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The Complex Landscape of HIV-1: Clinical Correlates, Genomic Mutations, and Evolutionary Alignments

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KEYWORDS

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Glycoprotein 120;
Subtypes;
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Mutational analysis,
Clinical correlates

Abstract:

O The HIV-1 epidemic in Pakistan remains to be a danger to the public's health. The number of individuals afflicted with HIV-1 has been steadily rising since the discovery of the first case in 1987. We used 148 HIV-1 infected patient specimens with high viral loads and were involved in the study. Out of 148, we included 37 patients 26 Men and 11 women. There were more men than women among the HIV-positive participants in this study (n = 79.27%). Patients older than 38-45 made up the largest percentage of positive cases (56.7%). We go through the patient's medical record including, treatment, and clinical parameters. In which we found newly positive patients n=28, 75.67% with no treatment. A significant difference was found in TLC of the untreated group and untreated group (8.42 ± 2.05, 5.41 ± 1.37), and PLT (192.7 ± 23.45, 165.4 ± 13.62) whereas a general decrease in the prevalence of HB (10.01 ± 0.77, 12.8 ± 0.92) HIV1 positive patients. The findings of this study suggest that a significant difference was found in untreated group and untreated group ALT (43.32 ± 36.78, 31.5 ±

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28.21), AST (35.11 ± 13.42, 29.7 ± 6.77), Urea (41.3 ± 5.45, 19.4 ± 3.21) Creatinine (1.12 ± 0.43, 0.8 ± 0.12). We identified mutations in the genomic regions of HIV-1 against sequence-1 (Pro118Met, Leu193phe, Ser440Tyr, and Thr499IIe). Sequence-2 (Glu47Lys, Cys131Glu, Lys231Glu, Thr455Val). Sequence-2 (Tyr7His, Pro120Arg, Ser143Pro, Glu321Thr, Asn386Tyr). In this study, 26 nucleotide sequences were used, and the finished sample contained 1670 locations altogether. In MEGA 11, evolutionary studies were carried out. Sequence 2 and Sequence 3 showed the closest evolutionary relationship. Our amplified sequences aligned their mutual evolutionary relationship with sequences such as AB773885, AB254156, A04321, AF042105, and AB032740 retrieved from China, Japan, France, Australia, and Thailand extended from the same node. The findings of this study might be beneficial for future large-scale investigations on subtyping, sequencing, distribution, and the manufacture of drugs.

Introduction

The human immunodeficiency virus (HIV) relates genetically to the genus Lentivirus and the Retroviridae family and subfamily Orthoretroviridae. It is the causative agent of a highly contagious disease i.e. Acquired Immunodeficiency Syndrome (AIDS) [1]. HIV invades CD4+ T helper lymphocytes in the human body thus reducing the number of CD4+ cells however certain in vitro studies showed pathogenesis of the human immunodeficiency virus in certain other body cells such as B lymphocytes, natural killer (NK) cells, CD8+ cells and dendritic cells in the blood that needs further confirmation of infection in vivo [2,3]. HIV is known for its high mutation rate and thus the emergence of drug-resistant and mutant strains has necessitated designing new antivirals [4].

The HIV-1 epidemic in Pakistan remains to be a serious danger to the public's health. The number of individuals afflicted with HIV-1 has been steadily rising since the discovery of the first case in 1987 [5]. Initially, it was restricted to singular occurrences, but more lately, it has expanded to include breakouts and localised infections. There were 180,000 affected individuals in Pakistan by the middle of 2020 [6]. An HIV infection pandemic was discovered in Larkana last year, which sparked a localised spread across the nation with a 57% rise in the total affected population [7].

The rapid spread of HIV in Pakistan has emerged as one of the most significant challenges facing the country's public health [8]. According to projections provided by UNAIDS in 2021, the number of people living with HIV in Pakistan was approximately 160,000, and approximately 5,000 new cases were diagnosed each year. Researchers estimate that 0.1% of adults between the ages of 15 and 49 are living with HIV. However, it's possible that the reported numbers are lower than they actually are since there wasn't enough screening done. Since the beginning of the 2000s, Pakistan has seen a consistent increase in the number of deaths caused by HIV. According to figures provided by the World Health Organization (WHO), HIV was the cause of 6,900 fatalities in Pakistan in 2019 [9]. Drugs that block HIV fusion or entrance into host cells have been developed. Due to the high rate of change and genetic variety of HIV, no medication has been developed to date that can completely eradicate the patient's HIV virus. However, antiretroviral drugs can somewhat control the infection and enable patients to live longer and healthy life [10]. Precise characterization of HIV-1using the Env gene can help determine the genetic diversity among the virus that will further aid in drug designing [11]. HIV testing is not widely available in Pakistan, particularly in the more rural parts of the nation. Testing for HIV is primarily done in hospitals because it is so difficult to find therapy for the disease. People in this country have a difficult time acquiring access to diagnostic testing and medical treatment, which contributes to the already high prevalence of HIV in the country.

