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Prevalence of HIV-1 Subtypes in Infected Concordant and Discordant Couples in Nigeria.

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ABSTRACT: The peptide binding immunoassay (PELISA) was used as previously described to determine HIV-1 subtypes among 60 married HIV-1 sero-positive individuals and their spouses identified at the University College Hospital, Ibadan in order to investigate the rate of heterosexual partners by different HIV-1 subtypes in Nigeria.

Out of the 60 couples whose blood samples were analysed, 33 (55%) were both positive HIV while only one spouse of the couples was sero-positive among the other 27. Using the McNemer test for discordant paired samples, an insignificant (P=0.0636) difference was obtained between male to female and female to male transmission. Subtypes A, B, C and E were detected among these individuals. Most (82.3%) of the subtype C and all of the subtype E (12) were detected among the transmitters (couples with concordant sero-positive status).

This study indicates that HIV-1 subtypes C and E may be more efficiently transmitted heterosexually than the other subtypes.

Key Words: HIV-1 subtypes; HIV-1 prevalence; Concordant couples; Discordant couples; Nigeria.

Introduction

The number of sexually acquired HIV-1 infection continues to increase rapidly in both male and female population groups worldwide. Infection with the virus has been estimated to reach over 35 million by the year 2000 (1). Although HIV-1 has been isolated from cervical and vaginal secretions of infected women as well as the semen of infected men (2), there appears to be a higher probability of male to female, than female to male transmission of the virus (3).

The global male to female ratio of infection with HIV-1 is about 5:3 (4). However, the epidemiology of the disease is different in sub-Saharan Africa where infection occurs almost equally between both sexes with a male to female ratio of approximately 1:1 (1, 4).

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It has been observed that the heterosexual route of transmission of HIV-1 is higher than that of HIV-2. Kanki *et al* (5) reported incidence of 1% for HIV-2 and 3% for HIV-1 over an 8 years period. It has also been suggested that HIV-1 subtype E circulating in Thailand may be more efficiently transmitted heterosexually than HIV-1 subtype B introduced into the same population from Europe and USA (6).

The significance of HIV subtypes in the rapid spread of the virus in sub-Saharan Africa where heterosexual mode of transmission predominates has not been established. This is important, considering the fact that most, if not all the various HIV-1 and HIV-2 subtypes identified to date have been found in this region. In Nigeria, previous studies have shown a male to female ratio of I:1 (7, 8, 9). However, the rate of heterosexual transmission of the various subtypesof the virus circulating in the country has not been determined.

In this study we determined the HIV-1 subtypes of 60 infected concordant and discordant couples in Ibadan, Nigeria to evaluate their differential rate of heterosexual transmission.

Materials and Methods

Subjects and Sample collection

Sixty married HIV-1 seropositive individuals and their spouses identified as the University College Hospital, Ibadan, Nigeria were analyzed for this study. The subjects were recruited among those referred for HIV screening from the medical out-patient clinic and the medical wards of the University College Hospital, Ibadan from 1993 to 1997. HIV seropositive individuals were informed about the study and requested to come with their spouses for HIV testing. Blood was collected only from spouse of initially identified seropositive persons who gave their consent to participate in the study.

Ten mls of blood was collected from each of the seropositive individuals as well as from their spouses. The plasma/serum samples was separated within 24 hours of sample collection and stored at –20°C until when tested. Demographic information was collected at the time of blood collection.

Analysis of Samples:

Samples from the spouse of each seropositive individual was tested using commercial HIV-1/2 kits (Murex and Double check EIA). The HIV1 or 2 status of repeatedly reactive samples was confirmed by Western immunoblotting (Biorad, Novapath).

Plasma/serum samples from these 60 individuals and their seropositive spouses were tested for the presence of specific HIV-1 subtype status using Peptide Binding Immunoassay (PELISA) as previously described (10, 11) as as modified for samples from regions with multiple HIV-1 subtypes (12).

Results

Plasma or serum samples from sixty heterosexual couples with the husband or wife or both HIV sero-positive were analysed for this study. Thirty-three of the couples were both positive giving a couple infection rate of 55% for both husband and wife. Twenty four (88%) of the 27 positive spouses with negative partners were symptomatic at the time of presentation indicating advanced stage of infection. Thus the couples would have had sexual interaction for some time after either one of the spouses or both have been infected. All the couples had been married for at least 21/2 years.

Table 1 shows the possible rate of male to female and female to male transmission. Out of the 48 HIV positive women, 15 (31%) of their husbands were negative. Similarly, out of the 45 positive men, 12 (27%) of their wives were negative. Using the Mc-Nemer test for discordant paired samples, an insignificant difference was obtained between the male to female and female to male transmission (P=0.0636).

Thirty-three (73.5%) wives of the 45 symptomatic husbands were asymptomatic but sero-positive at the time of presentation. On the other hand, only 3 (20.0%) husbands of 15 symptomatic wives were asymptomatic and the other husbands were sero-negative.

Specific antibodies to HIV-1 subtypes A, B, C and E were detected in the blood samples of these infectedcouples. None of the samples reacted with peptide of subtypesD and O includedin this study. Out of the 33 couples who were both HIV sero-positive, 27(81.8%) couples had homologous HIV-1 subtypes while in the other 6 couples, blood samples of the spouses were positive to two different subtypes (subtype C and E in all 6 cases). Infection with subtype A was found in 6 couples (22.2%) and subtype E in 3 couples (11.1%).

