Original Article

Detection of HIV-1 Genetic Variation in *Gag* Gene, Subtyping, and Phylogenetic Tree at Thi-Qar Province, South of Iraq

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Abstract

Background: HIV constitutes a major health problem for the individual and society and is a threat to public health. The World Health Organization aims to eliminate this threat by 2030. **Objectives:** This study aims to detect the genetic variation in *gag* gene and determine the subtype of HIV in Thi-Qar Province South of Iraq. **Materials and Methods:** The current experimental study extended from February 2022 to February 2023. HIV RNA was extracted from 39 samples infected of previously diagnosed HIV-infected patients by enzyme-linked immunosorbent assay (ELISA). assay. Positive samples were amplified, confirmed by polymerase chain reaction (PCR), and 11 samples were sent to Macrogen, South Korea, to conduct sequence to analysis by BLAST-NCBI and other bioinformatics application. **Results:** The current study showed the isolates belong to subtype A1, and the replacement of valine with isoleucine at position 16 in amino acid sequence. The current study was recorded the isolates in the Genbank with the accession numbers of the *gag* gene sequence (LC739756, LC739757, LC739758, LC739759, LC739760, LC739761, and LC7397-62), as well as the accession numbers of the gag amino acid sequence (BDU28062, BDU28063, BDU28064, BDU28065, BDU28066, BDU28067, and BDU28068). **Conclusion:** HIV-1 subtype A1 in Thi-Qar province, valine at position 16 instead isoleucine. Because of the absence of a previous study in Iraq on HIV gag gene variation, subtypes identification, there is a need to conduct more studies to assist public health plans in limiting the distribution of the virus, treating, and eliminating infected people.

Keywords: *gag*, HIV-1 subtype, PCR, phylogenetic tree, RNA, Thi-Qar

Introduction

Characterizing pathogen assemblies from well-defined and demographically diverse cohorts is crucial for understanding pandemics. Phylogenetic analysis can be used to trace the history of the virus and shed light on how and where different strains emerged. HIV-1 is genetically complex, with a number of distinct subtypes and circulating recombinant forms (CRFs) that circulate in the world. Studies have shown both continuous and frequent transmission of HIV-1 across country borders, despite the fact that diversity and distribution of subtypes vary across geographic regions, genome sequence that cover partial *gag*, *pol*, and full the *env* gene, Africa showed the greatest variation in subtype composition, whereas those from the United States were the most uniform, with transmission patterns for the four most common strains (A, B, C, and CRF01-AE).[1] Drug resistance profiles can be determined through HIV-1 genotyping, which is based on the use of a combination of consensus and degenerate primers targeting the HIV gene.[2] Support for antiretroviral therapy response, transmission of resistance mutations, disease progression, and viral spread can all be strengthened with data on HIV molecular epidemiology, which is necessary for verifying the dynamics of the HIV/AIDS epidemic in different regions. HIV epidemic prevention and control strategies rely heavily on molecular epidemiological data.[3] Improving the prognosis for ART patients through routine HIV-1 genotyping before ART, immediate ART, and individualized ART regimens.

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Subtypes differ in their incidence immune recovery or immunologic failure.[4] Iraq is one of the low-infection countries, but the community knowledge is at a medium level about ways to prevent the virus.[5] Because of the absence of previous studies in Iraq of HIV subtypes, this study aimed to detect the genetic variation in *gag* gene, identify HIV subtypes, and draw the phylogenetic tree in Thi-Qar Province, Iraq.

Materials and Methods

Collection of samples

In current experimental study, RNA was extracted from 39 plasma samples that appeared to be infected by enzyme-linked immunosorbent assay (ELISA) from 43,850 blood donors during the period from February 2022 to February 2023, in the main blood bank in Thi-Qar province, with the aim of confirming the infections by using polymerase chain reaction (PCR) (which showed 30 cases only, all of them males, their ages between 30 and 39 years), and determining the genetic variation in the gag gene, in addition to identifying the subtypes of HIV-1. Positive samples were collected from infected patients after their consent and stored in deep freeze at -70ºC. The samples were transferred to the genetic engineering laboratory at Al-Ameen Center for Advanced Biotechnology, Department of Virology in Najaf Province.