In Pakistan, both the infection and mortality rates due to HIV remain at very high levels. There is also a function for the constraints that are placed on the screening chances and the access to care. The gp120 protein is necessary for the pathogenicity of HIV and may play a role in the virus's ability to infect cells from a variety of species. Additional research is needed to completely understand the role that gp120 plays in the development of the HIV pathogenesis as well as its potential impact on zoonotic transmission [12,13].

HIV-1 is known for its highly diverse genome which makes it untreatable since it frequently develops drug resistance as a result of mutation [14]. This study aims to genetically analyze one of the most mutating regions of the HIV-1genome, i.e. Env gene (GP120) which carries five

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distinct variable regions, with a mutation rate of 40% among all of its structural genes [15]. Env gene is the primary gene that was studied to understand the mechanism of HIV entry to the host cell. The V3 loop, i.e., the third variable region in the Env gene (GP120) is the determinant of tropism of the virus by choosing the co-receptors (either CCR5 or CXCR4) for attachment of the virus to alternative host cells as the disease progresses. The increase in the population living with HIV-1countrywide requires rapid development of drugs to control the disease progression which makes this study more significant. Therefore, this research will provide a basis for the development of appropriate fusion inhibitors (drugs) with the most recent data from patients all over Pakistan [16].

Statement of a problem as HIV is known for its highest mutation rates, thereby causing resistance to the antiretroviral drugs making its treatment impossible. Therefore, the rate of mutation of HIV determines its genetic diversity which leads to the emergence of unique recombinant forms of HIV-1. Complete knowledge of all resistant strains is becoming difficult to trace the exact pathogenic mechanism of the virus. The study aimed to evaluate the blood parameter among treated group and untreated group, medication description and analyze the mutation rate of the Env gene Gp-120 of HIV-1 as well as determine the genetic diversity of the Env gene of HIV-1.

Materials and Methods

The study subjects and sample collection

To select the subjects for this study we defined an inclusion criterion which is as follows: (1) Predetermined viral load >1000 copies/ml was considered for this study [16], (2) we used >18 years subject's. (3) Informed written consents were taken from the subjects. After consents, the venous blood samples were collected from HIV-positive patients through venipuncture at National AIDS Control Program, Hayatabad Medical Complex, Peshawar and Punjab AIDS Control Program. One hundred forty eight samples were processed further, after being collected in EDTA tubes for plasma separation. Plasma was separated by centrifugation at 3000 rpm for 15 minutes, transferred to a biohazard bag and stored at -20°C according to the Undisa et al., [17].

RNA extraction and quantification

Plasma obtained from blood samples collected was thawed at room temperature and then viral RNA was extracted from the sample using QIAamp Viral RNA Mini Kit (Qiagen) [18]. RNA was quantified through NanoDrop and the absorption ratio A260/280 >=1.8 is considered for the next step studied by Chen et al., [19]. RNA was successfully extracted from seventeen samples as confirmed through quantification by using NanoDrop (Optizen NanoQ, Korea). Supplementary table S1 shows the results obtained after the spectrophotometric analysis of RNA.

cDNA synthesis

Thermo Scientific RevertAid cDNA synthesis kit was used for cDNA synthesis. cDNA synthesis conditions were applied as follows: 5 minutes at 25°C, 60 minutes at 42°C, and 70°C for 5 minutes as followed by Robins et al., [20]. The reverse primer i.e., HIVCR1 (*TGCTAGAGATTTTCCACACTGAC*) for cDNA synthesis from HIV-1RNA was used. The produced cDNA was either used right away for the intended gene's replication or it could be kept at -20°C.

PCR and sequencing of HIV-1Env gene GP120

To amplify the GP120 of the Env gene, three pairs of forward and reverse primers were designed to amplify the gene in three fragments as per Xiao et al., criteria [6]. The first gene fragment was amplified via polymerase chain reaction (PCR) with primers F1 *ATGAAAGTGAGGGGATCAGGAA* and R1 *TACCTCTCATGCTTGTGGTGATATTG* in a 50 μL reaction volume. In the first stage, the reaction conditions were as followed: 95°C for 5 mints in a single cycle; then 35 cycles of 95°C for 40s, 55°C for 40s and 72°C for 1 min; and followed by 72°C for 10 min, and lastly held at 4°C. Similarly, the second fragment of GP120 was amplified by using primers F2 *GGGGGGTACTAGGAACACG* and R2 *CCCTCGAAGGTGGATCGAG* by using same PCR conditions as for the first fragment. The third fragment of GP120 was also amplified using the same cycling conditions and primers F3 *TTGCATGTTGGGGACGAAGCC* and R3 *CTCGAACTTGGGCGGCGAG*. PCR products were validated using 1.5% agarose gel electrophoresis. The positive PCR products were sent for molecular sequencing. (Table 1)

