Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/jptcp.v31i1.3938

CLINICAL PROFILES AND PHYLOGENETIC ANALYSIS OF HIV-1 IN NEWLY DIAGNOSED PATIENTS FROM PUNJAB, PAKISTAN

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Abstract

This study explored the clinical profiles, and phylogenetically analysis of HIV-1 positive patients compared to a control group. The hematological analysis revealed significantly lower hemoglobin (HB) levels in HIV-1 positive individuals (11.31±0.63 g/dL) compared to the control group (11.84±0.57 g/dL). White blood cell counts (WBCs) were also notably reduced in HIV-1 positive patients (6.52±2.01x10³mm³) compared to the control group (7.85±0.88 x10³mm³), with reference values ranging from 4-11 x10³mm³. Platelet counts (PLTs) exhibited a significant decrease in HIV-1 positive patients (179.21±27.24 x10³mm³) compared to the control group (307.03±36.21 x10³mm³). Biochemical analysis indicated elevated levels of liver enzymes in HIV-1 positive patients, with ALT levels at (63.3±12.3 U/L), AST levels at (52.7±9.5 U/L). Additionally, renal function markers demonstrated increased levels in HIV-1 positive patients, including urea at 41.1±7.4 mg/dL and creatinine at 1.7±0.3 mg/dL compared to the control group. Furthermore, RNA analysis via 1% TAE agarose gel electrophoresis revealed distinctive bands in high viral load samples, providing insights into the quality and integrity of extracted RNA. The electrophoretic separation of three

Fragments (F1, F2, and F3) of the surface glycoprotein GP-120 of Human Immunodeficiency Virus type 1 (HIV-1) was visually represented through 1.5% TBE agarose gel electrophoresis. The graphical representations facilitated the analysis of specific characteristics of GP-120 across various samples, contributing to a comprehensive understanding of the viral genetic variations. This integrative approach, combining hematological, biochemical, and genetic analyses, offered a comprehensive perspective on the clinical and molecular aspects of HIV infection, providing valuable insights for further research and potential therapeutic interventions.

Keywords: HIV, Clinical Profiles, Biochemical Markers, Hematological Analysis, Genetic Variations, GP-120, Viral Load

Introduction

HIV-1 (Human Immunodeficiency Virus-1) continues to pose a significant global health problem (1). Its transmission dynamics are complicated by the presence of several subtypes and recombinant forms (2). Comprehending the genetic variability and epidemiological trends of the HIV/AIDS virus is vital in order to develop efficient measures for prevention and treatment in the ongoing fight against the disease (3).

The worldwide prevalence of the Human Immunodeficiency Virus-1 (HIV-1) continues to pose a significant and difficult problem in the field of public health, impacting a large number of people globally (4). Although Antiretroviral Therapy (ART) has made great progress in improving the prognosis and quality of life for individuals with HIV, the virus's deceptive characteristics require ongoing monitoring and evaluation to ensure constant vigilance. The comprehensive study of hematological and biochemical data plays a crucial role in this continuing assessment (5).

In addition to measuring viral levels, hematological and biochemical markers offer more insight into the complex interaction between HIV-1 and the human body. These measures are essential indications of the general health status, revealing possible problems, coexisting medical conditions, and side effects associated to treatment (6).

The hematological indicators, such as the Complete Blood Count (CBC) and white blood cell differentials, which provide insight into the immune system's reaction to HIV-1 infection (7). Changes in red blood cell (RBC) counts, hemoglobin levels, and platelet counts provide valuable information about common problems such as anemia, which is often linked to HIV-1 (8). Simultaneously, changes in the composition of white blood cells, particularly CD4+ T lymphocytes, serve as indicators of immune system deterioration (9).

Regarding biochemistry, which includes tests for liver function, renal function, and electrolyte levels, these measurements offer insight into the operational health of essential organs. Increased levels of liver enzymes can indicate disorders such as hepatitis associated with HIV or hepatotoxicity caused by antiretroviral drugs, whereas alterations in kidney function indicators, such as creatinine levels, can indicate possible renal problems (10).

Punjab, a highly populous area in Pakistan, offers a distinctive setting for examining the genetic composition of HIV-1. The region unique sociodemographic features, which encompass a combination of rural and urban groups, along with its strategically advantageous geographical position, make it an interesting focus point for studying the transmission dynamics and molecular evolution of the virus.

Methodology

Study Design:

This research was designed at Institute of molecular biology and biotechnology (IMBB), University of Lahore to carefully evaluate clinical parameters and phylogenetic analysis of HIV-1 positive

patients to gain insights into the virus-host interplay and inform effective patient management strategies.

