Bioscience Research Communications
Vol. 13, No. 4, August 31, 2001
Printed in Nigeria

0795-8072/2001 $6.00 + 0.00

BRC 2001063/13411

Multiple Presence and Heterogeneous Distribution of HIV-1 Subtypes in Nigeria

Georgina N. Odaibo[1], David O. Olaleye*[1,2], Horst Ruppach[2], Goddy O Okafor[3] and Urusula Dietrich[2].

[1]Department of Virology, College of Medicine, University of Ibadan, Nigeria.
[2]Department of Molecular Virology, Institute fur Chemotherapeutishe-forshung, Georg-Speyer Haus, Frankfurt, Germany.
[3]University of Nigeria Teaching Hospital, Enugu, Nigeria.

(Received April 19, 2001)

ABSTRACT: Human immunodeficiency viruys type I (HIV) subtypes circulating in Nigeria was determined by using the Peptide based Enzyme Immuno-Assay (PELISA) to analyze sera or plasma samples collected from 925 individuals with ELISA and Western blot confirmed HIV-I infection in the three broad geographical regions (southwestern, southeastern and northern) of Nigeria. The synthetic peptides used as the capture antigens in the PELISA were designed from the consensus sequence of the third hypervariable region.
(V3 loop) of HIV-1 subtypes A, B, C, D, E and O of HIV-1. The assay was initially validated using plasma samples from individuals infected with various genetically identified HIV-1 subtypes in Europe and Africa. Any serum or plasma sample that reacted with more than one peptide was re-tested using the same antigen panel in a limiting ELISA technique.
The result co-circulation of multiple HIV-1 subtypes including A, B, C, D, E, and O in Nigeria. Varying prevalence of specific antibodies to the six HIV-1 subtypes included in the PELISA panel were detected among infected individuals in Nigeria. Subtype C was the most prevalent, 48.3% (447) followed by A = 19.8% (183), D = 9.5% (88), E = 8.4% (74), B = 2.5% (23) and group O, 2.4% (20). Thirty-six (3.9%) of the samples tested were dually reactive while 52 (5.6%) did not react with any of the six HIV-1 subtype peptides included in the assay. A heterogeneous distribution of at least 5 HIV-1 subtypes was observed in all the regions with subtype C being the most prevalent in all the locations, states and regions, followed by subtypes A, D and E. None of the samples from the northern and southeastern regions reacted with HIV-1 subtype O and B peptides respectively.
As far as it can be ascertained, this is the first report of detection of HIV-1 subtypes B, D and E in Nigeria. Furthermore, the result of this work indicates widespread circulation of multiple HIV-1 subtypes in Nigeria. Therefore a polyvalent vaccine will be the best option for effective prophylactic immunization against HIV-1 infection in Nigeria.

Key words: HIV-1 Subtypes, heterogeneous, Nigeria.

*To whom correspondence should be addressed at the:
Department of Virology, College of Medicine, University of Ibadan, Ibadan, Nigeria.

Introduction

The incidence of AIDS continues to increase especially in sub-saharan Africa (1-4). Globally, it is estimated that 36.1 million people were infected with HIV at the end of December 2000 (4). About 90% of the people infected are in the developing countries (4). Some research groups, in particular the Harvard AIDS Institute, described the WHO figures as underestimated and that as many as 100 million infections may have occurred by the end of the 20th century.

HIV-1 shows heterogeneity among its isolates (5,6). Different isolates of the virus have been shown to differ in their biological properties (7) as well as immunological and molecular properties. The heterogeneity of HIV-1 strains has been studied by molecular characterization of the genomic sequences either by sequencing fragments amplified by polymerase chain reaction or by heteroduplex mobility assay (8,9). Although, these methods allow for direct subtype classification, they are time consuming, expensive and require specialized equipment and highly trained personnel (10). In addition, the method cannot be easily used for large number of samples and in places where facilities for such work are not available as in most developing countries.

A simple serological subtyping assay to facilitate determination of the distribution of HIV-1 genotypes circulating in a given population was developed recently (10, 11, 12). The assay compared well with genotypic analysis such as heteroduplex mobility assay (10). It was shown that the serological approach accurately detected the dominant subtype reactivity in more than 90-97% of the cases (10, 11). This technique has been used to determine the circulating HIV-1 subtypes in The Gambia (13), Israel (12) and Tanzania (14). These are countries with multiple HIV-1 subtypes as the situation in Nigeria (10, 11, 14).

HIV has spread extensively into both the rural and urban areas of Nigeria (15, 16, 17). The first HIV-1 strain partially characterized from the country was shown to belong to subtype A (18). Since then identification of isolates belonging to subtypes G and O from Nigeria have been reported (19, 20). In this paper, we report circulation of additional HIV-1 subtypes in Nigeria as determined by the PELISA technique.

