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ANALYSIS OF THE GENETIC DIVERSITY AND PHYLOGENETIC RELATIONSHIP OF HIV (GP-41) SEQUENCES FROM DIFFERENT REGIONS OF PAKISTAN

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Abstract

Background: The Human Immunodeficiency Virus (HIV) is a highly morphic, retrovirus that rapidly evolves through mutation as well as recombination. However, no significant data of sequenced glycoprotein (GP41) of HIV exist in Pakistan due to its fast mutation rate. The purpose of this study is to analyze the polymorphism rate in HIV envelop gene (GP41).

Methodology: The current study was conducted at The University of Haripur, Department of Microbiology. Initially, RNA extraction and reverse transcription were carried out under Punjab

AIDS Control Program, the molecular analyses was carried out at the microbiology lab of The University of Haripur. Molecular sequencing was done by the Center for Applied Microbiology (CAMB), Lahore and the blood parameters were analyzed at the Yahya Welfare Complex Haripur, KP, Pakistan.

Results: Out of 57, thirty seven (37) were identified as HIV positive by CMIA method and confirmed by PCR. Out of 37 isolated, 4 (10.8%) isolates carry GP41 gene. GP41 of HIV1 was divided into 2 fragments of 494 bp and 552 bp for PCR amplification, after sequencing process, the resultant sequences were merged to obtain a complete GP41 sequence. Bioinformatic analysis of sequenced data detected substitutions in 11 codon positions. Alanine substitution with threonine, arginine with cysteine, serine with proline, serine with leucine, valine with leucine, serine with alanine were detected. Some of these substitutions were related to enfuvirtide resistance.

Conclusion: This study serves as a platform for healthcare providers to monitor HIV-related clinical biomarkers, preventing early toxicity, improving patient quality of life, and reducing mortality rates. The study's polymorphism analysis reveals substitution points for refined drug design against the frequently mutating envelope gene, enhancing therapeutic resistance coverage.

Keywords: Human Immunodeficiency Virus, HIV associated biomarkers, polymorphism, envelop gene GP41.

Background

The Human Immunodeficiency Virus (HIV) falls within the retrovirus category known as lentiviruses. Retroviruses employ their RNA to synthesize DNA within the host cell. HIV primarily targets the immune system, particularly CD4 cells—a subset of white blood cells crucial for combating infections. By attacking these CD4 cells, HIV undermines the immune system, thereby rendering the body less capable of effectively defending against infections [1].

Over 1.8 million new infections emerge each year across the world. According to estimates,Since its initial emergence, HIV has affected more than 70 million individuals and tragically claimed nearly 35 million lives. Compelling evidence now suggests that the presence of stigma and bias against individuals living with HIV, as well as those susceptible to infection, plays a significant role in the scarcity of HIV treatment, screening initiatives, and access to essential drug therapies [2]. At the final moment of 2016, an approximately 11.2 million HIV-positive persons in the world were unconscious of their disease, another 6.0 million who were conscious of their illness were not on antiretroviral medication, and also another 3.6 million who had been on medication were not considered seriously [3, 4].

The escalating HIV-1 epidemic within Pakistan constitutes an emerging and pressing matter of public health importance. With the initial report of the inaugural HIV-1 infection case in 1987, a consistent upward trajectory in the incidence of infections has persisted. By the year 2020, the estimated population of individuals living with HIV in Pakistan had surged to approximately 180,000, underscoring the gravity of the situation. [5]

Several medications have been developed to impede the fusion or entry of HIV into host cells. [6, 7] However, it's important to note that none of these drugs possess the capability to entirely eradicate the virus from the body [8, 9]. The underlying reason for this lies in HIV's propensity for rapid mutation and genetic variability, enabling it to effectively elude the immune system's responses and cultivate resistance against therapeutic agents [10-13].

Methodology

The current study was conducted at The University of Haripur, Department of Microbiology. Initially, RNA extraction and reverse transcription were carried out under Punjab AIDS Control Program, the molecular analyses was carried out at the microbiology lab of The University of Haripur. Molecular

sequencing was done by the Center for Applied Microbiology (CAMB), Lahore and the blood parameters were analyzed at the Yahya Welfare Complex Haripur, KP, Pakistan.