Viral RNA extraction

Viral RNA was extracted from 1 mL of plasma for 39 samples that appeared positive by ELISA assay, by using the FavorPrep viral RNA/Viral Nucleic Acid Mini Kit (50 preps), FAVORGEN Biotech Corp., Cat. No: FAVNK001-1, Lot. No: CB806-122103, Taiwan, according to the manufacturer's instructions available at link: https://labturbo.com/wp-content/uploads/2022/07/FAVNK-000-1-001-001_1-001-2-1.pdf.

Synthesis of cDNA

RNA was converted to DNA by the following components: 5 µL of total RNA, 1 µL of dT, 1 µL of dNTP, 3 µL of nuclease-free water then incubated at 65°C for 5 min and immediately put on ice for 2 min. Has been added: 7 µL of nuclease-free water, 1 µL reverse transcriptase (RT) enzyme, and 2 µL RT reaction buffer then incubated at 85°C for 5 min after 60 min at 42°C.[6]

Nucleic acid amplification

Nucleic acid amplification protocol PCR as following: 95°C at 5 min for 1 cycle, 94°C at 50 s, 65°C at 40 s, 72°C at 35 s for 35 cycle and 72°C at 3 min for 1 cycle. Primer sequence of HIV-1 *gag* gene forward 5′-CAGAAGGAGCCACCCCACAAG AT-3′ and reverse 5′-TTCCTGCTATGTCACTTCCCCTTGG-3′ with size 189 bps.[7] Then, electrophoresis in agarose gel of the PCR product showed that there were only 30 infected samples.

Sequencing

The respective PCR products of HIV-1 positive for 11 samples were sent to South Korea, Macrogen Company, for sequencing. After completing the sequence by Macrogen Company and receiving the results, the Finch TV software was used to check the quality of the sequences. Seven samples that appeared to have a good sequence were recorded in the GenBank, with accession numbers obtained for sequence of *gag* gene. When the start of codon was determined, accession numbers were obtained from NCBI for the amino acids sequence of gag protein. After that, similar and most identical isolates were searched by using NCBI-BLAST database.

HIV subtypes

HIV subtypes were identified using REGA HIV-1 subtyping tool-version 3.0 by link http://dbpartners.stanford.edu:8080/RegaSubtyping/stanford-hiv/typingtool/ after entering accession number is followed by the nucleotide sequence, then press (ANALYZE).[8]

Statistical analysis

Chi-square was used and there were no significant differences between ELISA and PCR. It was expected that the chi-square would be used to analyze the results of subtypes in the event that they are different, and since the results of all isolates belong to the same subtypes, this does not enable us to perform the statistical analysis of subtypes.

Ethical approval

The ethics of scientific research was adhered to according to the Helsinki Declaration, and the approval of the health institution was obtained with document numbered 1387 in November 11, 2021 before the start of this study, in addition to written consents from patients to donate a sample to complete this article.

Results

The sequence of the HIV-1 *gag* gene for 11 samples that were sent to Macrogen Company, Korea, seven samples showed high similarity rates compared to the isolates in the GenBank database, where Nucleotide BLAST-NCBI was used. These isolates were similar to isolates KU921938 and KU921935 at 100% that were studied in Kenya, and were very close to isolates KU921937, KU921936, and KU921934 at a rate of close to 99.14%, with AF071473 and EF161036 at a rate of 98.27% that was also studied in the same country. The seven isolates were recorded in the GenBank with accession numbers (LC739756, LC739757, LC739758, LC739759, LC739760,

