

**Virology methods**

Semen and blood samples were centrifuged within 2 hours of collection and the plasma and cellular components stored at ~70°C. HIV-1 RNA was extracted from blood and semen plasma by a silica gel capture method previously observed to successfully remove inhibitors of the polymerase chain reaction (PCR) and quantified using an in-house, internally calibrated reverse transcribed PCR assay (RT-QPCR, Department of Virology UCL, London). The lower limit of quantification was 1000 copies/ml.

**Statistical methods**

Cases were compared with controls with respect to age, years since HIV diagnosis, ethnicity, median numbers of partners in previous 3 months, and most recent CD4 count and HIV-1 viral load before first visit. For comparisons of age, number of partners, CD4 count and time since HIV diagnosis the Mann-Whitney test was used. For viral loads before first visit, and partners, CD4 count and time since HIV diagnosis the Mann-Whitney test was used. In all analysis of HIV-1 RNA loads undetectable measurements were considered as 500 copies/ml (half the limit of detection), and log10 values were used. To compare ethnicity and HIV-1 RNA detectability the Mann-Whitney test was used, and their correlation assessed using Pearson’s correlation coefficient. Average changes in HIV-1 RNA loads across study visits were estimated for cases and controls, and these changes compared. This analysis was based on generalised estimating equations (GEE) of Stata 7, because of multiple measurements for patients, selecting an exchangeable working correlation structure, and using the robust standard errors. As planned subgroup analysis, comparisons with controls were made for all cases, for NSU cases alone, and cases with chlamydial urethritis (CU) or GU.

**RESULTS**

Twenty cases (nine GU, three CU, one combined CU and GU, and seven NSU) and 35 controls were recruited. In this study, all cases had polymorph counts of >10 p/hpf counts and all controls counts of <5 p/hpf. All cases were symptomatic, except one with NSU who had a polymorph count of 11 p/hpf. Three of the remaining NSU cases had polymorph counts of between 10 and 20 p/hpf and the other three, counts of >20 p/hpf. All cases of CU or GU had polymorph counts of >20 p/hpf except one with CU with a count of 15 p/hpf. Seven controls had symptoms of urethral discomfort, but were negative for chlamydia and gonorrhoea. One case with GU and two controls were receiving antibiotics for unrelated minor infections at presentation. Median age, years since HIV diagnosis, ethnicity, numbers of sexual partners in the previous 3 months, pre-study BPVL, and pre-study CD4 count were similar between cases and controls (table 1).

**BPVLs and SPVLs at study visit 1 and follow up (see table 1)**

HIV-1 RNA was detectable in 16/20 cases compared with 23/35 controls in semen (p = 0.36, Fisher’s exact test) and in 18/20 cases compared with 33/35 controls in blood (p = 0.62).

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**Table 1  Baseline characteristics and viral loads of cases and controls**

<table>
<thead>
<tr>
<th></th>
<th>Urethritis</th>
<th>Controls</th>
<th>P value**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>20 (9 GU, 1 GU/CU, 3 CU, 7 NSU)</td>
<td>35</td>
<td>—</td>
</tr>
<tr>
<td>Median age (years) [range]</td>
<td>33.5 (23.9–48.5)</td>
<td>35 (24.5–41.8)</td>
<td>0.345</td>
</tr>
<tr>
<td>Years since HIV diagnosis [range]</td>
<td>3.6 (0.41–14.18)</td>
<td>1.13 (0.11–16.19)</td>
<td>0.069</td>
</tr>
<tr>
<td>White ethnicity (n)</td>
<td>18</td>
<td>31</td>
<td>1.00</td>
</tr>
<tr>
<td>Median partners in last 3 months [range]</td>
<td>4 (1–21)</td>
<td>3 (0–51)</td>
<td>0.297</td>
</tr>
<tr>
<td>Mean pre-study BPVL [95% CI]</td>
<td>4.26 (3.90 to 4.62)</td>
<td>4.34 (4.06 to 4.63)</td>
<td>0.728</td>
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<tr>
<td>Median pre-study CD4 count [range]</td>
<td>475 (56–1220)</td>
<td>477 (44–1590)</td>
<td>0.937</td>
</tr>
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</table>

