

Rapid detection of HIV-1 subtypes in Ghana by heteroduplex mobility assay

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Abstract: Background: In Ghana, the HIV-1 profile has been quite dynamic. Previous reports identified HIV-1 subtypes A, D and G present and recently the CRF02_AG has been described as the predominant molecular form of HIV-1 in Kumasi, Ghana. This underscores the need for constant molecular characterization of HIV-1 species in the country. Objective: To provide current updates on the nature of HIV subtypes in Ghana, there is need for a user-friendly tool for routine monitoring of subtypes in the absence of cost-intensive and skill-demanding sequencing techniques. This work demonstrates the use of heteroduplex mobility assay (HMA) for rapid subtype detection of HIV-1 isolated from Ghanaian patients. Method: Viruses from 15 antibody-positive HIV-1 patients were isolated directly by co-culturing peripheral blood mononuclear cells (PBMC) with phytohemagglutinin (PHA)-stimulated donor PBMCs from an HIV seronegative individual and through HeLa cells positive for CD4/CXCR4/CCR5 (MAGIC-5A cells). That was followed by proviral DNA extraction. Heteroduplex Mobility Assay (HMA) technique was then performed on the HIV gag gene. Results: Using the HMA technique, newly isolated HIV-1 strains were subtyped as follows: There were seven subtype A (47%), two subtype G (13%) and six (40%) A/G recombinants. Conclusion: The HIV-1 CRF02_AG in Ghana has spread much more rapidly than the previously predominant subtype A over the years. Constant molecular characterization of HIV strains is necessary to enable clear elucidation of the prevailing HIV species in Ghana. This study presents the HMA as a useful tool for monitoring subtype emergence and distribution in the country.

Keywords: HIV-1 Subtype, HMA, Ghana

1. Introduction

Human immunodeficiency virus (HIV) is a virus species belonging to the family Retroviridae, subfamily Orthoretrovirinae and of the genus Lentivirus owing to the long time the virus takes to present symptoms. HIV is responsible for the development of acquired immunodeficiency syndrome – AIDS. Two related virus types, HIV-1 and HIV-2 are known to be the etiologic agents of AIDS 1, 2. HIV-1 largely accounts for the pandemic affecting nearly 50 million people worldwide in the past decade.³ Despite the development of antiretroviral drugs approved for clinical use, HIV-1 infection remains incurable. This is in part due to the tremendous evolutionary potential of HIV-1 and the complex interactions between the viral

populations and the host,⁴⁻⁶ driven largely by three major factors: the high virus replication rate in vivo, high mutation rate arising from the error-prone nature of the reverse transcriptase that copies the viral RNA into DNA and the proclivity for recombination arising from the presence of two RNA molecules copied alternatively.⁷⁻⁹

HIV strains have thus diversified extensively over the years.¹⁰ Four different divergent lineages of HIV-1 have been described to date, namely group M (main), O (outlier), N (non-M, non-O) and the recently identified group P; group M being responsible for the major HIV pandemic worldwide, while groups O, N and P appear to be mainly restricted to Central Africa.¹¹⁻¹³ HIV-1 group M is sub-divided into 9 subtypes (A to D, F to H, J and K) and at least 55 ‘circulating recombinant forms’ (CRFs), and multiple unique

recombinant forms (URFs). 14-18 Except in equatorial Africa, the HIV epidemic is genetically relatively homogeneous: B subtype viruses predominate in Europe and North and South America, E subtype viruses predominate in Southeast Asia.¹⁹ Subtype C viruses predominate in southern Africa and India. Subtypes B and F mixing have occurred and in South America B/F recombinants have been detected.²⁰ Subtypes B and C mixing is occurring in South Africa, and E/A subtypes and C mixing is occurring in Asia.²¹ As might be expected, in East Africa where the A and D subtypes co-circulate, several A/D recombinants have been detected.²² In western equatorial Africa, multiple HIV-1 subtypes (A, C, D, E, F, G and H) as well as the outlier “group O” HIV-1 strains are known to co-circulate, and studies suggest that recombinant forms are quite common in this region.²¹

Studies have also shown the increasing importance of CRFs in the global HIV-1 epidemic. They account for over 18% of infections and predominantly represent the local form in Southeast Asia (CRF01-AE) as well as in West and West Central Africa (CRF02-AG). In West Africa in particular, this recombinant form was found to be associated with up to 31% of new infections.²³⁻²⁶ In Ghana, the CRF02_AG has been described as the predominant molecular form of HIV-1 in Kumasi, which is near the northern part of the country, and Koforidua in the Eastern part, irrespective of clinical condition of subjects.^{27,28} This progression of genotype variabilities underscore the fact that has to be noted that the current geographic distribution of subtypes and recombinants may not remain static, and with the widespread occurrence and the dynamic emergence of HIV-1 recombinant variants, especially in neighbouring West African countries, it is of increasing importance for constant molecular characterization of HIV strains to enable clear elucidation of the prevailing HIV species in Ghana. Besides, the formulation of a human immunodeficiency virus (HIV) vaccine remains an important challenge,^{29,30} especially for the developing nations that are most threatened by HIV epidemics. It is therefore necessary to focus more research on HIV subtype determination in various geographic regions to inform developing a vaccine that exhibits cross-subtype activity despite sequence variation.