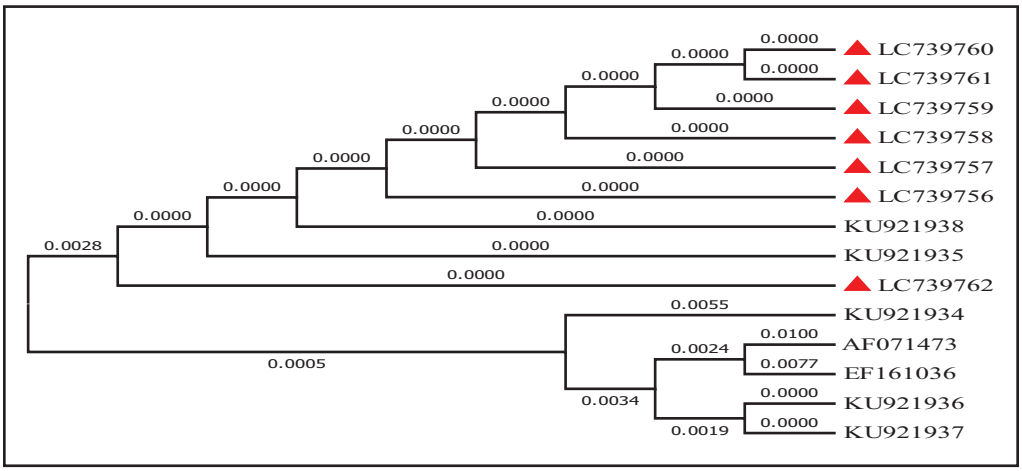


Figure 1: Phylogenetic tree of HIV by MEGA7 software depended on *gag* gene sequence

LC739761, and LC739762). The sequence analysis was shown by using the MEGA7 program to analyze the phylogenetic tree of the HIV, where there was a clear affinity for the nucleotides of the isolates of current study with the isolates in the database, which bear accession numbers (KU921938, KU921935, KU921934, AF071473, EF161036, KU921936, and KU921937), respectively [Figure 1].

HIV-1 subtyping was identified by REGA HIV-1 subtyping tool-version 3.0. REGA is characterized by high performance and scalability and is suitable for analyzing sequences with the identification of HIV-1 subtypes, using accession number and FASTA sequence in the HIV databases (REGA HIV Subtyping Tool) and link http://dbpartners.stanford.edu:8080/RegaSubtyping/stanfordhiv/typingtool/ it showed that all isolates belong to the subtype A1 as Figure 2.

HIV-1 gag protein sequence

The gag protein sequence was obtained by identifying the start codon when submitted to NCBI as confirmed by sequencing the nucleotides of the HIV-1 *gag* gene for isolates of current study by using blastx-NCBI. All nucleotides of the *gag* gene for seven isolates produced gag protein in blastp. It was registered in the GenBank with accession numbers (BDU28062, BDU28063, BDU28064, BDU28065, BDU28066, BDU28067, and BDU28068). It showed different matching percentages with the isolates recorded in the GenBank as in Table 1, and the alignment showed the transformation of valine (V) in isolates of current study at the position 16 with the isoleucine (I) in the isolates registered in pairwise form as in Figure 3.

Discussion

In current study, the results of sequence for seven HIV-1 samples in Thi-Qar province belonged to the A1 subtype.

A defining characteristic of the HIV-1 epidemic is the large genetic diversity.[8] Subtypes and recombinant forms of HIV-1 have emerged and spread in a non-uniform of the worldwide.[9] The rate of disease progression may vary within a geographical area according to subtypes.[10,11] Subtype A1 began in the Congo at the end of the 1950s.[12] Then expanded to other countries and diversified to lead to the emergence of combined forms.[13] The prevalent disseminated strain in Iran, the CRF35 AD subtype, has been identified.[14] But another study showed that the A1 subtype is more prevalent in patients among infected.[15,16] In contrast, subtypes B, CRF01-AE, and CRF02-AG are most common in Turkey, while subtypes C, A1 and F1 are of low prevalence.[17] Subtypes C, B, A, D, and G are most prevalent in Saudi Arabia,[18] and in Kuwait, subtypes B, C, and A are the most prevalent.[19] Subtype A1 is prevalent in Pakistan and parts of Eastern Europe, Russia, Africa and Latvia.[20,21] And is increasing among new infections in Portugal and other Western European countries.[22] Kostaki *et al*.[23] they mentioned that A1 represents 93.8% of the subtypes among those infected in Greece, by analyzing 57.2% of those infected with HIV-1. Thus, Greece is considered a major source of A1 transmission to Europe and other remote places as far as America and Australia.[24] The A1 subtype has the highest prevalence in Kenya.[8] There is a close relationship between the A1 subtypes in both Kenya and Pakistan.[20] Subtype A1 has been previously described as a virus subtype that can contribute to the slow progression of symptoms in infected people due to its low viral load and slow decrease in CD4 count compared to other subtypes such as B.[22]

Perhaps the infection reached Iraq through the American occupation soldiers or through African workers. Or because of the travel or emigration of Iraqis to European and other countries where the A1 subtype is widespread. Or through Pakistanis, because there is a close association between the subtype A1 in Kenya and Pakistan.[20] In Iraq, we did not find any study that dealt with the subtypes of HIV, whether in governmental or private institutions.