Table 1: Amplification primers for the identification and confirmation of GP120 of HIV-1

Name	Primer sequence	Primer Length	Product Length	Annealing Position
HIVSPF1	ATGAAAGTGAGGGGATCAGGAAG	24		ED31

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HIVSPR1	TACCTCTCATGCTTGTGGTGATATTG	26	502	
HIVSPF2	GAGGTATGGTGACAGAATATGCAC	24		ES7
HIVSPR2	CCCCTCCTGAGGAGTGACTAAA	22	597	
HIVSPF3	GAGGGGATCCTGAAGTTGC	19		ES8
HIVSPR3	TCTTTTTTCTCTCTGCACCACTC	23	440	

Molecular Sequencing

Molecular sequencing by Macrogen, Korea

Macrogen, headquartered in South Korea is a biotechnology firm that provides a full suite of molecular sequencing services (https://www.macrogen.com/en/main). The process begins with collecting samples and ending with the distribution of processed, quality-controlled data. After the DNA has been isolated from the sample, a library of fragments has been produced for sequencing. After the DNA has been processed, it is fed onto a sequencing equipment and raw sequencing data is produced. In order to align the reads and either construct a de novo genome or align to a reference genome, the data is processed and evaluated by specialist software. The sequencing data is checked for accuracy and completeness through quality control procedures. Information on the sequencing data quality, assembly statistics, and detected genetic variants or mutations are all included in the final report delivered to the client. In sum, Macrogen's molecular sequencing approach is a robust and thorough process that makes use of cutting-edge technologies and careful analysis to provide clients with accurate and trustworthy sequencing data and findings.

Bioinformatics Analysis

We aligned the frequency of mutation of HIV-1 through specific bioinformatics tools such as NCBI-BLAST, CLUSTAL-OMEGA and MEGAX-11 software. To determine the evolutionary past, the Maximum Likelihood Method and the Tamura-Nei model were used. The branch that had the greatest log probability had been established (-19865.95). Using the Maximum Composite Likelihood (MCL), the initial tree for the heuristic search were automatically obtained by applying the Nearest-Neighbor-Join and BioNJ algorithms to an array of paired distances calculated method. The structure with the best log likelihood value was then chosen. The branch lengths were counted in terms of replacements by worry site, and the tree was compressed to size.

HIV-1Alignment and subtyping

We obtained sequence fragments of the Env gene (GP 120) were edited and assembled. The assembled sequences were then aligned together with the reference sequence (HXB2) using the Clustal Omega program (available at: https://www.ebi.ac.uk/Tools/msa/clustalo/). We constructed a phylogenetic tree using the neighbor-joining method with the MEGA-11 application. Before analysis, the sequences acquired were subjected to the online BLAST to compare with all known HIV sequences in the Los Alamos National Laboratory database (https://www.hiv.lanl.gov/content/sequence/BASIC_BLAST/basic_blast.html). The HIV-1 subtypes of each individual were based on the most similar genotype identified. Samples with different subtypes were considered unique recombinant forms as per defined Hemelaar et al., [21].

Results

Demographic of patients

Total thirty seven whole blood samples of HIV1 positive patients were collected in EDTA-K3 vacuum tubes from various diagnostic centers in Islamabad, Peshawar and Lahore. These samples were processed in Punjab AIDS Control Programme for RNA extraction and cDNA synthesis which was then brought to Molecular Virology laboratory of The University of Haripur for further processing and analysis. Plasma was separated by centrifuging whole blood samples at 3000-4500 rpm for 15 mints.

Due to interpersonal and institutional impediments to health care, no transgender individuals participated in the study, which included 26 Men and 11 women. There were more men than women among the HIV-positive participants in this study (n = 79.27%). Patients older than 38-45 made up the largest percentage of positive cases (56.7%).

Table 2: HIV1 positive Patient's demographic (n=37)

Patients description		**n=37**	**%=37**
Genders	Men	26	79.27
	Women	11	29.7

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	22-37	5	13.5
Age Groups	38-45	21	56.7
	>45	11	29.7
	Divers	17	45.9
Work Status	Labor	8	21.6
	Unemployed	12	32.4
Qualification	Enlighten	13	35.1
	Illiterate	24	64.8

Patients grouping based their Medical Record

We go through the patient's medical record including, treatment, and clinical parameters. In which we found newly positive patient n=28, 75.67% with no treatment.