Conset Development

A Pictorial Questionnaire (PQ) was employed as a data collection tool to ascertain information pertaining to the subjects' living conditions, daily routines, travel history, and sexual behavior. Stringent measures were in place to ensure the confidentiality and anonymity of both data and samples. Prior to participation, all study subjects were provided with comprehensive details about the study's objectives and procedures, and their informed written consent was acquired.

Ethical Approval

Ethical clearance for this study was secured from the University of Haripur's Ethical Review Committee (Approval No: UOH/DASR/2021/4955) and the Punjab AIDS Control Program, which facilitated the collection of samples. Each HIV-positive patient involved in the study provided written consent after receiving comprehensive information regarding the study's purpose, objectives, and procedures, ensuring their informed participation.

Samples Collection

Blood specimens from individuals diagnosed with HIV were procured from various diagnostic centers affiliated with the National AIDS Control Program (NACP). The collection process ensued only after securing written informed consent from the patients, demonstrating their comprehension and agreement. Subsequently, a total of 196 samples underwent further processing as part of the study.

Inclusion criteria

The cohort of individuals living with HIV included participants aged 18 years and above, possessing CD4 cell counts surpassing 200 cells/mm^3, with a viral load exceeding 1,000 copies/mL. Additionally, their inclusion was contingent upon possessing ready access to comprehensive historical and contemporary medical, as well as laboratory records.

Exclusion criteria

Individuals below the age of 18 were excluded due to their lower prevalence of HIV positivity in the Pakistani context. The selection criteria encompassed those with CD4 cell counts below 200 cells/mm3, rendering them susceptible to bacterial and fungal opportunistic infections. Moreover, participants with an HIV viral load below 1,000 copies/mL were deliberately chosen to ensure a representation of lower viral load quantities.

Molecular Identification

RNA Extraction

The extraction of viral RNA from blood samples of HIV-positive individuals was conducted using the QIAamp Viral RNA Mini Kit (Qiagen, Valencia, CA) Catalog No. 740956. Out of the total 37 samples, RNA extraction was performed on 16 samples. The quality of the extracted RNA was evaluated through 1% agarose gel electrophoresis. Subsequently, the quantification of the extracted RNA was carried out using a NanoDrop instrument (Optizen, Mecasys, Korea), and the readings were captured at wavelengths of 260/280.

Figure 1: Quantification of RNA by NanoDrop

Table 1: Reverse Primer for cDNA Synthesis

Name	Primer Sequence	Primer Length	Melting Temperature	GC Content %
HIVCR1	TGCTAGAGATTTTCCACACTGAC	23	61℃	44

cDNA Synthesis

Subsequent to RNA extraction, a comprehensive array of components was employed for reverse transcription of the viral RNA into complementary DNA (cDNA). This encompassed complete viral RNA, reaction buffer, reverse transcriptase enzyme, a designated primer, deoxynucleotide triphosphates (dNTPs), and an RNase inhibitor. This process was conducted through the utilization of a commercially available kit, specifically Solis BioDyne's FIREScript® RT cDNA Synthesis kit. The ensuing cDNA was then integrated into a polymerase chain reaction (PCR) reaction. This entire sequence of actions took place within a Multigene Optimax thermal cycler (Labnet, USA). Post cDNA synthesis, the resulting cDNA could be promptly employed for the amplification of the target gene or stored at a temperature of -20℃ for future use.

Polymerase Chain Reaction

The molecular characterization of HIV was achieved through the application of polymerase chain reaction (PCR) methodology. To discern specific segments within the envelope gene of HIV, two distinct pairs of primers were employed. These primer sets were thoughtfully chosen to match particular conditions conducive to the accurate identification of the target sequences.

Primers Used in the Study

This study entailed the assessment of the HIV envelope gene (GP41) through the implementation of polymerase chain reaction (PCR). To facilitate the detection of the transmembrane protein or glycoprotein GP41, a set of four primers was employed. These primer sequences were meticulously designed using the Oligo Calculator tool and subsequently validated via Primer BLAST analysis (http://www.hiv.lanl.gov/) for their precision and suitability. Detailed primer sequences can be found in Table 3.2.