By sequencing the amino acids of the HIV-1 gag protein in the current study, the valine was found to be substituted

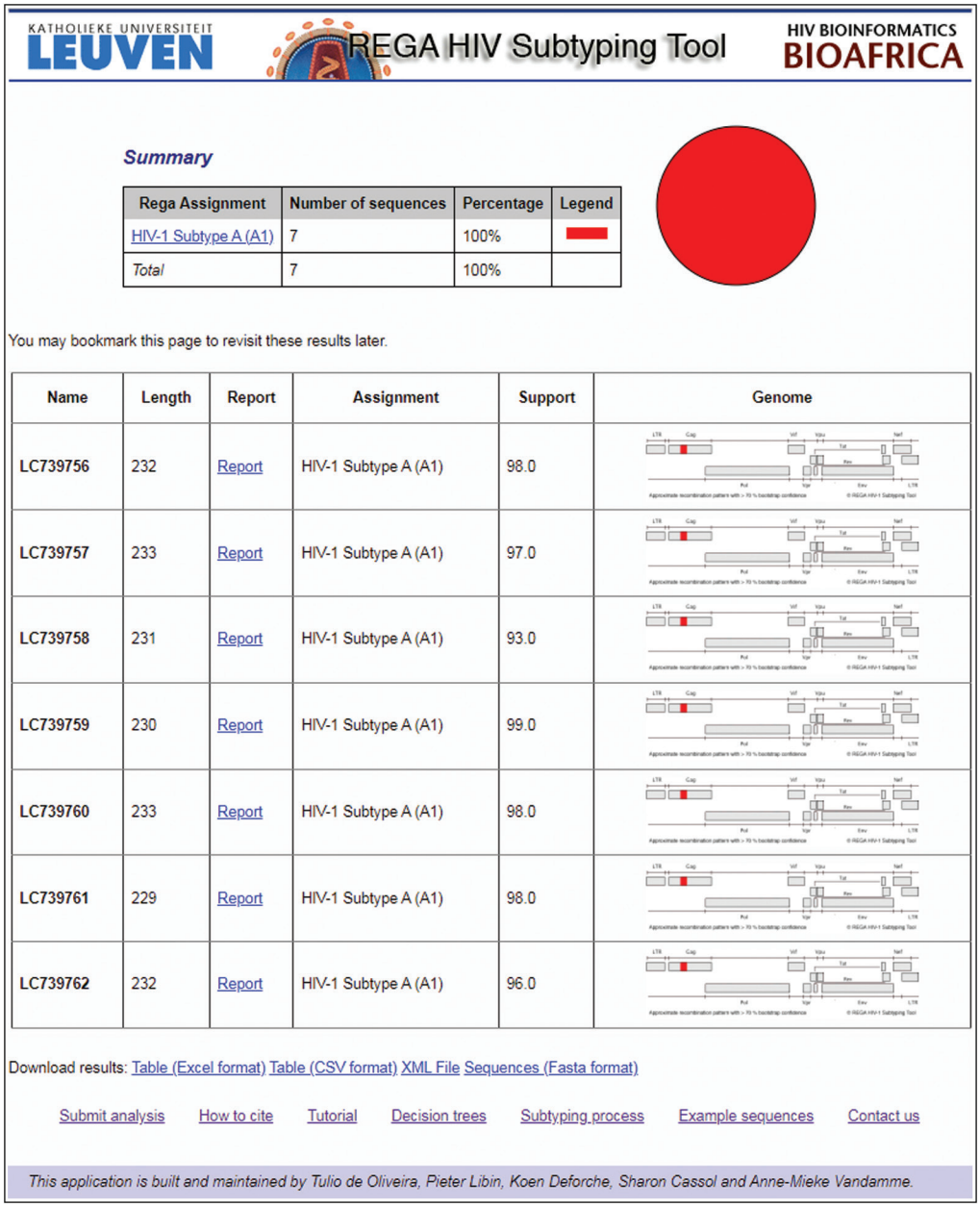


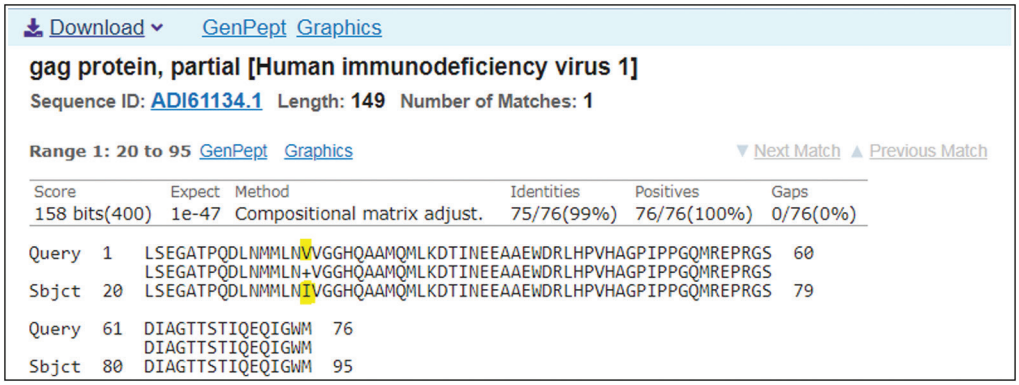
Figure 2: REGA HIV subtyping tool after input isolates accession number and sequence in FASTA forma

Table 1: Matching percentages with the isolates recorded in the GenBank

No.	NCBI homology gag protein sequence				
	Accession number	Location	Substitution amino acid	A.N*. of matching isolate	Identity
1	BDU28062	16	V/I	ADI61134	98.70%
2	BDU28063				
3	BDU28064				
4	BDU28065				
5	BDU28066	16	V/I	QIN89781	98.68%
6	BDU28067	16	V/I	ADI61134	98.70%

* Accession number

with isoleucine at position 16. This is consistent with the study of Meher *et al.*[25] which stated that replacing isoleucine with valine leads to the loss of the methyl group, which reduces the interaction with the central phenylalanine of some drugs, resulting in reduced binding to the drug or a decrease in the Van der Waals energy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gag protein, partial [Human immunodeficiency virus 1]

Sequence ID: ADI61134.1 Length: 149 Number of Matches: 1

Range 1: 20 to 95 GenPept Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Method	Identities	Positives	Gaps
158 bits(400)	1e-47	Compositional matrix adjust.	75/76(99%)	76/76(100%)	0/76(0%)

```
Query  1   LSEGATPQDLNMMLNVVGGHQAAMQMLKDTINEEAAEWDRLHPVHAGPIPPGQMREPRGS  60
           LSEGATPQDLNMMLN+VGGHQAAMQMLKDTINEEAAEWDRLHPVHAGPIPPGQMREPRGS
Sbjct  20  LSEGATPQDLNMMLNIVGGHQAAMQMLKDTINEEAAEWDRLHPVHAGPIPPGQMREPRGS  79

Query  61  DIAGTTSTIQEQIGWM  76
           DIAGTTSTIQEQIGWM
Sbjct  80  DIAGTTSTIQEQIGWM  95
```

Figure 3: Alignment of gag protein sequence it showed a correlation 99% with the isolation ADI61134.1 in GenBank database in pairwise form