Table 3: Medical record status of the HIV1 positive patients

Medical Status	n=37	%=37
Treated group	9	24.32
Untreated group	28	75.67

Table 4: Treatment prescribed by concern medical practitioner

Drugs Names	Drug State	Concentration (mg)	Tablets/pack	Drug prescribed (Age group)
Efavirenz, Lamivudine, Zidovudine	Tablets	150, 300, 600mg	90 tablets	22-37
Lamivudine, Neirapine, Zidovudine		150, 200, 300mg	60 tablets	
Efav renz, Lamivudine, Tenofovir		600, 300, 300mg	30 tablets	
Lamivudine/Tenofovir		300, 300mg	30 tablets	
Dolutegravir/Lamivdine/Tenofovir		50/300/300mg	30 tablets	
Zidovudine		300mg	60 tablets	>45
Lamivudine		150mg	60 tablets	
Nevirapine		200mg	60 tablets	
Efavirenz		600mg	30 tablets	

****All above drugs are locally prescribed by the specialists in the Pakistan under the guidelines and drug plan issued by the National AIDS Control Programme of Pakistan (https://nacp.gov.pk/whatwedo/treatment.html).**

Clinical Parameters of Treated group and untreated group

The findings of this study suggest that significant difference was found in leukopenia (TLC), and thrombocytopenia (PLT) whereas a general decrease in the prevalence of anemia (Hb) HIV1 positive patients as shown in (Table 2). The findings of this study suggest that significant difference was found Alanine Transaminase (ALT), Aspartate Ami-notransferase (AST), Urea and Creatinine.

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Table 5: Clinical variables among HIV1 positive patients

Clinical variables	Unit (SI)	Patients group	Treated group	Reference Ranges	P-value
Alanine Transaminase (ALT)	u/l	43.32 ± 36.78	31.5 ± 28.21	4-36	0.0108
Aspartate Aminotransferase (AST)		35.11 ± 13.42	29.7 ± 6.77	8-33	0.001
Total Bilirubin		0.9 ± 0.4	0.6 ± 0.2	0.1-1.0	0.598
Urea	mg/dL	41.3 ± 5.45	19.4 ± 3.21	5-20	0.000
Creatinine		1.12 ± 0.43	0.8 ± 0.12	0.7 to 1.3	0.390
Hemoglobin	g/dL	10.01 ± 0.77	12.8 ± 0.92	M=13.8 to 17.2 F=138 to 172	<0.0001
Leucocytes	10^3mm^3	8.42 ± 2.05	5.41 ± 1.37	4000-11,000	0.001
Platelets		192.7 ± 23.45	165.4 ± 13.62	150,000-350,000	0.001

RNA Extraction and Quantification

Plasma was then used to extract HIV1 RNA using QIAmp Viral RNA Mini kit (Qiagen, Germany).In order to evaluate the integrity of extracted RNA a part of it was run on 1% TAE agarose gel.

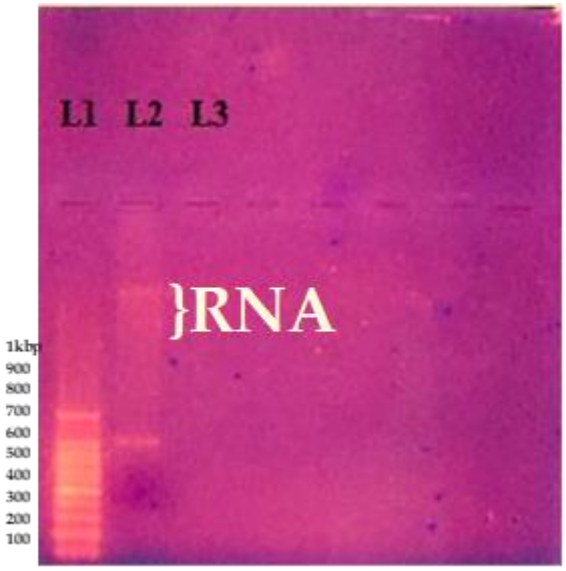

Figure 1: 1 % TAE agarose gel electrophoresis shows the results of RNA extracted from samples with high viral load. L1 shows 1kb DNA ladder and L2 shows RNA.

Amplification of GP-120 Of Human Immunodeficiency Virus TYPE-1 VIRUS

Surface glycoprotein GP120 was successfully amplified by the use of three set of primers. Amplificates were confirmed through fractionating on gel electrophoresis

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using 100 bp Thermo scientific ladder. Further molecular sequencing was done by (CAMB) Center for Applied Microbiology, Lahore.

PCR amplification of first fragment f1 of hiv1 GP-120 by using HIVSPF1/HIVSPR1 primers:

PCR amplification of first Fragment F1 of HIV1was performed. Out of 26, five strains showed presence of first segment of HIV1 as shown in (Figure 2)