**BPVL at study visit 1**

<table>
<thead>
<tr>
<th></th>
<th>Urethritis</th>
<th>Controls</th>
<th>P value**</th>
</tr>
</thead>
<tbody>
<tr>
<td>GU/CU only</td>
<td>4.11 (3.76 to 4.45)</td>
<td>4.21 (4.03 to 4.40)</td>
<td>0.550</td>
</tr>
<tr>
<td>NSU only</td>
<td>4.00 (3.22 to 4.77)</td>
<td>—</td>
<td>0.385</td>
</tr>
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</table>

**BPVL after study visit 1**

<table>
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<tr>
<th></th>
<th>Urethritis</th>
<th>Controls</th>
<th>P value**</th>
</tr>
</thead>
<tbody>
<tr>
<td>GU/CU only</td>
<td>4.19 (3.78 to 4.59) [n=22/15]</td>
<td>4.27 (4.02 to 4.52) [n=23/21]</td>
<td>0.752</td>
</tr>
<tr>
<td>NSU only</td>
<td>3.92 (3.24 to 4.60) [n=9/6]</td>
<td>—</td>
<td>0.306</td>
</tr>
</tbody>
</table>

**SPVL at study visit 1**

<table>
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<tr>
<th></th>
<th>Urethritis</th>
<th>Controls</th>
<th>P value**</th>
</tr>
</thead>
<tbody>
<tr>
<td>GU/CU only</td>
<td>3.99 (3.53 to 4.45)</td>
<td>3.55 (3.27 to 3.83)</td>
<td>0.078</td>
</tr>
<tr>
<td>NSU only</td>
<td>4.27 (3.66 to 4.87)</td>
<td>—</td>
<td>0.014</td>
</tr>
</tbody>
</table>

**SPVL after study visit 1**

<table>
<thead>
<tr>
<th></th>
<th>Urethritis</th>
<th>Controls</th>
<th>P value**</th>
</tr>
</thead>
<tbody>
<tr>
<td>GU/CU only</td>
<td>3.88 (3.54 to 4.23) [n=21/16]</td>
<td>3.59 (3.24 to 3.94) [n=24/23]</td>
<td>0.228</td>
</tr>
<tr>
<td>NSU only</td>
<td>4.12 (3.54 to 4.69) [n=12/10]</td>
<td>—</td>
<td>0.111</td>
</tr>
</tbody>
</table>

GU: gonococcal urethritis; CU: chlamydial urethritis; NSU: non-specific urethritis; BPVL: mean log10 blood plasma viral loads; SPVL: mean log10 semen plasma viral loads.

*Figures quoted are number of measurements/number of patients: **P value from comparison with controls.

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**Figure 1**  Mean log10 change in semen plasma viral load from visit 1 to follow up. GU/CU = gonococcal or chlamydial urethritis; NSU = non-specific urethritis (non-gonococcal and non-chlamydial). Level of detection = 1000 (3 log), and undetectable viral loads considered as 500 (2.7 log). Mean change in patients with GU/CU = −0.25 log (~0.47 to 0.03) p = 0.028.
BPVLs were higher than SPVLs in controls by 0.66 log (p<0.001) and there was a fairly good correlation between BPVL and SPVL (r = 0.46, p = 0.005 Pearson coefficient).

Among cases overall and in patients with GU or CU, BPVLs were similar to SPVLs (p = 0.58 and p = 0.52 respectively, paired t test) and there was again a good correlation between BPVL and SPVL (r = 0.61, p = 0.004 and r = 0.71, p = 0.006, respectively). SPVLs appeared to be lower than BPVLs, in those with NSU, by 0.52 log (p = 0.07).

There was little difference in mean log BPVL between cases and controls. Compared with controls mean log SPVL appeared higher in cases overall, (3.99 log for cases v 3.55 log for controls; p = 0.08), significantly higher in GU/CU cases (4.27 log; p = 0.014) but were similar in NSU cases (3.48 log; p = 0.82) (see table 1). Little difference was detected either in SPVL or BPVL in cases of GU compared with BPVL (4.5 log v 4.07 log; p = 0.266; mean SPVL; 4.58 log v 4.15 log; p = 0.44, respectively).

At follow up 16/24, 6/24, and 1/24 controls and 9/16, 1/16, and 5/16 cases provided semen samples at visit 2 only, visit 3 and at both follow up visits respectively. More specifically among the cases at follow up, semen samples were provided by 6/10, 2/10, and 2/10 with GU/CU and 3/6, 0/6, and 3/6 with NSU at visit 2, visit 3 and at both follow up visits respectively. Little difference was detected in mean BPVL or SPVL between cases or controls at follow up. Among those with GU/CU, mean SPVL remained approximately half a log higher compared with controls but this difference was not significant.