Materials and Methods

Study Sites and Blood Samples Collection

The samples used for this study included 925 sera or plasma samples collected from individuals with confirmed HIV-1 infections in eight states in both the northern and southern regions of Nigeria (Fig. 1). The samples were collected from Teaching Hospitals (University College Hospital, Ibadan – UCH, University of Nigeria Teaching Hospital, Enugu – UNTH, Lagos University Teachning Hospital, Lagos – LUTH, University of Port-Harcourt Teaching Hospital, UPTH, University of Maiduguri Teaching Hospital, Maiduguri – UMTH, Federal Medical Centre, Owerri, Imo State and Oyo and Osun State HIV screening centers/blood banks. The samples from the Teaching Hospitals included HIV positive sera in their storage during the period covered by this study (1993-1997) while those from other screening centers were based on available samples during monthly visit to sites for collection of samples.

HIV-1 Serotyping Procedure

The specific subtype of HIV-1 that infected the donors of positive serum or plasma sample analyzed for this study were determined using the serotyping technique developed at the Chemotherapeutische Forschung Institut, Frankfurt, Germany (14) 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 peptides used as antigens which were designed from amino-acid sequences of the specific HIV-1 subtypes (10, 11, 14).

The serotyping ELISA was initially developed to differentiate antibodies to HIV-1 subtypes B and non-B at the Chemotherapeutishes Forschung Institut, Frankfurt, Germany. For this study, the assay was modified for detection and differentiation of wider spectrum of HIV-1 subtypes based on relative binding of antibodies to specific antigens. A reactivity pattern of HIV-1 antibodies in sera of infected persons from

Fig 1. Map of Nigeria Showing source of samples by States

Uganda, Rwanda, Tanzania, Nigeria and Brazil whose isolates have been subtyped by nucleotide sequencing analysis was used to develop an algorithm for subtyping of isolates from areas with multiple subtypes (tables 1 and 2).

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 full-length cycle of the subtype B was used while all the others were short and linear. The specific amino acid sequences of the V3 synthetic peptides used are shown in Table 1. The peptides were synthesized and purified by high performance liquid chromatography in U.K. (10, 11) and obtained ready for use (through Urusula Dietrich). The selection of the HIV-1 subtypes that were included was based on the prevalent subtypes in Africa as of the time of the study (20) and known HIV-1 genetic subtypes based on previous sequencing of some HIV-1 isolates from Nigeria (18).

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 (specific serotypes) are shown in Table 2. Samples that reacted with antigens of more than one HIV-1 subtype were further analyzed by the limiting ELISA (11) to determine the exact status of such samples.

Table 1: Amino Acid Sequence of V3 Peptides

Subtypes	Amino Acid Sequence
A	KSVRIGPAFYAT
B	CTRPNNNTRKSIHIGPGRAFYTTGEIIGDIRQAHC
C	KSIRIGPQTFYAT
D	CTRPYNNTRQRTHIGPGQFYRTGDI
E	E_L: DTSITIGPGQVFYRRT
	Ecycl: CTRPSNNTRTSITIGPGQVFYRTGDIIGDIRKAYC
Group O:	CERPGIQTVEIRIGPMAWYSMGLGRSSGDSRAAYC

Table 2: Reaction Patterns of Different HIV-1 Subtypes in the V3 PEIA

Subtypes	V3 - Peptides						
	P1(IB)	P2(A)	IND	D	E_L	E_{CY}	O
HIV-B	+++	-	-	-	-	-	-
HIV-1A	+++	+++	+	-	-	+	-
HIV-1C	+++	+	+++	-	-	+	-
HIV-1D	+++	+	-	+++	-	-	-
HIV-1E	-	+	+	-	+++	+++	-
HIV-10	-	-	-	-	-	-	+++

+++ Strongly reactive
+ Weakly reactive
- Non-reactive

Results

Antibodies to the six HIV-1V_3 peptides included as antigen for this study were detected among HIV-1 positive sera from Nigeria HIV-1 subtype C infection was the most prevalent. It was detected in 447 (48.3%) of the 925 samples tested. This was followed by subtype A which occurred at a prevalence of 19.8% (n=183) then subtype E 8.0% (n=74). Prevalence of HIV-1 subtype B and O was low, occurring in only 23 (2.5%) and 22 2.4%) respectively of the samples tested (Table 3).

Thirty-six (3.9%) of the samples tested were dually reactive with peptides of more than one HIV-1 subtype (Table 3) while 52 (5.6%) did not react with any of the six peptides of HIV-1 subtypes used. Samples that had dual reactivity with peptides of subtypes A and C (A/C) were the most prevalent (11 of 36, 30.6%) among those with multiple reactivity. This was followed by those with the E and O (E/ O) combination (19.4%) [7/36]. Subtype A/D, and C/E, were the least prevalent, being only one of the 36 (2.8%) for each combination. Other HIV-1 subtype combination found among the samples tested included A/E (8.3%), B/D (5.6%), C/O (13.9%), D/O (8.3%) and E/D (5.6%).