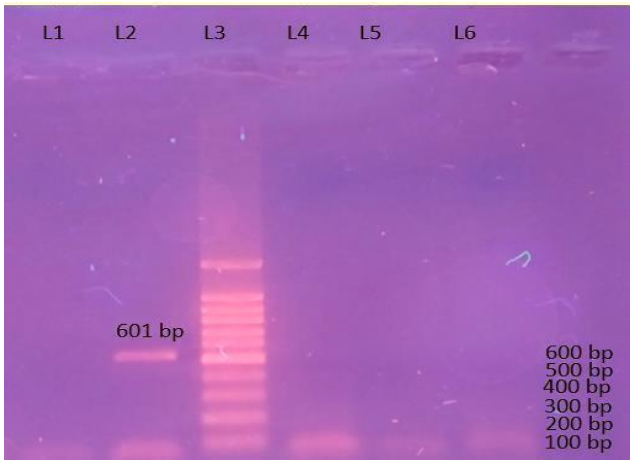

Figure 2: 1.5% TBE agarose gel electrophoresis shows the results of first Fragment F1of surface glycoprotein of Human Immuno Deficiency Virus type 1 (601 bp). Lane 1 refers to sample no 3, lane 2 refers to 4, lane 3 refers to 100bp ladder, lane 4 refers to sample no 10, and lane 5 refers to sample no 11, while lane 6 refers to sample no 12, respectively.

PCR amplification of second fragment F2 of HIV1GP120 by using HIVSPF2/HIVSPR2 primers

PCR Amplification of second fragment F2 of GP120 of HIV1was performed. Out of 26 strains, eight strains showed presence of second segment of HIV1as shown in (Figure 3).

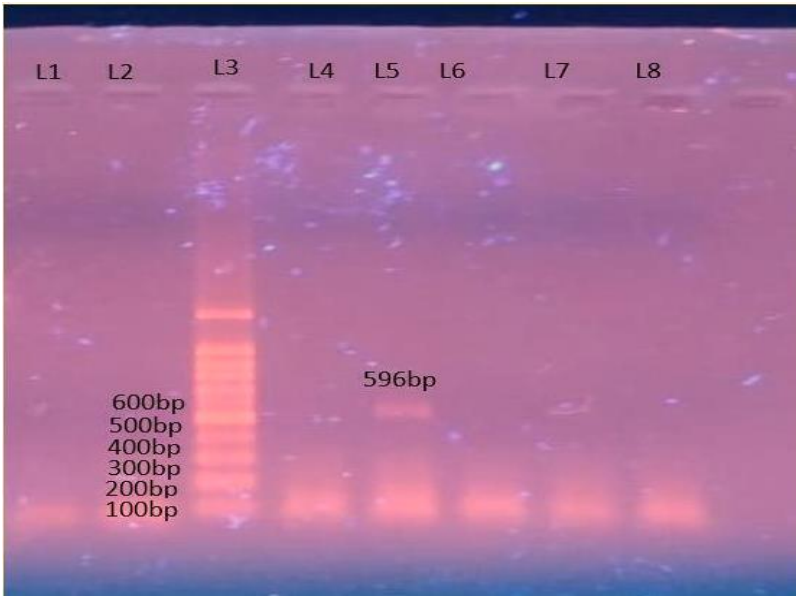

Figure 3: 1.5% TBE agarose gel electrophoresis shows the results of second Fragment F2 of surface glycoprotein of Human Immuno Deficiency Virus type 1 (596 bp). Lane 1 refers to sample no 3, lane 2 refers to 4, lane 3 refers to 100bp ladder, lane 4 refers to sample no 10, and lane 5 refers to sample no 11, while lane 6 refers to sample no 12, respectively.

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PCR Amplification oif Third Fragment F3 of GP-120 Of HIV1 By ising HIVSPF3/HIVSPR3 Primers

PCR Amplification of third fragment F3 of GP120 of HIV1 was performed. Out of 26 strains, 4 strains showed presence of third segment of HIV1is shown in (Figure 4).

