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Epidemiological evidence of recent introduction of HIV-1-Subtypes B and O into Nigeria

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ABSTRACT: Peptide based Enzyme Immuno-assay (PELISA) was used to determine HIV-1subtypes circulating in Nigeria. The synthetic peptide used as the capture antigen were designed from the consensus sequence of the third hypervariable region (V3loop) of 6 HIV-1 subtypes namely A, B, C, D, E and O. A total of 925 ELISA reactive and Western blot confirmed HIV-1 positive plasma or serum samples collected over a 5 year period (1993-1997) from the three broad geographical (south-western, south-eastern and northern) regions of the country were analysed for the study.

Specific antibodies to the six HIV-1 subtypes were identified among the seropositive samples tested. There was an overall increase in the incidence of all the subtypes over the 5 years covered by this study. However, the prevalence of subtypes B and O was relatively low being 2.5% and 2.4% respectively. In addition, subtype B was not detected among HIV-1 positive samples collected before 1995. Furthermore, subtypes B and O were not detected in the samples from the south-eastern and northern regions respectively. Also, the incidence of subtypes B and O decreased with age while there was an increase in the incidence of subtypes E and D with age. On the other hand, the incidence of subtypes A and C did not vary significantly with age.

Absence of antibodies to HIV-1 subtype B among blood samples from confirmed seropositive subjects that were collected during 1993 and 1994 together with low prevalence of subtypes B and O found in this study indicate their recent introduction. In addition, absence of antibodies to both subtypes in the south-eastern and northern regions respectively and decrease in their incidence with age are convincing indications of recent introduction of both subtypes into Nigeria. There is therefore a need for continuous monitoring of HIV infection to identify appearance/introduction of new variants of the virus into any particular geographical region.

Key Words: HIV-1, Subtypes B and O, Nigeria.

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Introduction

HIV-1 isolates have been classified into ten distinct subtypes (A-J (group M) and recently group O and the none-M none-O recently called N) based on the sequence of the *env* gene (1,2,3). There is also genetic evidence of at least five major subtypes of HIV-2 (A-E) (Sharp *et al.*, 1994). The various HIV subtypes have been reported in different geographical regions of the world. HIV-1 subtype B is the predominant subtype in W. Europe and N. American while subtypes E and C are the predominant subtypes in China and India respectively though the presence of other subtypes have been documented with minimal spread (5,6,7). In Africa multiple HIV-1 subtypes have been reported with subtypes A, D or C being the most prevalent subtypes in most of the countries (2,3).

With the high movement of people from one geographical region to another, introduction of new subtypes into other geographical regions occurs very frequently. The initial HIV-1 subtype B epidemic in Thailand was introduced from W. Europe/N. America (8,9). Similarly it has been reported that the subtype C circulating in India was introduced from South Africa (9). In this report we provided epidemiological evidence of recent introduction of HIV-1 subtypes B and O into Nigeria.

Study sites: The samples used for this study included 925 sera or plasma collected from individuals with confirmed HIV-1 infections in eight states in both the northern and southern regions of Nigeria. Samples were collected from teaching Hospitals (University College Hospital, Ibadan – UCH; University of Nigeria teaching Hospital, Enugu – UNTH; Lagos University Teaching Hospital, Lagos – LUTH; University of Port Harcourt teaching Hospital, Port-Harcourt – UPTH; University of Maiduguri Teaching Hospital, Maiduguri – UMTH), state government hospitals (Osun State Hospital, Osun – OSH, Federal Medical Centre, Imo) and state HIV screening centres and blood banks.

Samples: Plasma or serum samples were collected from HIV-1 seropositive individuals in Nigeria. Samples were collected from different HIV screening centers and teaching hospital over a five year period (1993-1997). A total of 925 samples were collected and analysed for the study.

HIV-1 Serotyping Procedure

The subtype status of each HIV-1 positive serum or plasma samples analysed for this study as determined using the serotyping technique developed at the Chemotherapeutishes Forschung Institut, Frankfurt, Germany (10) as modified for samples from areas with multiple HIV-1 subtype (Olaleye, 1997, Personal Communication). The assay is based on relative binding of antibodies to different HIV-1 subtypes to corresponding peptide antigens designed from amino-acid sequences of the specific HIV-1 subtype (10, 11, 12).

A panel of seven peptides corresponding to sequence in the V3 region of HIV-1 (A to E and O) were synthesized and used as the capture antigen in the indirect ELISA. Two peptides were derived from subtype E (short and linear, E_L and long and full-length cycle, E-cycle). The subtype B peptides used are full length and cycle while all the others are short and linear.

The specific amino acid sequences of the V3 synthetic peptides used are shown in Table I. The peptides were synthesized and purified by high performance liquid chromatography in U.K. (Cheingsong-Popov *et al.*, 1994; Barin *et al.*, 1996) and obtained ready for use (through Ursula Dietrich). The selection of the HIV-1 subtypes that were included was based on the prevalent subtypes in Africa (3) and known HIV-1 genetic subtypes based on previous sequencing of some HIV-1 isolates from Nigeria (4).

