PRELIMINARY REPORT

Genetic variation and monophyletic assembly of HIV-1 strains among infected patients within Libyan community

Mohamed A. Daw[1], Abdallah H. El-Bouzedi[2], Mohamed O. Ahmed[3]

[1]Department of Medical Microbiology and Immunology, Faculty of Medicine, University of Tripoli, Tripoli, Libya
[2]Department of Statistics, Faculty of Science, Tripoli University, Tripoli, Libya
[3]Department of Microbiology and Parasitology, Faculty of Veterinary Medicine, University of Tripoli, Tripoli, Libya

Abstract

A preliminary study was carried out among a total of 371 acquired immune deficiency syndrome (AIDS) patients from different regions of Libya, investigating the genetic variability of human immunodeficiency virus type 1 (HIV-1) strains. Patients studied were from four regions, including Western region ($n = 135$, 36.4%), Eastern region ($n = 98$, 26.4%), Middle region ($n = 85$, 22.9%), and Southern region ($n = 53$, 14.3%) of Libya. Phylogenetic analysis showed that 197 (53.1%) patients were sub-type B and 78 (21%) were sub-type A. In 96 (18.6%) patients, CRF02_AG strain was isolated in those from the Eastern region ($p < 0.001$). CRF02_AG strain was less distributed in other regions, while sub-types B and A showed equal distribution within other regions. The genetic diversity of HIV-1 strain was evident in Western, Central, and Southern regions, although strains from the Eastern region were found to be persistently monophyletic. Compared with other sub-Saharan and North African countries, the genetic sub-type distribution of HIV-1 strains in Libya is very heterogeneous in most of the regions, except the Eastern region. The strain isolated in patients from the Eastern region is unique due to several reasons, including being monophyletic, highly persistent, and rarely reported in other regions. Therefore, monitoring and following the continuity of HIV spread is essential for determining public health priorities, assessing the impact of interventions, and estimating current and future healthcare needs.

HIV AIDS Rev 2026; 25, 1: ?-?
DOI: https://doi.org/10.5114/hivar/172008

Key words: HIV-1, HIV-1 subtype A, HIV-1 subtype B, Libya.

Introduction

Human immunodeficiency virus (HIV)/acquired immune deficiency syndrome (AIDS) is a lethal threat, which had spread widely all over the world, particularly across developing countries since its emergence in the early 1980s. Despite all the scientific progress that has been made in the treatment and prevention of HIV/AIDS, over 40 million people in the world are infected with HIV, mostly in Africa [1, 2].

HIV type 1 (HIV-1), the main cause of AIDS, is regarded as one of the most unique viruses, not only due its infectivity, transmissibility, and social and economic impacts, but because of adherence to its genetic entity. It depicts a greater degree of genetic and antigenic variability than any other human-reported virus [3].

Address for correspondence: Mohamed A. Daw,
Department of Medical Microbiology and Immunology,
Faculty of Medicine, University of Tripoli, CC 82668, Tripoli,
Libya, e-mail: mohamedadaw@gmail.com

Article history:
Received: 04.09.2023
Revised: 07.09.2023
Accepted: 07.09.2023

International Journal of HIV-Related Problems
HIV & AIDS Review

HIV-1 is divided into four different genetic groups, namely M (main), O (outlier), N (non-M, non-O), and P. They have different geographic distributions but produce similar clinical symptoms [4, 5], although, group M accounts for the vast majority of HIV-1 infections worldwide. This group is further classified into 11 sub-types and sub-subtypes (A1, A2, B, C, D, F1, F2, G, H, K, and J). Re-combination between M sub-types has resulted in the generation of multiple circulating recombinant forms (CRFs), consisting of mosaic lineages of more than 70 CRFs with various types of unique recombinant forms [6, 7].

HIV-1 group O originates and continues to be an epidemic in Africa, particularly in Cameroon. Although, group M is found to be the leading cause of the AIDS pandemic and impacting millions of infections worldwide, group O has been estimated to have infected a total of around 100,000 individuals. Finally, two other groups of the HIV-1 lineage, N and P, have been reported in less than 20 and 2 individuals globally, respectively [8, 9].

The CRFs are widely distributed in the Fareast, particularly in Japan, China, and Singapore. However, in North America and Western countries, the B sub-type is a predominant strain, with is a steep increase in non-B sub-types and CRFs [10, 11]. However, human influx, including travelers, developing world migration, and civil wars, has contributed immensely to the spread and integration of HIV-1 variants, thus no strain can be easily considered specific to a certain region [12].