Table 2: PCR primers for the identification of Envelop gene (GP41) of HIV.

Name	Primer Sequence	Primer Length	Product Length (bp)	Melting Temperature (℃)	Annealing Temperature (℃)
TMF1	CATGGGCCAAGTTCCGAGC	21	494	65	59
TMR1	CGGACATCGGGAGGAGC	25		63	
TMF2	GGGGGGTACTAGGAACACG	22	552	61	56
TMR2	CCCTCGAAGGTGGATCGAG	23		61	

Conditions for Polymerase Chain Reaction

The Multigene Optimax thermal cycler (Labnet, USA) served as the instrumental platform for conducting polymerase chain reaction (PCR) to identify the transmembrane protein GP41 within the envelope gene glycoproteins of HIV-1. To execute a single PCR, a final reaction mix of 25 µL was meticulously prepared. This involved adding 2 µL of cDNA and 5 µL of pre-prepared master mix (Solis BioDyne), along with 1.5 µL of both forward and reverse primers. The remaining 15 µL was supplemented with autoclaved PCR water, culminating in the total volume of 25 µL. Subsequent to a brief centrifugation at 5000 rpm for 30 seconds to ensure thorough mixing, PCR amplification was carried out under the following conditions: an initial denaturation step at 95°C for 5 minutes, followed by 40 cycles, each consisting of denaturation at 95°C for 45 seconds, variable annealing temperatures for 30-45 seconds, initial primer extension at 72°C for 1 minute, and concluding with a final extension at 72°C for 10 minutes.

Gel Electrophoresis

The Multigene Optimax thermal cycler (Labnet, USA) was the chosen platform for conducting the polymerase chain reaction (PCR) aimed at identifying the transmembrane protein GP41 within the envelope gene glycoproteins of HIV-1. For each individual PCR reaction, a precisely crafted solution of 25 µL was prepared. This entailed the addition of 2 µL of cDNA, 5 µL of a pre-prepared master mix (Solis BioDyne), and 1.5 µL of both forward and reverse primers. The remaining 15 µL was complemented with autoclaved PCR water, culminating in a final volume of 25 µL. After a brief centrifugation at 5000 rpm for 30 seconds to ensure thorough mixing, the PCR amplification was executed under specific conditions: commencing with an initial denaturation at 95°C for 5 minutes, followed by a series of 40 cycles. Each cycle involved denaturation at 95°C for 45 seconds, variable annealing temperatures lasting 30-45 seconds, an initial primer extension at 72°C for 1 minute, and concluding with a final extension at 72°C for 10 minutes.

Statistical Analysis

Microsoft Excel 2019 (Microsoft Corporation, U.S) was used for statistical analysis. Quality metrics of data were percentage, mean, standard deviation. Comparison of basic and clinical parameters was done by independent sample t-test and chi-square test. Resulted P value < 0.05 was considered as a significant level.

Molecular Sequencing

Samples were sent to the Center for Applied Microbiology (CAMB), Lahore showed Envelop gene presence. Further mutation analysis was performed using the clustal omega multiple sequence alignment tool.

Sequence Homology of GP41 of HIV-1

The two fragments of GP41 were acquired after molecular sequencing were combined to obtain complete sequences of GP41 and named ABC-GP41. Sequence analysis revealed that the complete sequence size of Sequence 1 was 1046 bp. BLAST result shown 99.71 % homology with sequence ABC-GP41 figures 3.2 and 3.3.

Results and Disscussion

Thirty-seven (37) blood samples of HIV positive patients were collected from Ayub Medical Complex Abbottabad, Armed Forces Institue of Pathology Rawalpindi, Pakistan Institue of Medical Sciences Islamabad, Hayatabad Medical Complex Peshawar, DHQ Hospital Attock, Mayo Clinic Lahore, Teaching Hospital Sarghoda Rawalpindi, linked with National AIDS Control Program (as per concerned authorities' approval). Twenty normal patients were included as controls. Detail of the study participants is given in (Table 4.1).

