Asymptomatic urethritis and detection of HIV-1 RNA in seminal plasma

A J Winter, S Taylor, J Workman, D White, J D C Ross, A V Swan, D Pillay

Objective: To define risk factors for detection of HIV-1 RNA in semen in men attending the two largest HIV clinics in the West Midlands.

Methods: 94 HIV-1 seropositive men at any stage of infection donated matched semen and blood samples. 36 subjects (38%) were on no antiretroviral treatment, 12 (13%) were on dual therapy, and 46 (49%) were on three or more drugs. Median CD4 count was 291 cells × 10^3/μl. 87 subjects underwent a urethritis screen (Gram stained urethral smear and culture for gonococcus, and LCR for Chlamydia trachomatis on first pass urine). Quantitative cell free HIV-1 RNA was determined by commercial nucleic acid sequence based assay with a lower detection limit of 800 copies/ml for semen and 400 copies/ml for blood. Independent risk factors for seminal HIV RNA detection were defined by logistic regression.

Results: In univariate analysis, subjects not taking antiretrovirals were 11 times more likely to shed HIV RNA (21/36 (58%) vs 6/58 (10%); p<0.0001). Seven subjects (8%) had urethritis (including one C trachomatis infection). Urethritis was significantly associated with detection of seminal HIV RNA (adjusted OR, 80.2; p=0.006), as was blood plasma viral load (adj OR, 19.3 per factor 10 increase; p<0.001) and age (adj OR, 1.16 per 1 year older; p=0.001). Antiviral treatment status, absolute CD4 and CD8 count, clinical stage, treatment centre, ethnicity, and risk factor were not independent predictors. No subject with undetectable blood viral load had detectable seminal HIV RNA.

Conclusion: Asymptomatic urethritis is independently associated with seminal HIV RNA shedding.

(Sex Transm Infect 1999;75:261–263)

Keywords: semen; nucleic acid sequence based amplification; HIV transmission

Introduction

Sexual transmission of HIV by males is determined in part by the amount of infectious HIV in the semen. HIV-1 RNA can be detected in the seminal plasma of the majority of HIV infected men and in general correlates with infectious HIV determined by cell culture2 and with the amount of HIV RNA in blood plasma.2–4 Antiretroviral treatment reduces seminal HIV RNA5–8 and seminal HIV proviral DNA9 in most patients, but a significant minority continue to shed HIV RNA. Symptomatic genital tract inflammation greatly increases seminal viral load,2–4 but the possible role of asymptomatic urethritis in increasing seminal HIV RNA levels has not been explored. We investigated this by measuring the amount of HIV RNA in the semen of HIV seropositive men who were free of genital tract symptoms and agreed to be tested for urethritis.

Methods

STUDY POPULATION, URETHRITIS SCREENING, SEMEN, AND BLOOD SAMPLES

Seropositive men attending one of two teaching hospital HIV treatment centres in Birmingham, were eligible to enter the study if they were free of genital tract symptoms and willing to provide a semen sample. Subjects were screened for non-specific urethritis, gonorrhoea, and Chlamydia trachomatis as follows. A urethral smear for Gram stain was obtained by inserting a plastic loop 2 cm into the distal urethra. “Urethritis” was defined as the presence of more than five pus cells per high power field. Gonococcal isolation was attempted by direct inoculation of urethral material onto selective medium, and the presence of C trachomatis plasmid DNA was investigated by ligase chain reaction (LCR) on first pass urine. Semen samples were provided by masturbation into a dry sterile container. A matched blood sample was obtained within 4 hours. Absolute CD4 and CD8 lymphocyte counts were determined by routine flow cytometry on a sample taken within 1 month of semen sampling. Clinical stage was assigned according to the 1993 Centers for Disease Control and Prevention (CDC) criteria.10 All participants gave fully informed consent and the study was approved by the local research ethics committee.

SAMPLE PROCESSING, QUANTITATION OF HIV RNA

Samples of blood and liquified semen were separated by centrifugation (3000 rpm, 10 minutes) within 6 hours. Aliquots of plasma were stored at −70°C and analysed within 12 months. Quantitative HIV RNA in seminal and blood cell free plasma was determined with a commercial nucleic acid sequence based assay (NASBA; NucliSens HIV-1 QT; Organon Teknika). Samples were processed according to the manufacturer’s instructions, except that an additional microcentrifugation step (13 000 rpm, 20 minutes) was required to pellet all seminal cells, and, to reduce inhibition, only 100 μl of seminal plasma was used. The lower
limit of detection was 800 copies/ml for semen and 400 copies/ml for blood. Values below the lower limit of detection were set to 799 or 399 copies/ml respectively.