All the multiple reactive and the none-reactive samples were not included in the analysis of the serotyping results presented in this report. The exact status of these samples will be resolved by other methods. Fig 2 shows the proportion of clearly differentiated HIV-1 subtypes among HIV-1 infected persons in Nigeria.

Subtype C was found to be the most prevalent among samples collected from all the locations. It occurred with a prevalence of 42.3% to 83.2% in the different locations followed by Subtype A which was found in 11 of the 13 locations. Subtype B was detected in only 6 locations with about half of the samples (43.5%) from Maiduguri. On the other hand, out of the 87 samples that were positive for subtype D, 52 (59.8%) were from Ibadan in the southwest. However, none of the samples collected from Kaduna, Ejigbo and Samaru respectively were positive for subtype D, while subtype E was detected in samples from 10 of the locations. All sera collected from Owerri, Kaduna and Samaru were negative for subtype E. Overall twenty-two (2.6%) of the samples was reactive with subtype O peptide. Twelve (54.5%) of the positive subtype O samples were collected from Ibadan and one each (4.5%) from Enugu and Owerri. On the other hand, none of the samples from Ilesha, Ejigbo, Port Harcourt, Maiduguri, Ashaka, Kaduna, Shekina and Samaru reacted with subtype O peptide.

When the results were analysed by state of residence of donors. Subtype C was found to be the most prevalent (37.7%1 in Borno-83.2% in Enugu state) in all the states followed by subtype A with prevalence ranging from 5.3% for Enugu State to 35.7% in Rivers State. On the other hand, subtype E was more prevalent than subtype A in Enugu State but the difference was not significant. Subtype B was most prevalent in Borno State (47.8%) followed by Oyo State (26.1%). None of the samples from Enugu, Rivers and Imo States was positive for subtype B. Antibodies to HIV-I subtype D was detected in all the states with prevalence ranging from 1.1% in Kaduna and Imo State to 59.8% in Oyo State. Although antibodies to HIV-I subtype E was detected in the 8 states where samples were collected, it was prevalent in Oyo (37.8%) followed by Osun (33.8%). Twelve out of the 22 (54.5%) subtype O positive samples found in this study were from Oyo State followed by 5(27.3%) from Osun state and the least was (4.5%) found among samples from Enugu and Imo States. None of the samples from Borno, Kaduna and Rivers States reacted with subtype O peptide. For the purpose of further analysis, the country was divided into three geographical regions including South Western (SW), South Eastern (SE) and Northern (N) as shown in Table 4.

Discussion

HIV-1 isolates have been compared and subtyped by PCR amplification, sequencing, heteroduplex mobility assay (HMA) or serologically detection of antibody to the V3 region specific of the various subtypes (10, 12, 22, 23, 24). Although nucleotide sequencing and HMA are more direct and accurate methods for subtyping, serological subtyping is a rapid, simple and inexpensive method for studying the geographical distribution of HIV-1 subtypes using large number of samples (10, 11, 24).

Using the consensus V3 peptide for each subtype (A, B, C, D, E and O) as antigen, an indirect ELISA technique (11, 12) was employed to identify some of the subtypes of HIV-1 strains in Nigeria using sera collected from 925 HIV-1 seropositive persons from different parts of Nigeria. Although, identification of

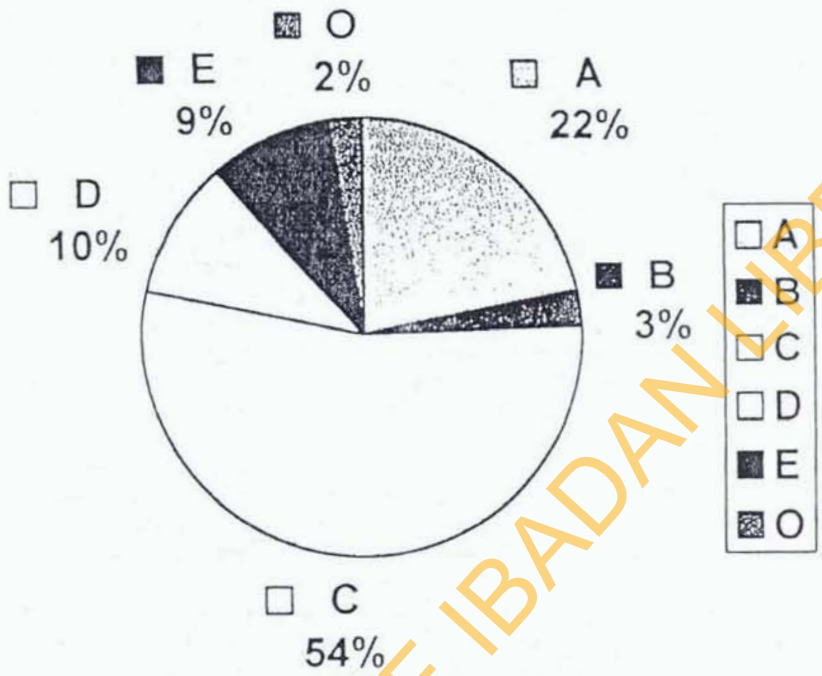

Fig. 2: Proportion of six HIV-1 Subtypes among infected individuals in Nigeria (1993-1997) n=831