Mutation Analysis GP41 of HIV-1

To check the mutations in GP41 of HIV-1, the sequence was aligned with six reported sequences (MK148546.1, MK148476.1, MK148541.1, MK145778.1, MK148535.1 and MK148499.1) using CLUSTAL Omega Multiple Sequence Alignment tool figure 3.4.

```
MK148499.1      ATAGTGCTGTTAGCTTGTTTAACGCCATAGCTATTGCAGTAGCTGAGGGGACAGATAGGG    957
MK145778.1      ATAGTGCTGTTAGCTTGTTCAACGCCATAGCTATTGCAGTAGCTGAGGGGACAGATAGGG    957
MK148535.1      ATAGTGCTGTTAGCTTGTTCAACGCCATAGCTATTGCAGTAGCTGAGGGGACAGATAGGG    957
MK148476.1      ATAGTGCTGTTAGCTTGTTCAACGCCATAGCTATTGCAGTAGCTGAGGGGACAGATAGGG    957
MK148541.1      ATAGTGCTGTTAGCTTGTTCAACGCCATAGCTATTGCAGTAGCTGAGGGGACAGATAGGG    957
ABC-gp41        ATAGTGCTGTTAGCTTGTTCAACGCCATAGCTATTGCAGTAGCTGAGGGGACAGATAGGG    960
MK148546.1      ATAGTGCTGTTAGCTTGTTCAACGCCATAGCTATTGCAGTAGCTGAGGGGACAGATAGGG    957
                ******************* ****************************************

MK148499.1      TTATAGAACTAATACAAAGAACTTTTAGAGCTATTCTCCACATACCTAGAAGAGTGAGAC    1017
MK145778.1      TTATAGAACTAATACAAAGAACTTGTAGAGCTATTCTCCACATACCTAGAAGAGTGAGAC    1017
MK148535.1      TTATAGAACTAATACAAAGAACTTTTAGAGCTATTCTCCACATACCTAGAAGAGTGAGAC    1017
MK148476.1      TTATAGAACTAATACAAAGAACTTGTAGAGCTATTCGCCACATACCTAGAAGAGTGAGAC    1017
MK148541.1      TTATAGAACTAATACAAAGAACTTGTAGAGCTATTCGCCACATACCTAGAAGAGTGAGAC    1017
ABC-gp41        TTATAGAACTAATACAAAGAACTTGTAGAGCTCTTCTCCACATACCTAGAAGAGTGAGAC    1020
MK148546.1      TTATAGAACTAATACAAAGAACTTGTAGAGCTCTTCTCCACATACCTAGAAGAGTGAGAC    1017
                ************************ ******* *** ***********************

MK148499.1      AGGGCTTGGAAAGGGCTTTACTATAG      1043
MK145778.1      AGGGCTTGGAAAGGGCTTTACTATA-      1042
MK148535.1      AGGGCTTGGAAAGGGCTTTACTATAG      1043
MK148476.1      AGGGCTTGGAAAGGGCTTTACTATAG      1043
MK148541.1      AGGGCTTGGAAAGGGCTTTACTATA-      1042
ABC-gp41        AGGGCTTGGAAAGGGCTTTACTATAG      1046
MK148546.1      AGGGCTTGGAAAGGGCTTTACTATAG      1043
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Figure 2: Mutation analysis of ABC-GP41 with six other reported sequences of GP41. Several mutations may have been observed throughout the sequence.

gga agg gat act cac cat tat cgt tgc aga ccc tcc tcc caa ccc aga ggg gac ccg aca
G R D T H H Y R C R P S S Q P R G D P T
ggc ccg aag aaa tcg aag aag gag gtg gag aag gag aca gag gca gat cca ttc gac tag