Changes in log viral loads from visit 1 to follow up
(seefig1)
No significant changes in BPVL or SPVL from visit 1 to follow up were detected in cases overall, or controls. However, among those with GU/CU alone, SPVLs, but not BPVLs, decreased following antibiotic treatment by an average 0.25 log (95% CI 0.03 to 0.47; p = 0.028). When compared with the changes observed among controls, this effect appeared to be broadly maintained with a relative reduction in SPVL in GU/CU cases of 0.34 log (−0.01 to 0.68; p = 0.056). Little change in SPVL was observed in those with NSU alone.

DISCUSSION
This study of 55 homosexual men is the largest as yet from the developed world examining effects of sexually transmitted infections on seminal plasma viral load in those not on ART. Compared with controls without STIs, SPVLs were approximately fivefold higher in those with GU or CU whereas SPVLs were not higher in those with NSU. Additionally, SPVLs were similar to BPVLs among those with GU or CU whereas SPVLs were approximately half a log lower than BPVLs among controls and those with NSU. Treatment of GU or CU resulted in reduction in SPVLs by a small but significant amount over controls and those with NSU. Treatment of GU or CU resulted in a relative reduction in SPVL in GU/CU, mean SPVL remained approximately half a log lower compared with controls but this difference was not significant.

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Previously, similar studies in the developed world have been small and few in number. A case report of a 2 log reduction in SPVL following treatment of CU28 did not comment on changes in BPVL and in a study of four patients not on ART, compared with controls without STIs, SPVLs were approximately fivefold higher in those with GU or CU but were not higher in those with NSU. Additionally, SPVLs were similar to BPVLs among those with GU or CU whereas SPVLs were approximately half a log lower than BPVLs among controls and those with NSU. Treatment of GU or CU resulted in reduction in SPVLs by a small but significant amount over 1 to 2 weeks. Thus, these results indicate GU and CU, though not NSU, increase SPVL in those not on ART.

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It is important to note that of those who attended for follow up only 7/24 controls and 7/16 cases attended study visit 3 (at 2 weeks after first presentation). Among cases, similar follow up patterns were observed in those with either
GU/CU or NSU. The African studies suggest that the maximum reduction of SPVL was seen in GU/CU cases may have an underestimate.

We previously demonstrated in a separate study that in a group of men similar to those of this study but receiving fully suppressive ART and with GU or CU, SPVLs remained undetectable. In a small subset of patients in whom virus was not suppressed in blood, high amounts of drug-resistant virus were detected in seminal plasma, though in only one case did treatment of gonorrhea result in reduction of SPVL. Our current study would thus strengthen the notion that antiviral therapy attenuates effects of STIs on genital shedding of HIV-1. As ART becomes more widely used, these attenuating effects, need to be confirmed in developing world settings because of high rates of STIs there and potential for widespread transmission of drug resistant HIV-1.

This study has demonstrated that gonococcal and chlamydial urethritis among homosexual men in the United Kingdom increases shedding of HIV-1 in semen and treatment of urethritis reduces its shedding. Controlling STIs in HIV-1 infected homosexual men may be critical in controlling the spread of HIV-1 among them.

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CONTRIBUTORS

STS, ST, DP, and IVDW conceived the study; STS wrote the study protocol and together with AJC designed the study; STS, ST, and SMD recruited patients for the study; STS performed viral load analysis and with JB; SKa and SKi validated the semen viral load assay. AJC performed statistical analysis; STS wrote the paper, which was principally reviewed by IVDW and AJC. All authors reviewed and contributed to the final draft.

Ethics approval for this study was received by Camden and Islington Community Health Services local research ethics committee.

Authors’ affiliations

STS, A J Copas, and J V D Weller, Centre for Sexual Health and HIV Research, Department of Primary Care and Population Sciences, Royal Free and University College Medical School, University College, London, UK.

S Taylor, S M Drake, Department of Sexual Medicine, Birmingham Heartlands Hospital, Birmingham, UK.

J Bennett, S Kaye, S Kirk, D Pillay, Centre for Virology, Division of Infection and Immunity, Royal Free and University College Medical School, University College, London, UK.

S Jadad, HIV/GUM, Department of Cellular and Molecular Medicine, St George’s Hospital Medical School, London, UK.

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REFERENCES


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