some HIV-1 subtypes had been previously reported from Nigeria ((19, 25, 26), we have employed the V3 serotyping method to analyze large number of HIV-1 seropositive samples from different parts of Nigeria in this study. The technique we used has been previously used to correctly subtype HIV seropositive samples from France, Thailand, West Indies (10) and some African countries including Central African Republic (10), the Gambia (13) and Tanzania (14).

Table 3: Distribution of six HIV-I Subtypes among infected individuals in Nigeria.

Subtype	Number tested	Percentage (%)	Cumulative Frequency (%)
A	183	19.8	19.9
B	23	2.5	22.3
C	447	48.3	70.6
D	88	9.5	80.1
E	74	8.0	88.1
O	22	2.4	90.5
DR	36	3.9	94.4
NR	52	5/6	100
TOTAL	925	100%	

DR - Dually reactive (reacting with Peptides of more than one subtypes)

NR - Non-reactive (Did not react with any of the six peptides used).

Table 4: Distribution of HIV-1 Subtypes among infected persons in the three geographical regions of Nigeria (1993-1997).

Subtypes	Western	Eastern	Northern	Total
A	127 (70.2) [22.7]	22 (12.2) [13.7]	32 (17.7) [29.4]	181 (21.8)
B	10 (43.5) [1.8]	0 (0.0) [0.0]	13 (56.5) [11.9]	23 (2.8)
C	272 (61.5) [48.7]	119 (26.9) [73.9]	51 (11.5) [46.8]	442 953.3)
D	73 (83.9) [13.1]	10 (11.5) [6.21]	4 (4.6) [3.7]	87 (10.5)
E	57 (77.0) [10.2]	8 (10.8) [5.0]	9 (12.2) [8.3]	74 (8.9)
O	20 (90.9) [3.6]	2 (9.1) [1.2]	0 (0.0) [0.0]	22 (2.7)
TOTAL	559	161	109	829

( ) Percentage of a particular subtype in a region
[ ] Percentage of sample from a region positive for a subtype

The result of this work showed that multiple HIV-1 subtype including A, B, C, D, E and O co-circulate in Nigeria. The work also indicates for the first time, the presence of HIV-1 subtypes B, C, D and E in Nigeria. Identification of large variety of HIV-1 subtypes circulating in the country is similar to the situation previously reported from many other African countries. Nkengasong *et al* (27) reported detection of HIV-1 subtypes A, B, E, F, G, H and O in Central Africa. Similarly, Ariyoshi *et al*. (13) also used the peptide enzyme immunoassay and found co-circulation of subtypes A, B, C, D and F in the Gambia. Furthermore, at least five different HIV-1 subtypes have also been reported in Europe and the Americas though with subtype B constituting almost 90% of the circulating HIV-1 subtypes in the region (27, 28). The result of this study further showed that HIV-1 subtype C may be the most prevalent (48.3%) subtype circulating in Nigeria. This finding is also similar to earlier reports from some African countries such as The Gambia where 57% of HIV subtypes were found to be C (13) and Malawi where subtype C was the predominant subtype identified among pregnant women (29). In addition, subtype C has been reported to be the most prevalent subtype of HIV-1 in South Africa (30, 31). This is contrary to other reports which indicate that subtype A is the most prevalent subtype in many of the countries of West Africa (9, 32). However, some workers have shown that most serotype C and A/C genetically cluster with subtype A-IbNg.

HIV-1 subtype B has sparingly been reported in Africa, previously from the Gambia (13), Cameroon (27) and South Africa (30). Specific antibodies to subtype B were detected for the first time in Nigeria in this study and were later confirmed by sequencing (Olaleye *et al*, in preparation). The prevalence of subtype B found in this study (2.5%) was lower than previously reported in some other African countries. Using the peptide Enzyme Immunoassay, Ariyoshi *et al*. (13) found a prevalence of 8% for HIV-1 subtype B in The Gambia, mainly among male patients who have traveled or lived in North America or W. Europe (12). The history of sexual contract between individuals tested in this study and people from places with high prevalence of HIV-1 subtype B is not known.