This study was set out to apply heteroduplex mobility assay (HMA) to a group of 15 new HIV isolates obtained from HIV-1 antibody-positive Ghanaians for subtype determination. The aim was to assess the sensitivity and reliability of the technique to HIV subtype determination especially in resource-limited settings like Ghana where the rarely available DNA sequencing requiring sophisticated facilities and procedures is not practical as a routine survey method.

2. Methodology

Initially, viruses were isolated directly by co-culturing

isolated peripheral blood mononuclear cells (PBMC) with phytohemagglutinin (PHA)-stimulated donor PBMCs from an HIV seronegative individual and through HeLa cells positive for CD4/CXCR4/CCR5 (MAGIC-5A cells). Proviral DNAs in these cells were extracted and two pairs of primers targeting the HIV gag gene were then used in a nested polymerase chain reaction (PCR) to amplify the gag fragment for HMA according to the procedure by Heyndrickx and colleagues³¹. The HMA technique for subtype determination was then performed. The technique is based on the relative mobilities of heteroduplex and homoduplex DNAs formed after subjecting a mixture of target and reference DNAs to denaturation and rapid cooling. Briefly, following the amplification of the gag fragment of the test samples, a corresponding reference strain fragment was also amplified in a single second-round PCR. Target amplicons were mixed with appropriate reference amplicons, denatured by heating to 94°C and rapidly cooled to 4°C for duplex formation in a thermocycler. The mixtures, containing both the homo- and hetero-duplexes were then stained in 1 µg/ml ethidium bromide solution, separated by polyacrylamide gel electrophoresis and visualized under ultra violet illumination. Subtype declaration was based on the consideration of the genetic closeness or divergence between the target and reference DNAs as revealed by the electrophoresed DNA bands.

3. Results

The conditions of the samples when received for the assay process, the results of the initial virus isolation attempts and the outcome of the HIV-1 gag gene amplification for HMA subtyping have been presented in Table 1. The initial virus isolation was done for the purpose of this investigation to virologically confirm HIV positivity prior to DNA subtyping. By the HMA technique all isolates tested were subtypable. Of the 15 HIV-1 isolates used in this investigation, seven (47%) were subtype A, two (13%) were subtype G and six (40%) were the circulating recombinant form CRF02_AG.

The polyacrylamide gel electrophoresis results of the classification of Ghanaian HIV-1 strains by the method of heteroduplex mobility assay are shown in Fig. 2. Subtype determination by the HMA technique is based on relative mobilities of heteroduplex and homoduplex DNAs formed after subjecting a mixture of target and reference DNAs to denaturation and rapid cooling. The figure presents the heteroduplex migration patterns for HIV-1 subtype determination. Each target amplified gag fragment was annealed with the reference amplicons of the prevalent subtypes (A, G and CRF02_AG). Identical target and reference fragments annealed best and the resultant heteroduplex DNA showed mobility closest to that of the homoduplex.

Table 1. Sampling and Virus Isolation for Subtyping by HMA

Sample Code	Sampling1 Condition	Virus Isolation2 by PBL	Virus Isolation3 by MAGIC-5A	HMAgag Amplification	Subtype by4 HMA
NJ-10-173	hemolysis+	-	+	OK	AG
NJ-10-175	hemolysis+	+	+	OK	G
NJ-10-176	hemolysis++	+	+	OK	AG
NJ-10-179	hemolysis+	+	+	OK	A
NJ-10-180	hemolysis+	+	+	OK	AG
NJ-10-181	hemolysis++	+	+	OK	A
NJ-10-182	hemolysis++	+	+	OK	G
NJ-10-185	hemolysis+	+	+	OK	A
NJ-10-188	hemolysis+	+	+	OK	A
NJ-10-189	hemolysis+	+	+	OK	AG
NJ-10-194	hemolysis+	-	+	OK	A
NJ-10-195	hemolysis+	+	+	OK	AG
NJ-10-196	hemolysis+	-	+	OK	A
NJ-10-197	hemolysis+	+	+	OK	A
NJ-10-198	hemolysis+	+	+	OK	AG