G P K K S K K E V E K E T E A D P F D -
tag ccg gat tct tgc ctc tct tct ggg acg acc tga gga gcc tgt gcc tct tca gct acc
- P D S C L S S G T T - G A C A S S A T
acc gct tga gag act tac tct tga ttg tag cga gga ttg tgg aac ttc tgg gac gca ggg
T A - E T Y S - L - R G L W N F W D A G
ggt ggg aag ccc tca aat att ggg gga aca tcc tgc agt att ggg gtc agg aac taa aga
G G K P S N I G G T S C S I G V R N - R
ata gtg ctg tta gct tgt tca acg cca tag cta ttg cag tag ctg agg gga cag ata ggg
I V L L A C S T P - L L Q - L R G Q I G
tta tag aac taa tac aaa gaa ctt gta gag ctc ttc tcc aca tac cta gaa gag tga gac
L - N - Y K E L V E L F S T Y L E E - D
agg gct tgg aaa ggg ctt tac tat
R A W K G L Y Y

Figure 3: Amino Acids of ABC-GP41

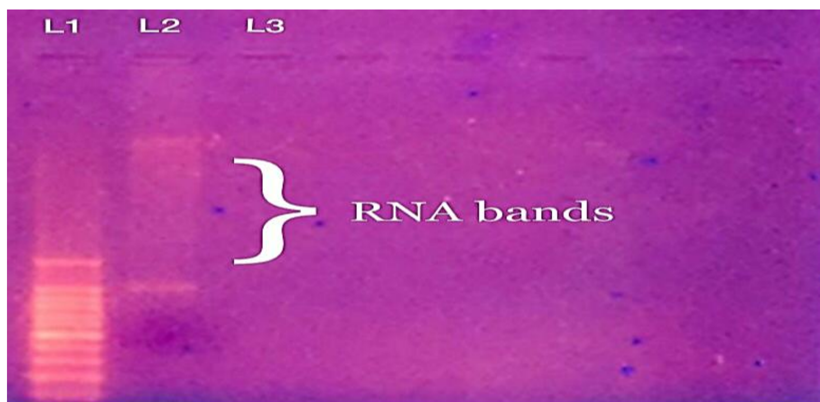

Figure 4: Agarose 1 % gel electrophoresis of HIV-1 RNA. Lane is denoted by L. L1 shows 100 bp RNA Ladder. L2 shows bands of RNA.

Table 3: Absorbance ratio of 260/280 and concentration of RNA in all HIV Positive Subjects.

S. No.	Sample ID	A260/ 280	RNA (ng/μL)	S. No.	Sample ID	A260/ 280	RNA (ng/μL)
1	Sa.1	1.64	84.7	20	Sa.20	2.31	653.8
2	Sa.2	1.45	77.5	21	Sa.21	2.08	1521.4
3	Sa.3	1.99	1318.2	22	Sa.22	1.56	121.5
4	Sa.4	2.52	1318.2	23	Sa.23	1.53	223.5
5	Sa.5	2.21	897.2	24	Sa.24	2.36	2235.2
6	Sa.6	1.23	1021.2	25	Sa.25	1.12	54.2
7	Sa.7	2.15	765.2	26	Sa.26	1.55	546.6
8	Sa.8	1.95	798.9	27	Sa.27	1.62	365
9	Sa.9	2.56	1698.3	28	Sa.28	1.55	652.2
10	Sa.10	2.1	1243.1	29	Sa.29	1.08	255.9
11	Sa.11	2.34	908.1	30	Sa.30	2.12	670.7
12	Sa.12	2.11	2329.2	31	Sa.31	1.34	908.1
13	Sa.13	2.24	1933.2	32	Sa.32	1.56	347.5
14	Sa.14	1.34	2226.2	33	Sa.33	1.82	230.9
15	Sa.15	2.18	706.7	34	Sa.34	1.43	257.3
16	Sa.16	2.11	838.2	35	Sa.35	1.15	89.4
17	Sa.17	1.54	345.2	36	Sa.36	1.56	320.6
18	Sa.18	1.43	84.2	37	Sa.37	1.62	564.3
19	Sa. 19	1.90	493.1				

Polymerase Chain Reaction of GP41 Fragment 1

A comprehensive analysis was conducted on a total of 37 samples to detect the presence of the envelope gene (GP41). These samples were derived from individuals diagnosed as HIV positive. Through a meticulous PCR analysis performed on these 37 samples, the genomic presence of the envelope gene (GP41) was elucidated. Remarkably, the outcome of this analysis revealed the identification of the envelope gene (GP41) within the genomes of four of the HIV positive patients.

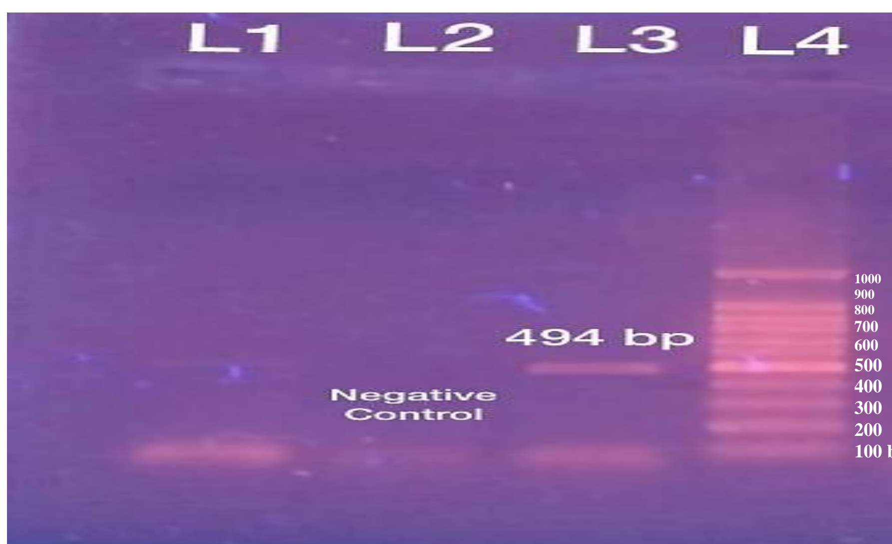