between valine and the drug. While Meher and Wang,[26] mentioned that replacing valine with isoleucine leads to an increase in the interaction between the methyl group with the central phenylalanine of the drug, which increases the separating barrier (unfavorable interactions) and reduces the affinity of the drug's binding, so the entropy increases as a result of this modification. Therefore, entropy eventually stresses the binding affinities and leads to lower binding free energies and causes drug resistance.[27]

Amino acid change in gag protein leads to increased sensitivity to protease inhibitor drugs. Changing from valine to isoleucine produced little viral replication capacity.[28] Gag proteins are complex proteins that regulate many different processes in the viral life cycle and the interactions with proteins in the host cell,[29] drug-induced modifications to gag may mediate enhanced sensitivity to inhibitors by altering multiple gag functions. A decrease in the virus's proliferative capacity, rather than a hypersensitivity to the drugs, would be the expected result.[30]

Conclusion

The current study has shown the replacement of the valine at the position 16 in the isolates of this study with the isoleucine in the isolates registered in the GenBank. It was also shown that the HIV-1 subtype in Thi-Qar province through seven samples belonged to A1 subtype. Through the phylogenetic tree, it was shown that the isolates of this study are very similar to the isolates studied in Kenya and recorded in the GenBank. All of which call for further studies to determine the subtypes throughout the country and overcome the obstacles that faced, such as the inability to know the history of infection for each patient or to know the risk group that could be the cause of infection.

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Conflicts of interest

There are no conflicts of interest.

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