Libya is the second largest country in Africa bordered by six different countries, having the longest coast in the Mediterranean Sea facing Europe. It has been considered the gateway to Africa and the main entry of African immigrants to Europe. HIV/AIDS in Libya has gained international interest when Bulgarian nurses were accused of injecting the virus in over four hundred children in Benghazi. A death penalty was issued by the Libyan court, which than changed for life-long impressment; however, lately, the nurses were released by efforts of the European Union. Since then, the infected children have been raised alone and no studies were carried out by western or Libyan scientists as agreed upon the release of accused nurses. If such an incident had taken place in any of the western countries, the world would be up in arms to find out the consequences of the outbreak [13, 14].

Understanding the epidemiology and diversity of this virus in Libya, it is important to improve the diagnosis, monitoring, and treatment of this infection in the country, especially as Libya has been evoked in internal armed conflicts as a result of the NATO intervention in 2011 [15, 16]. The conflict, however, had a great impact on HIV/AIDS in Libya and other Northern African countries, as previously demonstrated by our research team [2]. The current report aimed to determine and follow-up on the genetic diversity of HIV-1 strains within the Libyan community.

Methods and results

In this study, we investigated and traced HIV-1 genetic variability of 371 AIDS patients from different regions of Libya. HIV-1 genotyping and genetic analysis were carried out using partial PCR amplification performed among the study sample. A multiple alignment of the newly derived protease/RT sequences and full-length genome sequences with selected reference sequences was constructed, and the origin of sub-types was traced and analyzed as described in previous studies [17, 18]. In brief, phylogenetic trees were generated, and consistency of branching order was evaluated using phylogeny inference package version 3.5 c. Recombinant analysis employed SimPlot software, version 3.4, and alignment examination determined precise breakpoints. After breakpoint identification, each segment was extracted and analyzed phylogenetically to confirm the assignment of the sub-type [17, 19].

The overall results from the sub-typing approaches are summarized in Table 1. A total of 371 patients from different Libyan regions were examined, including 135 (36.4%) patients from the Western region, 98 (26.4%) from Eastern region, 85 (22.9%) from Middle region, and 53 from Southern region (14.3%) of Libya.

Of the studied population, 239 (64.4%) were males and 132 (35.6%) were females, with M : F ratio of 1 : 1.8. Over 50% of HIV-infected patients were individuals aged between 31 and 45 years, although the prevalence was less among young individuals aged 18-30 years (n = 82, 22.1%) and those aged over 45 years (n = 94, 25.3%).

Our analysis identified three HIV-1 sub-types. Of the 371 specimens tested, sub-type B was isolated from 197 (53.1%) HIV-infected individuals, followed by 78 (21%) patients with sub-type A, while CRF02_AG strain was present in 96 (18.6%) patients. Analysis of epidemiological and sub-typing data demonstrated no association between sub-type distribution and sex, age, or transmission risk factors (Table 1).

Regional-geographic tracing of the studied HIV-1 sub-types (CRF02_AG, A, B) showed a strong association between the distribution of these sub-types and geographic origin of the seropositive individuals. CRF02_AG strain was isolated in sixty nine (70.4%) patients from the Eastern region (p < 0.001), and it was less distributed within other territories, including Western region (n = 11, 8.1%), Middle region (n = 9, 10.6%), and Southern region (n = 7, 13.2%). B and A sub-types indicated equal distribution within other regions (Table 1). Patients infected with CRF02_AG strain revealed no co-infection with other sub-types. Multiple infections with more than one strain were reported in 57% of patients with both B and A sub-types.

Discussion

In Libya and other Middle East and North African countries (MENA), there is a paucity of information on molecular characterization of HIV strains due to limited research. Studies are mainly restricted to serological investigations, determining the level of exposure to HIV infection. A comprehensive epidemiological study within 25 years was carried out by Daw *et al.* [19], reporting that HIV/AIDS in Libya is condensed in certain regions of the country,

Table 1. Distribution of HIV-1 sub-types among Libyan patients infected with HIV

Demographic characteristics	No. of patients (%)	HIV-1 sub-types		
		CRF02_AG	B	A
	371 (100.0)	96 (18.6)	197 (53.1)	78 (21.0)
Gender, *n* (%)				
Male	239 (64.4)	67 (28.0)	119 (49.8)	53 (22.2)
Female	132 (35.6)	29 (22.0)	78 (59. ???)	25 (19.0)
Age (years)				
18-30	82 (22.1)	27 (32.9)	34 (41.5)	21 (25.6)
31-45	195 (2.6)	61 (31.3)	91 (46.7)	43 (22.1)
> 45	94 (25.3)	8 (8.5)	72 (76.6)	14 (14.9)
Libyan regions, *n* (%)				
West	135 (36.4)	11 (8.1)	93 (68.9)	31 (23.0)
East	98 (26.4)	69 (70.4)	17 (17.3)	12 (12.2)
Middle	85 (22.9)	9 (10.6)	57 (67.1)	19 (22.4)
South	53 (14.3)	7 (13.2)	30 (56.6)	16 (30.2)
Transmission risk factor, *n* (%)				
IVDUs	156 (42.0)	27 (17.3)	98 (62.8)	31 (19.9)
Heterosexual contact	87 (23.5)	15 (17.2)	52 (59.8)	20 (23.0)
Hospitals	59 (15.9)	29 (49.2)	21 (35.6)	9 (15.3)
Others	69 (18.6)	25 (36.2)	26 (37.7)	18 (26.1)