Figure 5: A 2% agarose gel electrophoresis was carried out to analyze the PCR products corresponding to the glycoprotein GP41 F1 of the HIV envelope gene. The lanes on the gel are designated as "L" In Lane 2 (L2), the negative control depicted. Positive bands indicative of the presence of the 1st fragment of GP41 are visible in Lane 3 (L3). In Lane 4 (L4), a 100 bp ladder is displayed, serving as a size reference for the fragments.

Polymerase Chain Reaction of GP41 Fragment 2

A comprehensive analysis encompassed the examination of 37 samples to ascertain the presence of the envelope gene (GP41). By conducting PCR analysis on the specimens collected from 37 patients

diagnosed with HIV, it was determined that a subset of four patients exhibited the presence of the envelope gene (GP41) within their genomic material.

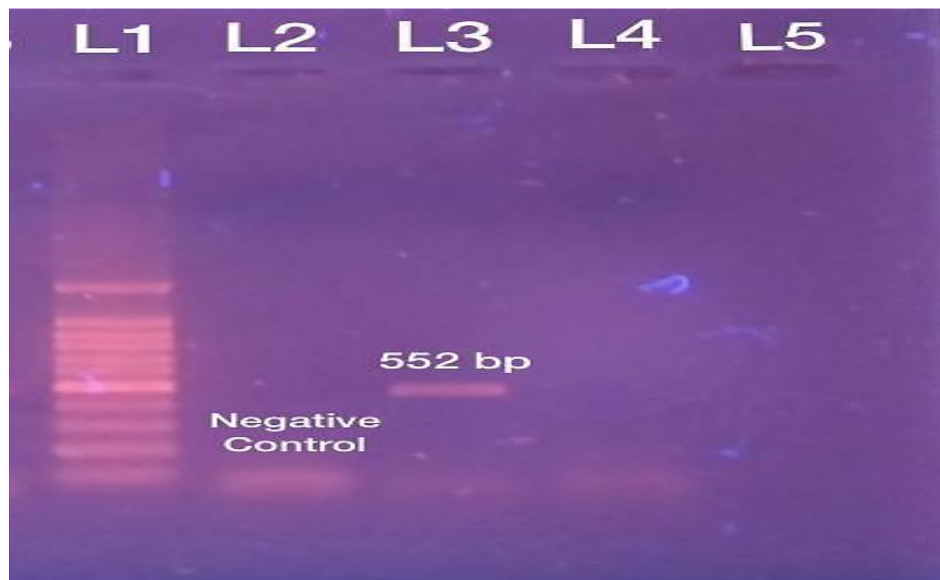

Figure 6: A 2% agarose gel electrophoresis procedure was employed to visualize the PCR products corresponding to glycoprotein GP41 F2 of the HIV envelope gene. The lanes on the gel are designated as "L" Lane 1 (L1) shows a 100 bp ladder used as a size reference. Lane 2 (L2) displays the negative control. Notably, positive bands indicative of the presence of the 2nd fragment of GP41 are discernible in Lane 3 (L3).

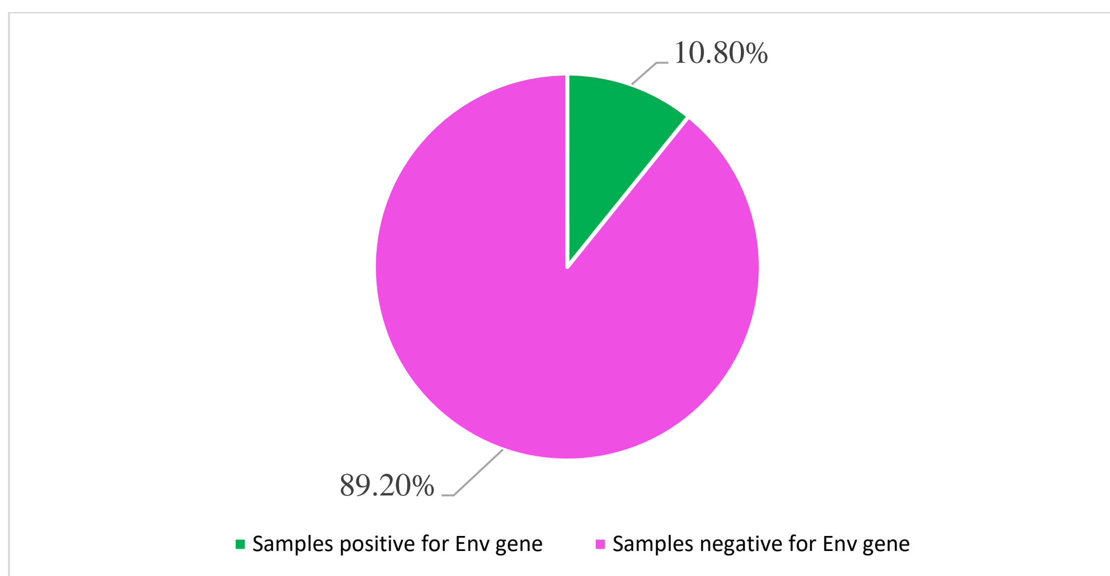

Figure 7: Graphical representation of samples showed bands in all two sets of HIV Envelop (GP41) gene primers. Out of 37 HIV-positive samples, the evelop gene GP41 was found in four (10.8%) samples that were sent to be sequenced.

This study was carried out to analyze the presence of polymorphism in GP41 of HIV-1. The substitutions obtained from the current study were shown in Table 4.16. Although only a minority of these substitutions had been previously reported as mutations related to enfuvirtide resistance [14, 15].

In current study 10 mutations and 1 polymorpm was detected through bioinformatic analysis on sequenced HIV envelop gene GP41. Obtained mutations were replacement of threonine with alanine on codon no. 161, cystine with arginine on codon no. 189, phenylalanine with cystine on codon no.

190, proline with serine on codon no. 193 and 194, Leucine with serine on codon no. 260, leucine with valine on codon no. 299, on codon no. 303 alanine with serine. *Only one polymorph identified at codon no 301. The highlighted mutations and polymorph from this study will help the therapeutics to cover resistance against envelop gene.

