Viral subtype and heterosexual acquisition of HIV infections diagnosed in Scotland

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Objective: As at December 1998, 87% of the estimated 33 million people living with HIV throughout the world resided in Africa and South East Asia.1 In Scotland (and the United Kingdom), a major public health concern has been that non-B subtypes of HIV which predominate in the regions above might enter the country and spread heterosexually among the indigenous population. The authors conducted an investigation to determine if, and to what extent, such transmission had occurred.

Methods: Stored blood samples from people who were diagnosed as HIV positive in central Scotland during 1995–7 and who were reported to have acquired their infection heterosexually, were identified. Sequence data were sought from each sample and, where obtained, viral subtype was assigned. For each case, viral subtype was linked to corresponding epidemiological details on heterosexual risk.

Results: Viral sequence was obtained from specimens for 53 of 59 cases. For 43 of the 53 cases, information on region of sexual contact was known. All 19 cases who had a sexual risk in Africa or Asia had a non-B subtype (A, C, or E) while 20 of 24 cases who did not report sexual contact in these regions had a B subtype (p <0.0001). Of the remaining 10 cases, nine had a subtype B and one a subtype C virus.

Conclusion: There is no evidence that non-B viral strains from developing countries have yet disseminated appreciably among indigenous homosexual men and women within Scotland. Continuing to collect both demographic and molecular data from indigenous heterosexuals who are newly diagnosed with HIV would improve the chances of detecting rapidly any appreciable dissemination of non-B subtypes among this population if it were to occur. Such information would be helpful in informing HIV prevention strategies.

Keywords: heterosexual transmission; HIV-1 subtypes; DNA sequencing; genotyping; Scotland
seropositivity to the Scottish Centre for Infection and Environmental Health (SCIEH).\textsuperscript{11} Reporting is near 100% complete and the information held on each case includes sex, date of birth, soundex code of surname, first part of postal code of residence, date of earliest positive specimen, and risk category; these data are collected through the use of a national HIV request form. Where heterosexual intercourse is the only risk activity indicated, an active system of surveillance is implemented to gather additional information and verify the probable route of transmission. A letter is written to the patient’s attending physician asking specific questions to determine if the cases or any of their sexual partners belonged to a high risk category and if they or their partners had sexual exposure abroad; up to 25% of cases are recategorised as IDUs or homosexual/bisexual males.

In Scotland, 154 people who were categorised as probably having acquired their HIV infection through heterosexual intercourse had an earliest positive specimen between 1 January 1995 and 31 December 1997. Of these, 55 were diagnosed in Lothian Health Board (includes Edinburgh), 41 in Greater Glasgow, 22 in Tayside (includes Dundee), and 36 in the rest of Scotland.

OBJECTIVES

(1) To identify a single, stored blood sample from each of a selection of patients, diagnosed as HIV positive during 1995–7, who had been recorded on the SCIEH database as having probably acquired their infection through heterosexual intercourse.

(2) To ascertain the HIV subtype in each sample.

(3) For each patient, to link the details of viral subtype with corresponding epidemiological information on heterosexual risk.

SAMPLE SELECTION

Eligible individuals were restricted to those who (a) met the criterion as indicated in objective (1) above, and (b) were undergoing clinical follow up in either Edinburgh or Glasgow because over 60% of heterosexual cases in Scotland were from these two areas; furthermore, immunology laboratories in both cities were able to provide blood specimens which were residual following routine CD4 count analysis.

In Edinburgh, an aliquot of plasma from all CD4 count specimens from each of a selection of patients, diagnosed as HIV positive during 1995–7, that had been recorded on the SCIEH database as having probably acquired their infection through heterosexual intercourse.

(a) To identify a single, stored blood sample from each of a selection of patients, diagnosed as HIV positive during 1995–7, who had been recorded on the SCIEH database as having probably acquired their infection through heterosexual intercourse. A letter is written to the patient’s attending physician asking specific questions to determine if the cases or any of their sexual partners belonged to a high risk category and if they or their partners had sexual exposure abroad; up to 25% of cases are recategorised as IDUs or homosexual/bisexual males.

(b) To ascertain the HIV subtype in each sample.

(c) For each patient, to link the details of viral subtype with corresponding epidemiological information on heterosexual risk.

LABORATORY METHODS

Viral RNA and proviral DNA were extracted from plasma and whole blood samples respectively. The RNA was subjected to reverse transcription to produce cDNA. Proviral DNA and cDNA were then amplified by nested polymerase chain reaction (PCR) in the p17 region of the gag gene (and in some cases additionally in the v3/v4 region of the env gene) and sequenced directly on an ABI 373A automated sequencer as described elsewhere.\textsuperscript{12} The resulting sequences were aligned using the GDE package and then neighbour-joining phylogenetic trees were constructed, using reference subtype strains from international databases as comparisons, so that a viral subtype could be assigned to each case.\textsuperscript{13}

RESULTS

SAMPLES

From the 55 identified patients from Lothian Health Board, stored plasma specimens were located for 48 and sequence material from the gag region was obtained in 42 of these. Of the 41 identified patients from Greater Glasgow, 11 whole blood samples were obtained and all yielded gag sequence data.