IVDUs – intravenous drug users

particularly in the Eastern and Middle regions. However, this was exacerbated further by the internal armed conflict, which resulted in the re-emergence and migration of HIV strains within Libya [20]. In this brief report, we tried to determine the impact of human genetic variation on HIV virus and its pathogenesis in Libyan population by evaluating the diversity of HIV-1 envelope sub-types circulating in the country.

HIV-1 sub-type B is the predominant strain, followed by sub-type A and to a lesser extent, CRF02_AG strain. This is in agreement with other studies carried out in African and European countries. In South Africa, sub-type B was the predominant sub-type isolated from 96% of HIV-infected population, followed by sub-type C. Similar results were reported in Portugal, where a total of 217 HIV-1-positive blood samples were genetically characterized, revealing that sub-type B was the most prevalent (50.2%), followed by sub-types G (21.7%), A (17.5%), and F (5.5%) [21, 22].

The geographical distribution of sub-types and circulating recombinant forms reflects the complexity of HIV-1 molecular epidemiology [23]. In this study, the distribution of these sub-types was unique within the Libyan geographical region, and CRF02_AG strain was mainly isolated in the Eastern region patients, while those infected with CRF02_AG in other regions primarily immigrated from the Eastern region; although strains A and B were distributed evenly within the West, South, and Middle regions. This indicates that monophyletic consistency is an assembled phenomenon associated with the emergence of HIV-1 strain in the Eastern region of Libya, with genetically variable strains from other regions and emerging multiple strains.

Furthermore, the isolation of these sub-types from HIV-infected patients was variable. CRF02_AG strain was persistently isolated from the same patient, with no co-infections with other sub-types. Multiple infection or co-infection with other strains of the same person was reported. Therefore, this genetic study provides useful information, highlighting monitoring the dynamics of the spread of HIV-1 sub-types and CRFs on regional levels as well as information of potential importance for the diagnosis and treatment of HIV/AIDS [24].

Conclusions

This is one of the few studies investigating the impact of human genetic variation on HIV-1 infection and pathogenesis in Libya or North Africa, an area with the world's highest prevalence of HIV-1 infections. The presence of monophyletic virus is one of the keys to the spread of HIV-1 in Libya. Apparently, this is most likely coming from an iatrogenic source rather than spread among patients, while very unlikely that the virus originated from a single human being and spread, as the case of other strains. Phylogenetic analysis of circulating viruses is useful in tracking the spread of HIV in different populations and geographic regions. Therefore, this information is fundamental in defining strategies for the prevention and control of infection as well as the implementation of proper diagnostic tests and viral load assays.

Disclosures

1. Institutional review board statement: Not applicable.
2. Assistance with the article: The authors would like to thank the Department of Medical Microbiology, Faculty of Medicine, University of Tripoli, the Libyan Society of Hospital Infection, and Libyan Study Group for Hepatitis and HIV/AIDS for their continuous support.
3. Financial support and sponsorship: None.
4. Conflicts of interest: None.