¹Condition of whole blood from patients upon receipt at NIID, Tokyo

²Initial virus isolation was not successful with three samples (indicated by '-')

³Virus isolation by MAGIC-F5A was successful for all the samples

⁴Subtyping of amplified HIV-1 gag gene by HMA was successful in all cases

⁵Subtypes were confirmed by sequencing (results not shown)

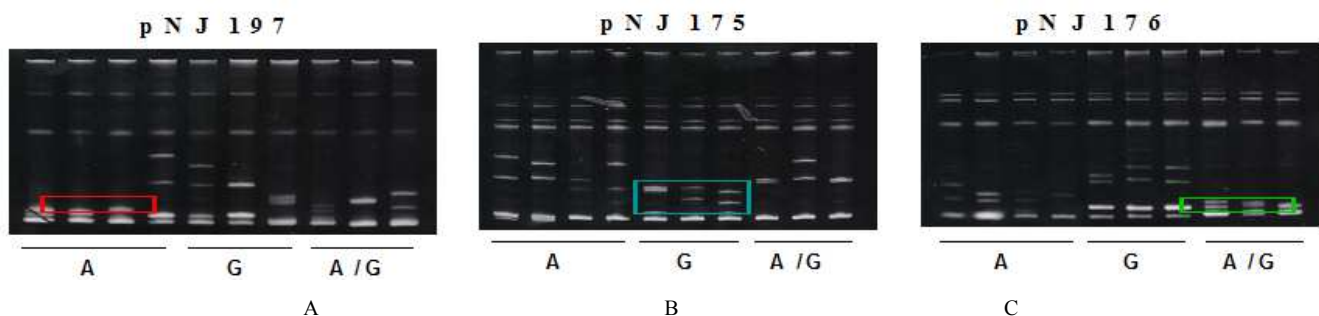


Fig 1. Representative HMA polyacrylamide gel electrophoresis showing homo- and heteroduplex DNA migration patterns for HIV-1 subtype determination. **A:** Patient HIV-1 Subtype is A; **B:** Patient HIV-1 subtype is G; and **C:** HIV-1 subtype is CRF02_AG.

Migration of a homoduplex in the polyacrylamide gel is less restricted and therefore proceeds faster (to the bottom of the gel) as compared to the heteroduplex. Mobility of heteroduplex is closest to that of the homoduplex if both are of the same subtype. Accordingly, the positions of the rectangle (Fig. 1 A, B and C) indicate HIV-1 subtypes identified.

4. Discussion

Using the HMA technique, fifteen these newly isolated Ghanaian HIV-1 strains were subtyped. Having thus confirmed, prevalent Ghanaian HIV strains could be subtyped directly by HMA following proviral DNA extraction without prior isolation. Findings of this study confirm the increasing importance of the CRF02_AG and the dynamic nature of HIV-1 subtype variation in Ghana.

The HIV-1 CRF02_AG is known to be circulating in West Africa. Reports have shown prevalence of this molecular form ranging between 38% and 82% in the sub-region, 26, 32,33 In Ghana, the HIV-1 profile has been quite dynamic. Previous reports identified HIV-1 subtype A dominating with

subtypes D and G also present.³⁴ The occurrence of the CRF02_AG form has not been extensively studied yet in Ghana. Despite the low number of samples analysed in this study, the findings confirm previous reports indicating the epidemiological importance of CRF02_AG in the Ashanti Region of Ghana. The occurrence of CRF02_AG in Ghana might not be a chance event due to the fact that this subtype predominates in West Africa; and in Ghana, it has spread much more rapidly than the predominant subtype A over the past decade^{14,29} This raises concerns that such recombinant forms might have emerged through natural selection based on their transmission efficiency.

Recombination therefore may be an important fitness search strategy in the ongoing evolution of HIV. It may be an important mechanism by which HIV evades drug or immune pressures. Future epidemiologic and clinical trials should consider and examine the role of recombination in HIV evolution and adaptation, and response to treatment especially in non-B subtype prevalent countries including Ghana. Furthermore, with the potential for inter-country spread of HIV recombinants in the West African sub-region³⁵, there is the need for constant molecular

characterization of HIV strains in the country to provide current updates on the nature of HIV infections in Ghana.

5. Conclusion

This study presents HMA as a useful tool for monitoring subtype distribution in the country. Further analyses are necessary to clarify the clinical significance of CRF02_AG in West Africa. This is of high relevance for involvement in future vaccine development efforts and vaccine trials in the West African sub-region.

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