Detention of specific antibodies to HIV-1 subtype E in this study is in agreement with findings in Central African countries of Cameroon (27) and Central African Republic (33) to the East of Nigeria but different from the situation in some countries to the west of Nigeria such as Cote D'Ivoire and The Gambia where subtype E has not been reported (13, 31). Detection of subtypes A and D in this country is similar to findings in Uganda (34), Rwanda (20), Kenya (20, 32) and The Gambia (13). The first HIV-1 strain isolated from Nigeria was shown to belong to subtype A (35). Furthermore, the result of this study confirmed previous reports of serological evidence of circulation of HIV-1 group O in Nigeria (26, 26). However, the prevalence of group O found in our series is higher than previously reported (26, 36) suggesting wide spread of group O viruses than previously reported. In addition to the Central African countries of Cameroon, Gabon and Equatorial Guinea, HIV-1 group O infections have also been reported in Benin, Senegal, Togo, Niger, Chad and Kenya (9, 27, 31, 32, 37, 38) showing that it is wide spread in Africa, but with limited dissemination to other parts of the world (20).

Apart from the six HIV-1 subtypes investigated in this study, other HIV-1 subtype such as F, G, H, J and I have been identified (19, 22, 27, 29, 31, 32). The result of this study showed that 52 of 925 (5.6%) of the HIV seropositive samples analysed did not react with any of the six peptides used as the capture antigen. This observation is similar to experience from other parts of the world (10, 39). Barin *et al* (10) analyzed HIV-1 seropositive samples from Africa, Thailand, West Indies and France and found that 2.6% of the serum samples tested did not react with any of the five peptides (A, B, C, D and E) used as antigen. It is therefore possible that the seropositive samples that did not react with any of the six HIV-1 peptides used may be due to infection with any of the other HIV-1 subtypes. Abimiku *et al* (19) have previously reported genetic evidence of presence of HIV-1 subtype G from blood samples collected from infected persons in Jos area of Nigeria. It is possible also that the individuals whose infecting virus could not be subtyped in our series were infected with yet unidentified subtypes or variants of HIV-1 in Nigeria. Previous report by Odemuyiwa *et al* (40) indicated circulation of several variants of HIV-1 in Nigeria. It is also possible that non-reactivity of those samples with any of the six peptides antigens used for this study may be due to low level of antibody to the V3 region of the gp 120 of HIV-1 in the infected individuals as earlier observed by Gaywee *et al* (24).

Thirty-six of the 925 (3.9%) HIV-1 seropositive samples examined in this study were positive for more than one HIV-1 subtypes. This phenomenon could be due to cross reactivity with the different subtype peptides could not be differentiated by the limiting ELISA technique. This problem is one of the limitations of HIV subtyping by peptide enzyme immunoassay (10, 11, 28, 29, 31, 32, 41). However, it is known that an individual can be infected with multiple HIV types or subtypes in a region where several

HIV types and subtypes co-circulate (41, 42, 43). Dual infections of the same person with distinct *env* subtypes and divergent HIV-1 and HIV-2 strains, which share only 40% homology have been reported (44). Furthermore results of experimental studies have shown possibility of super-infection by different HIV-1 strains (45, 46, 47). In addition, dual infections with different HIV-1 subtypes have been further supported by numerous reports of identification of HIV-1 and HIV-2 subtype recombinants (48, 49, 50, 51). A case of triple HIV-1 subtype infection involving group M (A and D and group O was found in a Cameroonian AIDS patient by Takehisa *et al* (43).

Cross antibody reactivity of subtypes A and C (A/C) antibodies found in this study was also observed by previous workers who used peptides and recombinant protein as antigen to differentiate HIV-1 subtypes (10, 12). Most of the dually positive samples, found in this study, 11 of 36 (30.6%) belonged to the A/C group. The V3 consensus of subtype C differs only slightly from that of A which may be responsible for the high incidence of A/C dual reactivity. A similar situation was found among HIV-1 seropositive samples from Tanzania subtypes by the V3 peptide ELISA (14). Dual infection and recombination have great implication for development of effective vaccine against the virus.

Overall, the results of this work have shown that polyvalent vaccines will be required for effective prophylactic immunization against HIV-1 infection in Nigeria and hence the need for continuous monitoring and molecular characterization of circulating HIV-1 subtypes in different geographical regions.

ACKNOWLEDGEMENT: We are grateful to the staff of Department of Virology, University of Ibadan for their contribution to the success of this study. We are particularly thankful to Mr. M.A. Ibeh for assistance in sample collection and processing. G.N. Odaibo was partly supported for this study with Bashorun Abiola University of Ibadan Postgraduate Scholarship. Some aspects of the work were carried out at GSH with a fellowship to D.O. Olaleye by the Alexander von Humboldt of Germany.