References

1. Piot P, Bartos M, Ghys PD, Walker N, Schwartländer B. The global impact of HIV/AIDS. Nature 2001; 410: 968-973.
2. Daw MA, Ahmed MO. Epidemiological characterization and geographic distribution of human immunodeficiency virus/acquired immunodeficiency syndrome infection in North African countries. World J Virol 2021; 10: 69-85.
3. Sharp PM, Hahn BH. The evolution of HIV-1 and the origin of AIDS. Philos Trans R Soc Lond B Biol Sci 2010; 365: 2487-2494.
4. Giovanetti M, Ciccozzi M, Parolin C, Borsetti A. Molecular epidemiology of HIV-1 in African countries: a comprehensive overview. Pathogens 2020; 9: 1072. DOI: 10.3390/pathogens9121072.
5. Santoro MM, Perno CF. HIV-1 genetic variability and clinical implications. ISRN Microbiol 2013; 2013: 481314. DOI: 10.1155/2013/481314.
6. Lau KA, Wong JJ. Current trends of HIV recombination worldwide. Infect Dis Rep 2013; 5 (Suppl 1): e4. DOI: 10.4081/idr.2013.s1.e4.
7. Chow WZ, Takebe Y, Syafina NE, Prakasa MS, Chan KG, Al-Darraji HA, et al. A newly emerging HIV-1 recombinant lineage (CRF58_01B) disseminating among people who inject drugs in Malaysia. PLoS One 2014; 9: e85250. DOI: 10.1371/journal.pone.0085250.
8. D'arc M, Ayouba A, Esteban A, Learn GH, Boué V, Liegeois F, et al. Origin of the HIV-1 group O epidemic in western lowland gorillas. Proc Natl Acad Sci U S A 2015; 112: E1343-E1352. DOI: 10.1073/pnas.1502022112.
9. Faria NR, Rambaut A, Suchard MA, Baele G, Bedford T, Ward MJ, et al. HIV epidemiology. The early spread and epidemic ignition of HIV-1 in human populations. Science 2014; 346: 56-61.
10. Ahn I, Son HS. Epidemiological comparisons of codon usage patterns among HIV-1 isolates from Asia, Europe, Africa, and the Americas. Exp Mol Med 2006; 38: 643-651.
11. Parczewski M, Urbanska A, Grzeszczuk A, Maciejewska K, Witak-Jedra M, Leszczyszyn-Pynka M. Phylogeographic analysis on the travel-related introduction of HIV-1 non-B subtypes to Northern Poland. Infect Genet Evol 2014; 27: 121-130.
12. Daw MA, El-Bouzedi A, Ahmed MO, Dau AA, Agnan MM, Drah AM, Deake AO; in association with Libyan Study Group of Hepatitis & HIV. Prevalence of human immune deficiency virus in immigrants crossing to Europe from North and Sub-Saharan Africa. Travel Med Infect Dis 2016; 14: 637-638.
13. Mysko B. Libya: foreign healthcare workers sentenced to death for deliberate infection. HIV AIDS Policy Law Rev 2004; 9: 61.
14. Parish LC. Freedom for the Bulgarian nurses and a Palestinian doctor. Clin Dermatol 2008; 26: 574. DOI: 10.1016/j.clindermatol.2007.11.009.
15. Daw MA, El-Bouzedi AH, Dau AA. Trends and patterns of deaths, injuries and intentional disabilities within the Libyan armed conflict: 2012-2017. PLoS One 2019; 14: e0216061. DOI: 10.1371/journal.pone.0216061.
16. Daw MA. Libyan healthcare system during the armed conflict: challenges and restoration. Afr J Emerg Med 2017; 7: 47-50.
17. Daw MA, El-Bouzedi A, Ahmed MO, Dau AA; in association with the Libyan Study Group of Hepatitis & HIV. Molecular and epidemiological characterization of HIV-1 subtypes among Libyan patients. BMC Res Notes 2017; 10: 170. DOI: 10.1186/s13104-017-2491-2.
18. Daw MA, Shabash A, El-Bouzedi A, Dau AA; in association with the Libyan Study Group of Hepatitis & HIV. Seroprevalence of HBV, HCV & HIV co-infection and risk factors analysis in Tripoli-Libya. PLoS One 2014; 9: e98793. DOI: 10.1371/journal.pone.0098793.
19. Daw MA, Daw AM, Sifennasr NEM, Draha AM, Daw AA, Daw AA, et al.; in association with Libyan Study Group of Hepatitis & HIV. Spatiotemporal analysis and epidemiological characterization of the human immunodeficiency virus (HIV) in Libya within a twenty five year period: 1993-2017. AIDS Res Ther 2019; 16: 14. DOI: 10.1186/s12981-019-0228-0.
20. Daw MA, El-Bouzedi AH, Ahmed MO. The impact of armed conflict on the prevalence and transmission dynamics of HIV infection in Libya. Front Public Health 2022; 10: 779778. DOI: 10.3389/fpubh.2022.779778.
21. van Harmelen J, Wood R, Lambrick M, Rybicki EP, Williamson AL, Williamson C. An association between HIV-1 subtypes and mode of transmission in Cape Town, South Africa. AIDS 1997; 11: 81-87.
22. Esteves A, Parreira R, Venenno T, Franco M, Piedade J, Germano De Sousa J, Canas-Ferreira WF. Molecular epidemiology of HIV type 1 infection in Portugal: high prevalence of non-B subtypes. AIDS Res Hum Retroviruses 2002; 18: 313-325.
23. Taylor BS, Sobieszczyk ME, McCutchan FE, Hammer SM. The challenge of HIV-1 subtype diversity. N Engl J Med 2008; 358: 1590-1602.
24. Huang A, Hogan JW, Istrail S, DeLong A, Katzenstein DA, Kantor R. Global analysis of sequence diversity within HIV-1 subtypes across geographic regions. Future Virol 2012; 7: 505-517.