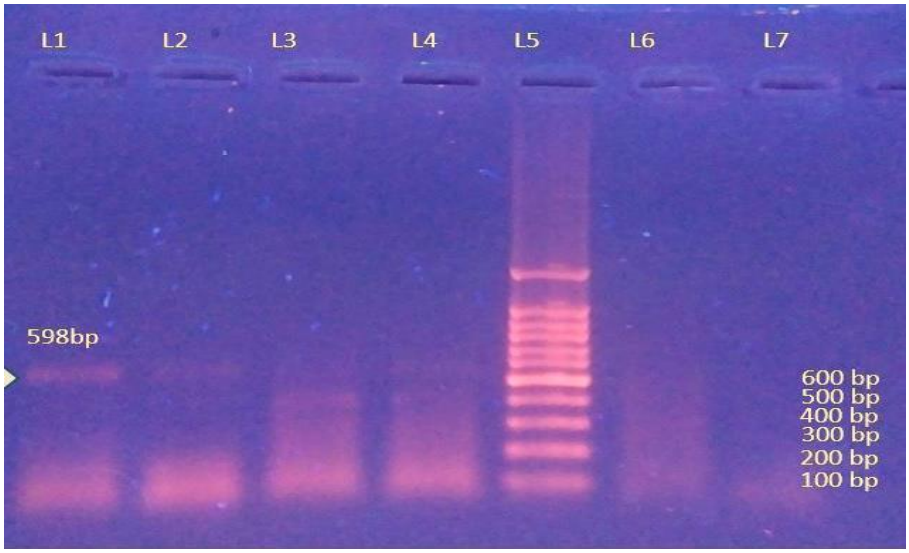

Figure 4: 1.5% TBE agarose gel electrophoresis shows the results of third Fragment F3 of surface glycoprotein of Human Immuno Deficiency Virus type 1 (598 bps). Lane 1 refers to sample no 3, lane 2 refers to 4, lane 3 refers to 100bp ladder, lane 4 refers to sample no 10, and lane 5 refers to sample no 11, while lane 6 refers to sample no 12, respectively.

Sequencing of GP120 of HIV1 Fragments

The three fragments GPF1, GPF2 and GPF3 of GP120 of HIV1 of three sequences acquired after molecular sequencing were combined to obtain complete sequences of GP120 and named as Sequence1, Sequence2 and Sequence3 respectively. Sequence analysis revealed that the complete sequence size of Sequence1 was 1567 base pairs, Sequence2 was 1563 and Sequence3 was 1546 respectively.

Sequence homology of GP120 of HIV1

The obtained sequences were analysed through BLAST and the sequence identity of Sequence1, Sequence2 and Sequence 3 with other sequences on NCBI is shown in Figure 14, 15 and 16 respectively. However, sequence homology of the sequences obtained is shown in Figure 5, & 6.

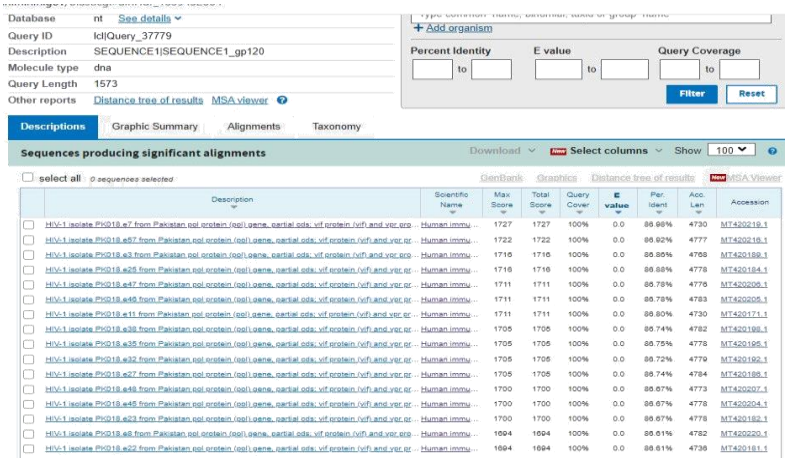

Figure 5: Sequence identity of Sequence1 of GP120 of HIV1 with already reported sequences on NCBI

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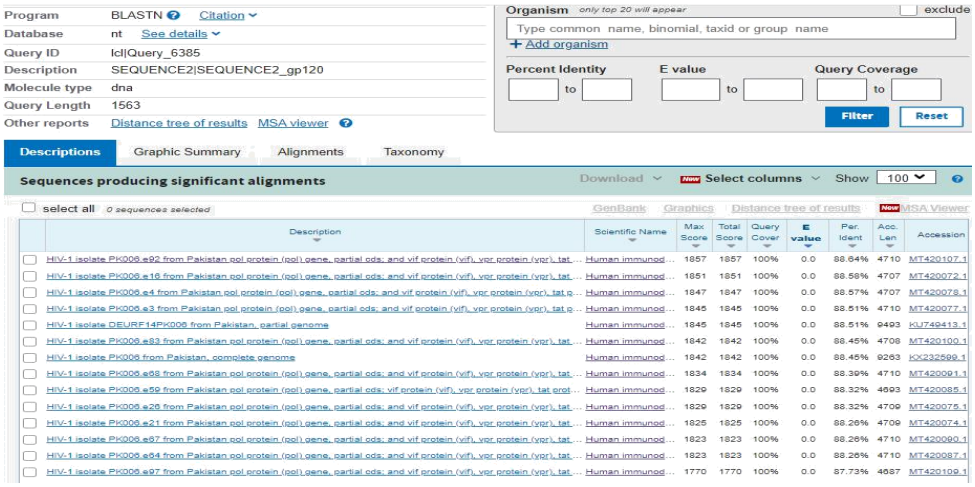

Figure 6: Sequence identity of Sequence2 of GP120 of HIV1 with already reported sequences on NCBI

HIV-1Mutation Analysis

The sequences acquired were analyzed for any mutation by using the online codon alignment tool at Los Alamos HIV database. The acquired sequences was aligned with already reported sequences or most similar sequences with molecular identity up to 99% retrieved from NCBI. We identified mutations after analysis and the genomic regions of HIV-1 as summarized in Table 6, 7, & 8. In this study, 26 nucleotide sequences were used, and the finished sample contained 1670 locations altogether. In MEGA 11, evolutionary studies were carried out. Sequence 2 and Sequence 3 showed the closest evolutionary relationship. Our amplified sequences were aligned their mutual evolutionary relationship with sequences such as AB773885, AB254156, A04321, AF042105 and AB032740 retrieved from China, Japan, France, Australia and Thailand extended from the same node.