References

1. Meterns, T.E. and Burton, A. (1996). Estimate and trends of the HIV/AIDS epidemic. AIDS 10 (Suppl. A) S221-S228.
2. W.H.O. (1998a) Global AIDS surveillance. Wkly Epidemiol. Rec. 73 June (26) 193 – 200.
3. W.H.O. (1998b). Global AIDS surveillance Part II. Wkly Epidemiol. Rec. 73 November (48) 373 – 380.
4. UNAIDS (1977). Implication of HIV variability for transmission. Scientific and Policy.
5. Shaw, G.M.; Hahn, S.K. and Arya, S.K. (1984). Molecular characteristic of Human T-Cell leukemia (lymphotropic) virus type III in the Acquired Immunodeficiency Syndrome. Science 226. 1165 – 70.
6. UNAIDS (1977). Implicationof HIV variability for transmission. Scientific and Policy.
7. Tersmette, M., de Goede, R.E.Y.; Bert, J.M.; Al, I.N.; Winkel, R.A.; Gruters, H.T.C.; Huizman, H.G. and Mieldema, F. (1988). Differential syncytium-inducing capacity of human immunodeficiency syndrome (AIDS) – related complex. Journal of Virology 62: 2026 – 2032.
8. Delwart, E.L.; Shpaer, E.G.; McCutchan, F.E., Louwage, J.; Grez, M.; Rubsamen-Waigmann, H. and Mullins, J.I. (1993). Genetic relationship determined by DNA heteroduplex mobidity assays. Science 262: 1257 – 1261.
9. Heyndrickx, L.; Janssesns, W.; Zekeng, L.; Musonda Ro; Anagonou, S., Van der Ajuwera G.; Coppens, S.; Vereeken, K., De Witte K., Van Rampelbergh, R.; Kahindo, M.; Morison, L.; McCutchan, F.E.; Carr, J.; Essex M.; Goudsmit, J.; Asjo, B.; Salminen, M.; Buve, A. (2000). Study Group of HIV Epidemics in African cities, van der Green G. Simplified Strategy for Detection of Recombinant HIV Group M Isolates by gag/env Heteroduplex Mobility Assay. J. Virol. 74: 363 – 370.
10. Barin, F.; Lahbabi, Y.; Buzelay, L.; Lejeune, B.; Baillou-Beakufills, A.; Denis, F.; Mathiot, C.; M'Boup, S.; Bothaunshi, V.; Dietrich, V. and Gondeau, A. (1996). Diversity of Antibodies Binding to V3 peptides representing concensus sequences of HIV type 1 Genotypes A to E. An approach for HIV type 1 Serological subtyping. AIDS Res. And Hum. Retroviruses 12: (13) 1279 – 1289.
11. Chengsong-Popov, R.; Bobkov, A.; Gsrsev, M.M.; Kaleebu, P.; Callow, P.; Rzhiminova, A.; Saukhat, S.K.; Burdojev, P.; Kolomijets, N.D., and Webber, J. (1994). Identification of human immunodeficiency virus type 1 subtypes and their distribution in the Commonwealth of Independent States (former Soviet Union) by serological V3 peptide binding assays and Ve sequence analysis. J. Infect. Disease. 168: 292 – 297.
12. Dietrich, U.; Grez, M.; Von Briensen, H.; Panhans, B.; Geissendorfer, M.; Kuhnel, H.; Maniar, J.; Rebsamen-Waigmann, H.; (1993). HIV-1 strains from India are highly divergent from prototropic African and US/European strains but are linked to a South African isolate. AIDS 7: 23 – 27.
13. Ariyoshi, K.; Chiengsong-Popov, R.; Wilkins, A.; Corrah, T.; Weber, J. and Whittle, H. (1996). HIV-1 subtype B in West Africa. Lancet 347: 328.