Table 4: List of polymorphs identified in ABC-GP41 of HIV-1 in comparison with 6 reported sequences.

S. No.	Codon No.	MK 148499.1	MK 145778.1	MK 148535.1	MK 148476.1	MK 148541.1	ABC-GP41	MK 148546.1
1	158	AC- -	AC- -	AC- -	AC- -	AC- -	ACG Thr	-
2	159	--A -	--A -	--A -	--A -	--A -	ACA Thr	--A -
3	161	GCT Ala	ACT Thr	ACT Thr	GCT Ala	GCT Ala	GCT Ala	GCT Ala
4	189	CGT Arg	CGT Arg	CGT Arg	CGT Arg	TGT Cys	CGT Arg	CGT Arg
5	190	TGC Cys	TTC Phe	TTC Phe	TTC Phe	TTC Phe	TGC Cys	TGC Cys
6	193	TCC Ser	CCC Pro	CCC Pro	TCC Ser	TCC Ser	TCC Ser	TCC Ser
7	194	TCC Ser	TCC Ser	TCC Ser	CCC Pro	TCC Ser	TCC Ser	TCC Ser
8	260	TTA Leu	TCA Ser	TCA Ser	TCA Ser	Ser	TCA Ser	TCA Ser
9	299	TTA Leu	GTA Val	TTA Leu	GTA Val	GTA Val	GTA Val	GTA Val
10	*301	CTA Leu	CTA Leu	CTA Leu	CTA Leu	CTA Leu	CTC Leu	CTC Leu
11	303	TCC Ser	TCC Ser	TCC Ser	GCC Ala	GCC Ala	TCC Ser	TCC Ser

Nevertheless, the Env gene has emerged as a novel focal point for the initial class of antiretroviral medications targeting HIV-1, garnering substantial global attention and undergoing extensive investigation. A spontaneous CD4 independent entry phenotype was studied that suggests direct entry ans virus attachment by GP120 and GP41. They co-related this phenotype to seven mutations in various regions of GP120 of env gene of HIV-1 such as C2, C3, V3 loop, but no mutation in GP41 was reported [16, 17].

Compensatory mutations within the env gene precipitate amino acid substitutions that confer a wide-ranging capacity for evading defects in virus replication. Moreover, these mutations play a pivotal role in enabling a remarkably efficient mode of cell-to-cell transmission [18, 19].
A study has reported instances where recombination within the env region gives rise to defective mutants. This phenomenon not only fosters the virus's phylogenetic evolution but also amplifies the diversity within the viral population. Importantly, these seemingly nonfunctional genetic elements can potentially undergo transformation, transitioning from inert entities to functional components with valuable roles [20, 21].

Conclusion and Recommendations

Among the total of 57 subjects examined, a subset of 37 individuals were conclusively identified as HIV-positive through the CMIA method, a determination that was subsequently verified by PCR. From this pool of 37 confirmed isolates, a distinct subset of 4 isolates (constituting 10.8%) was found to harbor the GP41 gene. The genetic composition of HIV1's GP41 was partitioned into two segments, measuring 494 bp and 552 bp, respectively, to facilitate PCR amplification. Post the sequencing