The test is based on the method of indirect ELISA for detection of HIV-1 antibodies. The criteria used to determine the reactivity of each sample i.e. specific serotype are shown in Table II. Samples that reacted with more than one subtype antigen were further analysed by the antigen limiting ELISA (11) to determine the exact status of such samples.

Results

The samples used for this study were collected over a period of five years (1993-1997). With the exception of HIV subtype B that was not detected among samples collected in 1993 and 1994, the other

subtypes were detected throughout the five years of the study. Fig. 1 shows the occurrence of various HIV-1 subtypes by year of sample collection. Subtype C was the most prevalent during the five years covered by the study (P= 0.000)). This was closely followed by subtype A. Subtypes O and B were the least prevalent during the five years covered by study. There was an increase in the prevalence of subtype D from 1993 (11.4%) to the 1995 (26.1%) and then a decline in 1996 (20.5%) and then increase in 1997 (27.3%). Similarly the incidence of subtype E increased from 1993 through 1995 and then decreased from 1995 through 1997. All the other subtypes (A, B, C, E and O) had a slight decline in prevalence from 1996 to 1997 (Fig. 1). Specifically subtypes A, B, C and O were most prevalent in 1996 (39.3%; 43.5%; 35.0% and 40.9% respectively) while subtype E (33.8%) and D (27.3%) were most prevalent in 1995 and 1997 respectively (Fig. 1.

HIV-1 group O was not detected among infected individuals 50 years or older while subtypes D and E were not detected among persons in the 0-9 years age group. The prevalence of subtypes A and C among the different age groups was similar. On the other hand, the prevalence of subtypes B and O decreased with age of the individuals (Fig. 2).

Subtype C was found to be the most prevalent subtype among all the age groups (Fig. 2). This was followed by subtype A while Subtype O was the least prevalent.

Table 1: Amino Acid Sequence of V3 peptides.

Subtypes	Amino Acid Sequence
A	KSVRIGPAFYAT
B	CTRPNNNTRKSIHIGPGRAFYTTGEIIG DIRQAHC
C	KSIRIGPQTFYAT
D	CTRPYNNTRQTHIGPGQFYRTGDI
E	E_L : DTSITIGPGQVFYRRT
	Encycl: CTRPSNNTRTSITIGPGQVFYRTGDIIGDIRKAYC
Group O	CERPGIQTVQEIRIGPMAWYSMGLGRSSGDSRAAYC

Table II: Reaction Pattern of Different HIV-1 Subtypes in the V3PEIA.

Subtypes	V3- Peptides						
	P1(1B)	P2(A)	1N(C)	D	E_L	E_{CY}	O
HIV-1B	+++	+	+	+	+	±	+
HIV-1A	+++	+++	+	+	+	±	+
HIV-1C	+ + +	+	+ + +	+	(+)	±	+
HIV-1D	+++	+	+	+++	+	±	+
HIV-1E	+	+	(+)		+++	+++	
HIV-1O	+	+	+	+	+	+	+++

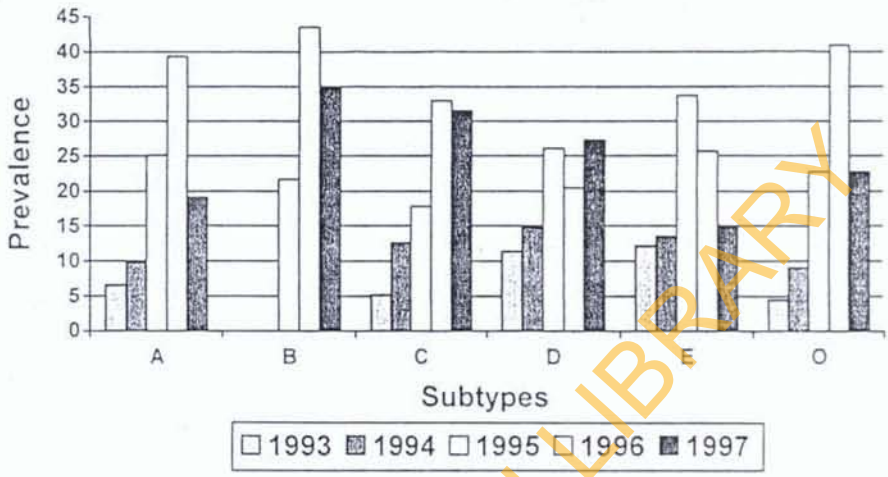

Fig. 1: Occurrence of HIV-1 subtypes by year of sample collection among HIV-1 seropositive individuals in Nigeria.