14. Hoelscher, M.; Sabitie, H.; Barin, F.; Cheingsong-Popov, R.; Dietrich, U.; Jordan-Harder, B.; Olaleye, D.; Nagele, E.; Markuzi, A.; Nwakagile, D.; Minja, F.; Weber, J.; Gurtler, L. and Sonnenburg, F. N.M (1998). HIV-1 V3 serotyping in Tanzanian samples: propable reasons for mismatching with genetic subtyping. AIDS Res. And Hum. Retroviruses. 14(2): 139 – 149.
15. Olaleye, O.D.; Bernstein, L.; Ekwezor, C.C.; Zhijuansheng, Omilabu, S.A.; Xiu-Yan Li Sulliva-Halley, J. and Rasheed, S. (1993). Prevalence of Human Immunodeficiency virus type 1 and 2 infections in Nigeria. J. Infect. Dis. 167 (3): 710 – 714.
16. Odaibo, G.N.; Olaleye, O.D. and Tomori, O. (1996). Human Immunodeficiency Virus Type 1 and 2 infection in some rural areas of Nigeria. 1st International Virology and Microbiology Conference. Nov., 1996, Yaounde, Cameroon.
17. Federal Ministry of Health (1999). HIV/Syphilis Sentinel Sero- Prevalence Survey in Nigeria. Technical Report.
18. Olaleye, D.O.; Zheg, Z.; Howard, J. and Rasheed, S. (1994). Isolation and Biological Characterization of a new Human Immunodeficiency Virus Type 1 strain in Nigeria. J. Virol.
19. Abimiku, A.G.; Stern, T.L.; Zwandor et. Al. (1994). Subtypes G. HIV type 1 isolates from Nigeria. AIDS Res. And Hum. Retroviruses. 11: 427 – 429.
20. Subbarao, S. and Schochetman, G. (1996). Genetic Viariability of HIV-1 AIDS 10 (Suppl. A): 513 – 523.
21. Zhu, T.; Mo, H.; Wang, N.; Nam, D.S.; Cao, Y.; Koup, R.A. and Ho, D.D. (1993). Genotype and phenotypic characterization of HIV-1 in patients with primary infection. Science, 261: 1179 – 1181.
22. Bobkov, A.; Garaev, M.W.; Rzhaninova, A. et. Al. (1994). Molecular epidemiology of HIV-1 in the former Soviet Union analysis of env. V3 sequences and their correlation with epidemiological data. AIDS, 8: 619 – 624.
23. Howard, T.M.; Olaleye, O.D. and Rasheed, S. (1994). Sequence analysis of glycoprotein 120 coding region of a new HIV type 1 subtype A strain (HIV-1 IbNg) from Nigeria. AIDS Res. And Hum. Retroviruses. 10: 1755 – 1757.
24. Gaywee, J.; Artenstein, A.W.; Vancott, T.C.; Trichavaroj, R.; Sukchammong, A.; Amlee, P. de Souza M.; McCutchan, F.E.; Carr, J.K.; Markowitz, L.E.; Muichael, R. and Nittayaphan, S. (1996). Correlation of genetic and serologic approaches to HIV-1 subtyping in Thailand. J. AIDS and Hum. Retrov. 13 (5): 1495 – 1497.
25. Heyndrick, X.L.; Janssens, W.; Alary, M.; Fransen, K.; Vereecken, K.; Coppens, S.; Williams, B.; Davo, N.; Guedeme, A.A.; Banganizi, E.; Joly, J. and Vander, Groen, G. (1996). Genetic Variability of HIV type 1 in Benin. AIDS Res. And Hum. Retrovir. 12 (5): 1495 – 1497.
26. Mulanga, K.C.; Williams, E.E.; Ani, E.; Peeters, M.; Saman, E. and Delaporte, E. (1995). Evidence of HIV-1 group O infection in Nigeria. Lancet 346: 308.
27. Nkengasong, J.N.; Janssens, W.; Heydrickx, L.(1994). Genotypic subtypes of HIV-1 in Cameroon. AIDS 8: 1405 – 1412.
28. Arnold, C.; Balow, K.L.; Parry, J.V. *et al* (1995). At least five HIV-1 sequence subtypes (A, B, C, D, A/E) recur in England. AIDS Res. And Hum. Retroviruses. 11: 427 – 429.
29. Orloft, S.L.; M.S. Kennedy, A.A. Belperron, P.J. Maddon and J.S. Mcdougal (1993). Two mechanisms of soluble CD4 (sCD4) Mediated inhibition of human immunodeficiency virus type 1 (HIV-1) infertility and their relation to primary HIV-1 isolates with reduced sensitivity to sCD4, Journal of Virology, 67: 1461 – 1471.
30. Williamson, C.; Elnglebvrecht, Lambrick, M. (1996). HIV-1 subtypes in different risk groups in South Africa (Letter) Lancet 346: 782.
31. Janssens, W.; Buve, A. and Nkengasong, J.N. (1997). The puzzle of HIV-1 subtypes in Africa. AIDS 11: 705 – 712.
32. Janssens, W.; Buve, A. and Nkengasong, J.N.; Heydrick, L.; Fransen, *et al*. (1994). Further evidence of the presence of genetically aberrant HIV-1 strains in Cameroon and Gabon. AIDS 8 (7): 1012 – 1013.
33. Murphy, E.; Korbor, B.; Georges-Coubot, M.; You, B.; Pinter, A.; Cook, D.; Kieny, M.; Alain, G.; Mathiot, C.; Barre-Sinoussi, F. and Girard, M. (1993). Diversity of V3 region sequences of HIV-1 from the Central African Republic. AID Res. And Hum. Retroviruses. 9: 997 – 1003.
34. Bruce, C.; Clege, C.; Feathersome, A.; Smith, J.; Biryahawaho, B.; Downing Robers and Oram, J. (1994). Presence of multiple genetic subtypes of Human immunodeficiency virus type 1 proviruses in Uganda. AIDS Res. Hum. Retrov. 10(11): 1543 – 1550.
35. Olaleye, D.O.; Sheng, Z.; Howard, T. and Rasheed, S. (1995). Biological Characterization of a new subtype- A variant of human immunodeficiency virus type 1 from Nigeria. Biokemistri 5(1): 51 – 59.
36. Williams, E.E. (1997). HIV Types and Subtypes within Nigerian communities. National symposium in HIV/AIDS in Nigeria. NIMR 10th to 17th November.
37. Songok, E.M.; Libando, D.K.; Rotich, M.C.; Oogo, S.A. and Tukei, P.M. (1996). Surveillance for HIV-1 subtype O and M in Kenya (letter). Lancet 347: 1700.
38. Peeters, M.; Mboups, Gueye *et al*. (1997). Geographical distribution of HIV-1 group O viruses in Africa. AIDS 11: 493 – 498.
39. Sherefa, K.M.; Sallberg, Sonnerborg, A. (1994). Evidence of no change in V3 loop antibody and recognition pattern in HIV type 1 infected Ethiopians between 1988 and 1993. AIDS Res. Hum. Retrovir. 10: 1551 – 1556.