Among the 27 couples with only one of the spouses being HIV sero-positive, 12 (44.4%), 9 (33.3%) and 3(16.2%) were infected with subtypes A, C and B peptides respectively. Blood samples from the other 3 individuals did not react with any of the peptides included as antigen in the protocol used for this work.

In the absence of information on extra-marital sexual partners of our subjects, the couples with concordant HIV sero-positive status are regarded as transmitters, while those with discordant HIV status are non-transmitter. Table 3 shows the distribution of the HIV-1 subtypes among the transmitters and non-transmitters. There was no difference in the prevalence of subtype A among the transmitters and the non-transmitters. On the other hand, all 3 subtype B detected were among the non-transmitters, while all the subtype E (12) detected were among the transmitters. Subtype C was detected among the two groups, however with a higher percentage (82.3%) among the transmitters ($P<0.05$).

Table 1: Transmission Rate of HIV-1 to Spouse by some HIV Seropositive Individual in Nigeria.

HIV STATUS	SPOUSES	
	Husband (%)	Wife (%)
Positive	45 (75)	48 (80)
Negative	15 (25)	12 (20)
Total	60	60

McNemer Test, $P = 0.0636$

Table 2: Correlation of Stage of Infectionwith HIV-1 Status of Spouse Among Couples in Nigeria.

Symptomatic	HIV Status of Spouse		
Spouse	No. Tested	Positive asymptomatic	Negative
H	45	33 (79.0%)	12 (21.0)
W	15	3 (20.0%)	12 (80.0)
Total	60	36	24

H = Husband; W = Wife.

Table 3: Distribution of Different HIV-1 Subtype Among Couples with concordant and discordant HIV-1 sero-positivity in Nigeria.

Status	Sub types No. (%)							
	A	**B**	**C**	**D**	**E**	**O**	**NR**	**Total**
Concordant	12(50)	0(0)	42(82.3)	0(0)	12(100)	0(0)	0(0)	66
Discordant	12(50)	3(100)	9(17.7)	0(0)	0(0)	0(0)	3(100)	27
Total	24(25.8)	3(3.2)	51(55)	0	12(12.8)	0	3(3.2)	93

Discussion

A good understanding of the virus, host and environmental factors influencing the heterosexual transmission of HIV-1 is necessary for development of intervention strategies to prevent its sexual transmission. In this study, the role of vital subtypes in heterosexual transmission of HIV-1 was investigated. The couples with concordant HIV sero-positive status among those included in this study were to have transmitted the virus while those with discordant HIV sero-positivity were considered non-transmitters. Subtypes A, B, C and E were detected among the couples. Infection of six of the couples by discordant subtypes may be due to independent infection of each of the spouses. A similar observation had been previously reported by Barin *et al* (13).

Out of the 27 couples among whom only one of the spouses was sero-positive, 12, 3, 9 of them were infected with subtypes A, B and C respectively. The other individual who did not react to any of the peptides may have been infected by any of the other subtype not included in this study, especially among the subtypes previously reported from Nigeria (14). In addition, the possibility of low level of V3 antibody below detectable level cannot be ruled out.

Comparison of the prevalence of the subtypes found among the 'transmitters' and non-transmitters' showed no significant difference in the prevalence of subtype A among the two groups. Subtype E was detected only among the transmitters while 82.3% of the transmitters were infected with subtype C. This result indicates that subtypes E and C may be more efficiently transmitted heterosexually than the other subtypes. Subbarao and Schochetman (15) had earlier suggested that the rapid spread of HIV-1 subtype C during the past few years may imply a selective advantage in its heterosexual transmission. The results of this study thus support this earlier observation (16). In addition, subtype C had previously been reported among 81% of heterosexually infected people in Cape Town, South Africa (16). Similarly, subtype E has been shown to be responsible for the widespread of HIV infection in the heterosexual population in Thailand (6). The ability of subtypes C and E to replicate rapidly especially in Langerhan cells (17) which have been suspected to be the cells through which HIV vaginal infection occurs (18) may have accounted for the propensity of these subtypes for heterosexual spread.

A couple infection rate of 55% for both husband and wife was found in this study. Although there was no significant difference in the number of sero-positive husbands and wives included in this study, if it is assumed that the symptomatic partner became infected first, there is a significant difference ($P<0.05$ in the number of symptomatic husbands (73.6%) and wives (20.0%) with sero-positive but asymptomatic spouse. This result suggests a higher probability of male to female than female to male transmission of the predominant HIV-1 subtypes circulating in Nigeria.

Factors such as stage of disease and immunosuppression have been shown to promote sexual transmission of HIV-1 (19, 20). It has also been found that individuals who are homozygous for a deletion in the CCRS/CXR4 gene are less frequently infected with HIV, whereas individuals who are heterozygous for the same mutation become infected. However, the later group can be protected against rapid progression to disease compared with HIV infected individuals homozygous for the normal CCRS/CXCR4 gene (21, 22). This may offer a reasonable explanation for the 9 individuals who were negative even though their spouses

were HIV sero-positive. However, the presence and prevalence of this genetic defect in Nigeria has not been studied.

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