SUBTYPE ASSIGNATION

The 53 gag sequences were compared by phylogenetic analysis with homologous sequences from international databases and assigned to subtype (table 1). Twenty three (43%) of the specimens examined were HIV-1 subtypes other than B; 17 were subtype C and six subtype A. No other gag subtypes were identified. However, since subtype E is a recombinant virus with a subtype A gag gene, further sequencing of gag subtype A viruses in the v3/v4 region of the env gene (data not shown) was performed to differentiate between true A and E subtypes. This further analysis revealed that one was indeed subtype E.\textsuperscript{13}

ASSOCIATION OF SUBTYPE WITH CONTACT DATA

The 53 gag sequences were compared by phylogenetic analysis with homologous sequences from international databases and assigned to subtype (table 1). Twenty three (43%) of the specimens examined were HIV-1 subtypes other than B; 17 were subtype C and six subtype A. No other gag subtypes were identified. However, since subtype E is a recombinant virus with a subtype A gag gene, further sequencing of gag subtype A viruses in the v3/v4 region of the env gene (data not shown) was performed to differentiate between true A and E subtypes. This further analysis revealed that one was indeed subtype E.\textsuperscript{13}
heterosexual risk for the 53 cases of whom 18 (seven from Greater Glasgow and 11 from Lothian) were female. For eight cases, all of whom had a B subtype, no information was available. Two cases, one who had a B and the other a C subtype, reported sexual risk in many, unspecified, countries. Of the nine cases who reported sexual contact with an IDU (in the United Kingdom or elsewhere in Europe), eight had a B and one an A subtype; this latter case indicated sexual contact with an IDU in Austria. Of the remaining 34 cases, 10 (eight with B and two with C subtype) reported sexual contact only in the United Kingdom, four (three with B and one with C subtype) contact outside the United Kingdom but only in Europe, 17 (13 with C and four with A subtype) contact in sub-Saharan Africa, two (one with C and one with E subtype) contact in Asia, and one (B subtype) contact in the United States.

Both cases who had subtype C viruses which were reportedly acquired in the United Kingdom had an African connection; one was a Zambian national who claimed not to have been sexually active before his arrival in Scotland and the other had sexual contact with an African partner in the UK. The only non-B subtype for which information was available, heterosexual risk was confined to the United Kingdom, United States, or Europe. Thus, in this series of cases, molecular typing to distinguish B from non-B subtypes was shown to be highly predictive of the region (developed or developing world) where HIV was probably acquired. This is an important finding because in instances where risk and demographic information cannot be obtained from the patient, molecular data can assist in tracing the likely origins of infection. No information was available from eight of the 53 cases and since they were all subtype B, the chances of them having been infected outside the United Kingdom, United States, or Europe are low.

If, as this study suggests, non-B subtypes have not disseminated into Scotland’s heterosexual population it is not because people infected with non-B subtypes have only come to Scotland recently. Indeed, HIV infected heterosexuals in Scotland who acquired their infection in Africa have been a potential source of HIV since the mid to late 1980s; between 1985 and 1990, 37 cases were diagnosed in central Scotland, the area covered by this study. It is possible that some dissemination has occurred but, as yet, remains undetected since HIV infected heterosexuals tend to be tested only after their HIV disease.19 A possible explanation for the lack of dissemination is that there has been insufficient heterosexual mixing between those infected in either Africa or Asia and indigenous people for cultural reasons and/or the transient nature of the former group’s stay in Scotland.

To date, those who have been most at risk of acquiring HIV heterosexually in Scotland have been the sexual partners of IDUs. During the late 1980s and the 1990s, however, needle/syringe exchange programmes reduced the spread of HIV among IDUs and thus the risk of HIV transmission from them to their partners.20–22 Few cases of heterosexual transmission in Scotland have occurred among
females who have had sex with an infected bisexual man. It would therefore seem reasonable to predict a decline in the number of HIV transmissions among Scotland’s indigenous heterosexual population. However, the global prevalence of HIV is continuing to increase and together with the ever increasing frequency of foreign travel, it is possible that these factors might alter the dynamics of HIV transmission among heterosexual men and women in Scotland (and the rest of the United Kingdom) in the future.

Continuing to collect both demographic and molecular data from indigenous heterosexuals who are newly diagnosed with HIV would improve our chances of detecting rapidly any appreciable dissemination of non-B subtypes among this population if it were to occur. Such information would be helpful in informing HIV prevention strategies.

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Contributors: DV designed the project, performed the viral subtyping, and wrote the paper; DG designed the project and wrote the paper; CMcS and JW provided the samples for testing and wrote the paper; FR and GC performed the epidemiological investigations to ascertain risk factor information on cases of heterosexual transmission, and wrote the paper.

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