Table 6: Sequence 1 mutations identified in GP120 of HIV-1.

Codon number	Reference WT codon (HXB2)	Mutated codon	Reference WT amino acid (HXB2)	Mutated amino acid	Mutation Analyzed	Genomic Region
118	CCA	ATG	Proline (P)	Methionine (M)	Pro118Met	Cys119 link to Cys205
193	TTG	TTC	Leucine (L)	Phenylalanine (F)	Leu193phe	V2 hyper variable region
440	AGT	TAT	Serine (S)	Tyrosine (Y)	Ser440Tyr	Co receptor binding site out of V3
499	ACC	ATT	Threonine (T)	Isoleucine (I)	Thr499Ile	Rev Responsive Element (RRE Region)

Table 7: Sequence 2 mutations identified in GP120 of HIV-1.

Codon number	Reference WT codon (HXB2)	Mutated codon	Reference WT amino acid (HXB2)	Mutated amino acid	Mutation Name	Genomic Region
47	GAA	AAA	Glutamic acid (E)	Lysine (K)	Glu47Lys	Buried Surface Area
131	TGC	GGC	Cysteine (C)	Glutamine (G)	Cys131Glu	V1 Hyper Variable Region
231	AAG	GGA	Lysine (K)	Glutamine (G)	Lys231Glu	Glycosite 230
455	ACA	GTA	Threonine (T)	Valine (V)	Thr455Val	CD4 contact residue (side chain contact only)

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Table 8: Sequence 3 mutations identified in GP120 of HIV-1.

Codon number	Reference WT codon (HXB2)	Mutated codon	Reference WT amino acid (HXB2)	Mutated amino acid	Mutation Name	Genomic Region
7	TAT	CAT	Tyrosine (Y)	Histidine (H)	Tyr7His	VPU, Cytoplasmic Domain, ENV Signal Peptide
120	CCA	CGT	Proline (P)	Arginine (R)	Pro120Arg	Core receptor binding site outside V3
143	AGT	GTC	Serine (S)	Proline (P)	Ser143Pro	V1 Hyper Variable Region
321	GGA	ACA	Glutamine (G)	Threonine (T)	Glu321Thr	V3 Loop, core Receptors specific R5/X4
386	AAT	TAT	Asparagine (N)	Tyrosine (Y)	Asn386Tyr	V4 Loop, Glycan contact

Discussion

The majority of newly confirmed HIV-positive patients (75.67%) were not receiving treatment, according to the findings. There were also noteworthy variations between the treatment and control groups in terms of total leukocyte count (TLC), platelet count (PLT), and HB (likely hepatitis B) prevalence. TLC and PLT levels were higher in the untreated group, although HB prevalence was reduced. In addition, the study found that the levels of ALT, AST, urea, and creatinine were all greater in the untreated group than in the treated group. These results suggest that untreated HIV-positive patients may have a higher risk of developing liver and renal problems. Untreated HIV can lead to a number of problems and comorbidities, as shown by this study, emphasizing the significance of early detection and treatment. Additionally, the results highlight the importance of routine HIV screening, particularly in high-risk populations, to guarantee prompt diagnosis and treatment.

After nearly 650 000 individuals was infected in which [510 000−860 000] deaths globally to date, HIV continues to be a serious general medical issues. However, with the increased availability of effective detection, treatment, and care, including for perceptive cases, HIV infection has evolved into a sensible chronic health problem, enabling those who are infected to live long and healthy lives. By the end of 2019, there were projected to be 38.0 million people living with HIV. The incorporation of governments has been steadily growing as a result of intentional worldwide efforts to combat HIV. According to a 2019 study, 53% of childrens and 68% of adults with HIV in the globe agreed to receive antiretroviral therapy (ART) for the remainder of their lifetimes [22].

Among three structural genes i.e., gag-pol and Env, the Env gene has not been studied in Pakistan much due to its fastest mutation rate. Saeed khan et al., 2018 retrieved already reported sequences of gag, pol and Env genes from Los Alamos and Stanford University HIV databases and conducted phylogenetic analysis for studying genetic diversity [23,24]. They reported that subtype-A was the most commonly found subtype in Pakistan. No mutation was reported in the Env gene of HIV-1. Our finding was identified A1 as the prevailing subtype among HIV patients in Pakistan. A very close homology with 02A1 has been observed through homology study. However, we found several mutations in the GP120 region of the Env gene of HIV-1in our study. The most important mutation to report in this study was identified in Sequence 2 i.e., Lys231Glu at Glycosite 230 of GP120 of Env gene which is associated with increased resistance to neutralization interface contact of GP120 to Gp41and is statistically significant sequence change in 1/5 patients following VRC01 infusion. T455E mutation of subtype B strain YU2 decreases binding of mAb B12 to <25% of WT binding.