procedure, the resultant sequences derived from these segments were meticulously integrated to yield a comprehensive and unified GP41 sequence. Bioinformatic analysis of sequenced data detected substitutions in 11 codon positions. Alanine substitution with threonine, arginine with cysteine, serine with proline, serine with leucine, valine with leucine, serine with alanine were detected. Some of these substitutions were related to enfuvirtide resistance as reported in HIV-1 can lead to death due to a compromised immune system and resistance to drugs being highly variable.
Numerous pharmaceutical agents have been developed with the aim of managing the disease progression resulting from HIV-1 infection. The scrutiny of HIV-1's GP41 has enabled a comprehensive grasp of the genetic constitution of its surface gene. While novel therapeutic interventions offer promising prospects for enhancing the lifespan of individuals grappling with AIDS, the intricate biological intricacies of HIV pose formidable challenges in the quest for discovering a definitive cure and/or vaccine.
Emerging pharmaceuticals instill optimism for extended lifespans among those affected by HIV, yet the persistent specter of rapid mutability remains a daunting obstacle to the development of an efficacious curative vaccine. The insights garnered from this research will yield substantial advantages for drug therapeutics and the realms of microbiology and molecular biology, catalyzing subsequent explorations into subtyping, distribution dynamics, and the intricacies of drug design and possible spontaneous remission. Given the recurrent mutational nature of the env gene (GP41) and its role in generating drug resistance, a thorough mutational analysis coupled with molecular insights will illuminate pivotal mutation sites, thereby refining the precision of drug design. Furthermore, the clinical biomarker data derived from this study will empower healthcare providers to adeptly monitor fluctuations in blood parameters, curbing the necessity for toxic interventions and ultimately enhancing well-being and reducing mortality risks among HIV-exposed patients.

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