**Table 1** Detection of semen and blood plasma HIV RNA according to antiretroviral treatment status

<table>
<thead>
<tr>
<th>Antiretroviral treatment (n=58)</th>
<th>No antiretroviral treatment (n=36)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median blood plasma viral load (log10 copies/ml; median (range))</td>
<td>2.6 (2.6 to 5.0)</td>
</tr>
<tr>
<td>Median semen plasma viral load (log10 copies/ml; median (range))</td>
<td>2.9 (2.9 to 4.4)</td>
</tr>
<tr>
<td>Proportion of subjects with detectable seminal plasma HIV RNA</td>
<td>6/58 (10%)</td>
</tr>
</tbody>
</table>

*Number of drugs: dual therapy (n=12), triple therapy or greater (n=46).
†by Mann–Whitney U test.
‡by Fisher’s exact test.

**Results**

**DEMOGRAPHICS**

Paired semen and blood samples were obtained from 94 men with a mean age of 36.2 (SD 9.6) years. Fifty eight subjects (60%) were taking antiretroviral therapy of whom 12 were on dual nucleoside therapy alone and the remainder were on regimens which included HIV protease inhibitors or non-nucleoside reverse transcriptase inhibitors. Median CD4 count was 291 cells ×10^3/l (range 10–935) and 24 subjects (26%) were asymptomatic. The study groups in each centre were similar in terms of age, CDC stage, risk factor for HIV infection, median blood viral load, and treatment history. Eighty seven men (93%) agreed to the urethritis screen.

**ASYMPTOMATIC URETHRITIS**

Seven of 87 subjects (8%) had asymptomatic urethritis including one with urethral chlamydial infection. Subjects with urethritis were younger (mean age 29.3 years v 36.9 years; 95%CI for difference 2.9–12.4 years; p=0.005 by Student’s t test) but did not differ by CD4 count, clinical stage, or duration of HIV. Among the 53 subjects on antiretroviral treatment tested for urethritis, those with urethritis were over eight times more likely to have either detectable (>799 copies/ml) or undetectable seminal HIV RNA (see table 1). Of the six subjects taking antiretrovirals who had detectable seminal HIV RNA all had detectable blood HIV RNA and two had urethritis. Median seminal HIV RNA in these subjects was 11 000 copies/ml (range 540–8100), compared with a blood viral load of 10^6 copies/ml (range 2700–25 000), compared with a blood viral load of 1060 copies/ml (range 540–8100).

**CORRELATION BETWEEN SEMEN AND BLOOD HIV RNA AND ANTIRETROVIRAL TREATMENT**

Overall, there was a significant correlation between blood and seminal plasma viral load (n=94; Spearman’s ρ 0.601; p<0.001) (fig 1). In no case did we find seminal HIV RNA when blood plasma HIV RNA was undetectable. Antiretroviral therapy significantly reduced the likelihood of detecting seminal HIV RNA (see table 1). Of the six subjects taking antiretrovirals who had detectable seminal HIV RNA all had detectable blood HIV RNA and two had urethritis. Median seminal HIV RNA in these subjects was 11 000 copies/ml (range 2700–25 000), compared with a blood viral load of 1060 copies/ml (range 540–8100).  All but one had initiated or changed therapy within the previous 6 weeks.

**LOGISTIC REGRESSION ANALYSIS**

Eighty seven subjects with complete data were included in the logistic regression analysis which allowed for urethritis, blood plasma viral load, antiviral treatment status, clinical stage, absolute CD4 and CD8 cell counts (stratified into quartiles), treatment centre, age, risk factor for acquiring HIV, and ethnicity. Seminal HIV RNA detection was strongly associated with urethritis (odds ratio 80.2; 95% confidence interval 2.2–2097; p=0.008), blood plasma viral load (odds ratio 19.3 per factor of 10 increase; 95% CI, 2–190).

**Figure 1** Scatter plot showing relation between seminal and blood plasma viral load in 94 male subjects. Cut off for detection of seminal HIV RNA by NASBA assay (marked by dotted lines) was 800 copies/ml for semen and 400 copies/ml for blood. Those with both values at the lower limit (n=42) are shown as a single large circle; one of these had urethritis. Open symbol treated (on dual (n=12) or triple or greater (n=46) antiretro viral therapy). Solid symbol untreated (on no antiretro viral therapy). Large grey symbol indicates asymptomatic urethritis detected on urethral smear or C trachomatis DNA detected in first pass urine by LCR.
Urethritis and seminal HIV RNA

Supported in part by grants from Glaxo-Wellcome, Roche Products (UK), the US National Institutes of Health and the UK Public Health Laboratory Service. We acknowledge the good humoured cooperation of all the participants and colleagues, especially Drs M Shahnamesh, S Drake, and P Cane. Additional assistance was provided by Gerry Gillaran and Maxine Owen.


Contributors: ST and AJW designed the study with assistance from JDRC, DW, and DP; and recruited patients; AJW analysed data and wrote the initial manuscript draft; DW and JDRC oversaw the study in each centre; JW developed techniques for semen analysis with help from DP; ANS undertook the multivariate analysis and modelling. All authors commented on the final manuscript.