Tariq et al., and Danboyi et al., reported a unique recombinant form (URF) of HIV-1i.e. URF_DG which is found closely related to URF_DG from the United Kingdom [25,26]. However, the Env gene has been widely researched around the globe and has been a new target of the first generation of antiviral medications against HIV-1 [27,28]. J Dumonceaux et al., 2020 reported a spontaneous CD4 independent entry phenotype that suggests direct entry of the virus by attachment of a co-receptor to GP120 [29,30]. They co-related this phenotype to seven mutations in various regions of GP120 of the Env gene of HIV-1such as the C2, C3 and V3 loop. In the Env gene, Rachel et al.

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described compensating mutations that result in amino acid changes that grant widespread freedom from errors in viral propagation. Additionally, it facilitates very effective cell-to-cell communication [31].

A typical retroviral genome comprising of gag, pol and Env genes flanked by long terminal repeats (LTRs) containing viral promotor constitutes the HIV genome. The protein capsid, matrix and nucleocapsid that are structural components are encoded by gag gene, however, glycoprotein 120(GP120) i.e., the surface proteins and glycoprotein 41 (gp41) i.e., the transmembrane proteins of viral envelope are encoded by Env gene. The functional components of HIV in the form of enzymes such as reverse transcriptase (RT), protease (PR) and integrase (IN) are encoded by pol gene [32]. Furthermore, there are six genes possessed by HIV that regulate several processes in HIV for which it is considered as a "complex" retrovirus. The transcription of provirus is boosted by tat gene, however rev aids in the export of unspliced RNA of HIV to the cytoplasm which is important phenomenon as it not only helps in the translation of essential retroviral proteins but also serves its role as genomic RNAs which are then assembled in new virion. Apart from these regulatory genes, some other genes are known as accessory genes because they do not play an absolute role in the replication of HIV as shown by the cell culture studies [33].

In our study, mutations Ser440Tyr and Pro120Arg in Sequence 1 and 3 respectively at the co-receptor binding site just outside the V3 loop may have an impact on the binding or attachment of HIV-1to the host CD4 cells [34]. In sequence 3, mutation Glu321Thr occurred in the V3-loop and may affect the light chain region as it was co-receptor specific R5/X4 site (49). Defective mu-tants are created by rearrangement in the Env region, according to a research by Huamian Wei et al. [9]. In addition to increasing the variety of the viral community, this encourages the phylogenetic development of the virus. Defective traits may change from being useless to being helpful.

Conclusions

The majority of newly confirmed HIV-positive patients (75.67%) were not receiving treatment, according to the findings. There were also noteworthy variations between the treatment and control groups in terms of total leukocyte count (TLC), platelet count (PLT), and HB (likely hepatitis B) prevalence. TLC and PLT levels were higher in the untreated group, although HB prevalence was reduced. In addition, the study found that the levels of ALT, AST, urea, and creatinine were all greater in the untreated group than in the treated group. These results suggest that untreated HIV-positive patients may have a higher risk of developing liver and renal problems. Untreated HIV can lead to a number of problems and comorbidities, as shown by this study, emphasizing the significance of early detection and treatment. Additionally, the results highlight the importance of routine HIV screening, particularly in high-risk populations, to guarantee prompt diagnosis and treatment.

In this study, 26 nucleotide sequences were used, and the finished sample contained 1670 locations altogether. In MEGA 11, evolutionary studies were carried out. Sequence 2 and Sequence 3 showed the closest evolutionary relationship. Our amplified sequences were aligned their mutual evolutionary relationship with sequences such as AB773885, AB254156, A04321, AF042105 and AB032740 retrieved from China, Japan, France, Australia and Thailand extended from the same node. The findings of this study might be beneficial for future large-scale investigations on subtyping, sequencing, distribution, and the manufacture of drugs. These findings may also be valuable for micro and molecular biologists working for pharmaceutical businesses. Because the envelope gene (GP120) has sections that frequently mutate and develop drug resistance, mutational research of the gene and its molecular information could identify the sites of mutation and further improve the design of medications. The clinical biomarker and molecular analysis data obtained from this study may also be helpful for healthcare practitioners in monitoring small alterations in the blood levels of their patients.

Thirteen new mutations were identified in all three sequences. Some of them are most likely to have an impact on CD4 receptor binding, antibiotic resistance and co-receptor binding which interrupts the entry of HIV-1 to the host cell. A1 is identified as the prevailing subtype among HIV patients in Pakistan. A very close homology with 02A1 has been observed through homology study.

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