40. Odemuyiwa, S.O.; Odaibo, G.N.; Tebit, D.M.; Taiwo, O. and Olaleye, D.O. (1996). Immunological determination of Human Immunodeficiency virus types 1 and 2 variants in south western Nigeria. Xth International Congress of Virology. August, 1996, Jerusalem, Israel.
41. Diaz, R.S.; Sabino, E.C.; Mayer, A.; Mooley, J.W.; Busch, M.O. and the Transfusion Safety Study Group (1995). Dual Immunodeficiency virus type 1 infection and recombination in a dually exposed recipient. Virol. 69: 3273 – 3281.
42. Artenstein, A.W.; Van Cott, T.C.; Mascola, J.R.; Carr, J.K.; Hegerich, P.A.; Gaywee, J.; Sanders-Buell, Robb, M.L.; Dayoff, D.E.; Thitivichianlert, S.; Nitayaphan, S.; Meneil, J.G.; Birx, D.L.; Mizhent, R.A.; Burke, D.S., McCutchan, F.E. (1994). Dual infection with human immunodeficiency virus type 1 of distict Envelope subtypes in Humans. J. Infect. Dis. 17: 805 – 810.
43. Takehisa, J.; Zekeng, L.; Miura, T.; Ido, E.; Yamashita, M.; Mboudjeka, I.; Gurtler, L.G., Hayami, M. and Kaptue, L. (1997). Triple HIV-1 infection with group O and group M of different clades in a single Cameroonian AIDS patient. J. AIDS and Hum. Retrov. 14(1): 81.
44. Grez, M.; Dietrich, U.; Balfe, P.; von Briesen, H.; Manin, J.K.; Mahambre, G.; Delwart, E.L.; Mullins, J.I. and Rubsamen-Waigmann, H. (1994). Genetic analysis of human immunodeficiency virus type 1 and 2 (HIV-1 and HIV-2) mixed infections in India reveals a recent spread of HIV-1 and HIV-2 from a single ancestor for each of these viruses. J. Virol. 68: 2161 – 2168.
45. Fultz, P.N.; Srinvasan, A.; Greene, C.R.; Butler, D.; Sweson, R.B.; McClure, H.M. (1986). Superinfection of a chimpanzee with a second strain of human immunodeficiency virus. J. Virol. 61: 4026 – 4029.
46. Lewis, P.M.; Emerman (1994). Passage through mitosis is require for on coretroviruses but not for the human immunodeficiency virus. J. Virol. 68: 510 – 516.
47. Howell, R.M.; Fitzgibbon, J.E.; Noe, M.; Ren, Z.J.; Gock, D.J.; Schwartzer, T.; A.; Dubin, D.T. (1991). In vivo sequence variation of the human immunodeficiency virus type 1 *env* gene: Evidence for recombination among variants found in a single individual. AIDS Res. And Hum. Retroviruses. 7: 869 – 876.
48. Kim, J.H.; Mosca, J.D.; Valley, M.T.; McLinden, R.J.; Burke, D.S. and Redfield, R.R. (1993). Consequences of human immunodeficiency virus type 1 superinfection of chronically infected cells. AIDS Res. Hum. Retroviruses 9: 875 – 882.
49. Groenink, M.; Andeweg, A.C.; Fouchier, R. *et al.* (1992). Phenotype Associated *env* gene variation among eight evidence for in-vivo recombination and determinants of cytotropism outside the V3 domain. J. Virol. 66: 6175 – 6180.
50. Gao, F.; Yue, Craig, S.; Thorton, C.L.; Robertson, D.L.; McCutchan, F.E.; Bradaa, J.A.; Sharp, Halin, B.H. and WHO Network for HIV isolation and chracterization (1994). Genetic variation of HIV type 1 in four WHO sponsored vaccine evaluation sites: Generation of functional envelope (glyco-protein 160) clone representative of sequence of subtypes A, B, C and E. AIDS Research and Human Retroviruses. 10: 1359 – 1368.
51. Zhu, T.; Wang, N.; Carr, A.; Wolinsky, S. and Ho, D.D. (1995). Evidence for co-infection by multiple strains of human immunodeficiency virus type 1 subtype B in an acute seroconvertor. Journal of Virology. 69: 1324 – 1327.