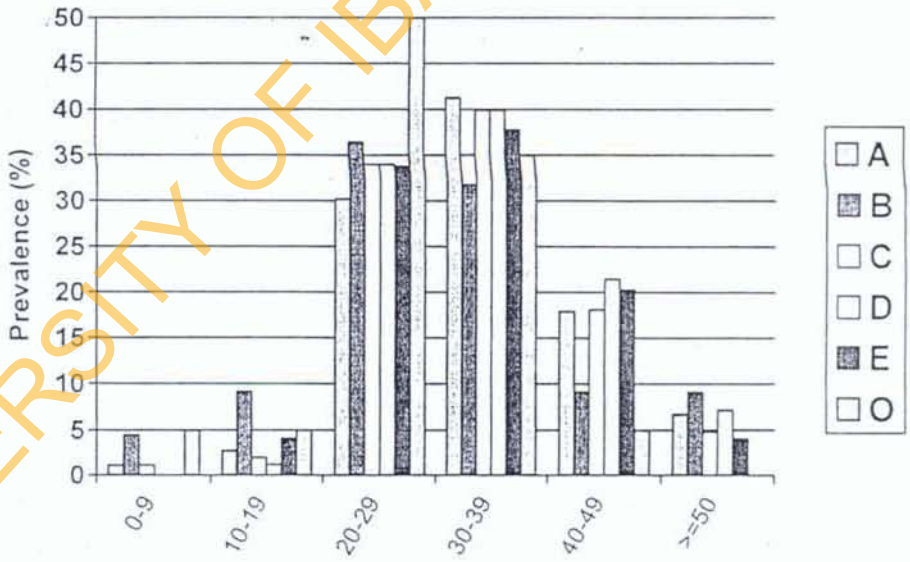

Fig. 2: Distribution of some HIV-1 subtypes within different age groups in Nigeria (n = 81).

Discussion

Using the consensus V3 peptide for the six HIV-1 subtypes (A,B,C,D,E and O) as antigen, PELISA was employed to determine the relative proportion of different subtypes of HIV-1 among sera or plasma collected from 925 infected persons from Nigeria.

The prevalence of subtypes B (2.5%) and O (2.4%) found in this study is lower than previously reported in some other African countries (2, 14). Using the Peptide Enzyme Immunoassay as in this study, Ariyoshi *et al*., (1996) reported a prevalence of 8% for HIV-1 subtype B in the Gambia. Similarly, prevalence rates of 6% and 10% have been reported for subtypes B and O respectively in Cameroon (2,3,15), a neighbouring country to the east of Nigeria. The relatively low incidence of subtypes B and O is suggestive of recent introduction of both subtypes into the country.

With the exception of subtype B that was not detected among samples tested before 1995, all the other subtypes included in the antigen panel for this study were detected throughout the five years of blood sample collection for this study. In addition, there was an overall increase in the prevalence of all the subtypes over the five years (1993-1997). This observation suggests an introduction of multiple HIV-1 subtypes at different times rather than a shift from one subtype to another. Comparatively, the results of this study also indicate that subtype C and A may have been in circulation for a longer period in Nigeria than subtypes E and D. This situation is similar to that reported from Kenya where subtype A was shown to be in circulation earlier than subtype D (1). The lack of detection of antibodies to subtype B among samples collected in 1993 and 1994 for this study also indicate that this subtype may have been recently introduced into the country.

Another interesting finding of this study is the steady increase in the prevalence of subtype E from 1995 after which it declined gradually from 1995 through 1996 to 1997. This is in contrast to the situation in Thailand where there was a steady and rapid increase in the rate of spread of subtype E in the heterosexual populace which led to the suggestion that it may be better transmitted sexually than subtype B (16).

Although, the reason for the decline in prevalence of subtype E in Nigeria is not well understood, it is possible that a less virulent strain of the virus is present in Nigeria, especially in relation to heterosexual transmission. Frequent mutation is a common phenomenon in retroviral replication, a mechanism that enables it to escape the immune system of the host (3). Mutations as well as recombination have been suggested to be responsible for the heterogeneous nature of human immunodeficiency virus (17, 18). Genetic characterization as well as transmission studies of subtype E strains circulating in Nigeria will provide better information on the nature of the variants of the virus circulating in the country. In Thailand, the early strains of subtype B in circulation was closely related to the North American subtype B strain. However, another genetic variant ("Thai B" or "B") later emerged.

HIV-1 subtypes A, B and C were detected among all the age groups. Subtype B has been shown to be transmitted more efficiently parentally than sexually (16, 19). In this study, subtype B was more prevalent among infected persons in the sexually active age group (20-39 years) thus suggesting that it may also be efficiently transmitted sexually. However, the variants of subtype B circulating in Nigeria may be different from that of W. Europe and N. America.

Subtype O was found to be the least prevalent in most of the age groups in this study. This finding together with the observation that none of the individuals 50 years or older was positive for subtype O, further support the suggestion that it may be recently introduced into the country. The group O strains have so far been shown to have very limited dissemination to other parts of the world (3). A survey conducted in France from 1990 to 1994 resulted in identification of only 9 patients infected with HIV-1 group O and, eight of these individuals were of Cameroonian origin (20).

The fact that subtypes D and E were not detected among persons 0-9 years may be due to limited number of samples from individuals in this age group. On the other hand, it may indicate preference of these subtypes for sexual mode of transmission since individuals of this age group are likely to be infected through other routes than sexual.

These findings have important implications for diagnostics, therapy and vaccine development.

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