Study Setting:
The study was conducted in collaboration with designated HIV treatment centers and healthcare facilities in Punjab, Pakistan chosen for their diverse patient demographics clinical parameters, molecular characterization and accessibility.

Participant Selection:
Inclusion Criteria:
- Patients newly diagnosed with HIV-1.
- Age 18 years and above.
- Willing to participate in the study.
- HIV newly positive patients

Exclusion Criteria:
- Individuals with known hematological or biochemical disorders unrelated to HIV-1.
- Those unwilling or unable to provide informed consent.

Sample Size Determination:
The sample size was calculated based on statistical power considerations, anticipated effect sizes, and precision requirements for key outcomes, ensuring the study's ability to detect meaningful associations.

Laboratory Analysis:
Hematological Analysis:
Procedure for the Mindray BC-5000 CBC Analyzer (11)
System Initialization:
- The Mindray BC-5000 analyzer was activated, and the proceeding of the initialization procedure was permitted to take place.
- It was made certain that the consumables and reagents did not exceed their expiration dates and that they were loaded correctly.

Selection of the CBC Test:
- The user interface of the analyzer was navigated in order to pick the CBC test.
- The specific criteria that were selected to be included in the complete blood count (CBC) test were the number of red blood cells (RBC), the number of hemoglobin (Hb), the number of white blood cells (WBC), the number of platelets, and the differential leukocyte count.

Automated Analysis:
- First steps were taken to initiate the automated analysis process. After aspirating the material, the BC-5000 then proceeded to perform a series of measurements, after which it mixed the sample with reagents.
- The analyzer utilized flow cytometry in addition to other optical approaches throughout the process of analyzing the components of blood cells.

Display of Results:
- The results of the electrocardiogram were displayed on the screen of the analyzer. RBC count, Hb concentration, neutrophil count, white blood cell count, platelet count, and differential leukocyte count were frequently included in the results.

Quality Control:

- In order to guarantee the analyzer's accuracy and precision, routine quality control checks were carried out in accordance with the guidelines provided by the manufacturer.

Biochemical Analysis:

Procedure for the Micro-lab 400 Plus Analyzer (12)

Sample Preparation

- In the first step of the process, standard venipuncture techniques were utilized to collect blood samples from HIV-1 positive patients.
- The blood samples that were taken were centrifuged in order to obtain plasma or serum, depending on the requirements for the examination of urea, creatinine, ALT, and AST.
- The sample loading container of the analyzer was opened, which is the third step in the sample loading process.
- The samples of plasma or serum from the HIV-1 positive individuals were loaded into the sample rack or tray that had been designated for that purpose.
- Information pertinent to the patient was successfully entered into the system.

Selection of Tests:

- The user interface of the analyzer was explored in order to pick the particular tests for urea, creatinine, ALT, and AST.
- The parameters that were selected and any necessary assay settings did not require any changes.

Automated Analysis:

- First steps were taken to initiate the automated analysis process. Aspirating the sample, mixing it with reagents, and carrying out the necessary assays for ALT, AST, urea, and creatinine were all steps that were carried out by the Microlab 400 Plus.
- For the measurement of ALT and AST, photometric or enzymatic methods were utilized, whereas colorimetric methods were utilized for the detection of urea and creatinine.

The collection of data:

- The analyzer gathered information regarding the levels of urea, creatinine, ALT, and AST that were present in the representative samples.

Display of Results:

- The results were seen on the screen of the analyzer, which displayed numerical values for different parameters such as urea, creatinine, ALT, and AST.

Quality Control:

- In order to guarantee the analyzer's accuracy and precision, routine quality control checks were carried out in accordance with the guidelines provided by the manufacturer.

Statistical Analysis:

Calculation of means, standard deviations, and interquartile ranges for continuous variables, and Frequency distributions for categorical variables.

Ethical Considerations:

- Ethical clearance was duly secured from the Institutional Review Board (IRB) of the University of Lahore and the Punjab AIDS Control Programme.
- Secured informed consent from all participants.
- Ensured the utmost confidentiality and privacy of participant information.
- Obtained ethical approval from the pertinent institutional review boards.

Molecular Identification and RNA Extraction Protocol for HIV-Positive Blood Samples
Procedure:

- In a 1.5mL Eppendorf tube, 500µL of AVL buffer, supplemented with carrier RNA, was meticulously combined with 200µL of the plasma sample. The resulting mixture underwent vortexing for 15 seconds and was subsequently allowed to stand at ambient temperature (15-25°C) for 10 minutes.
- To the prepared sample, 500µL of concentrated ethanol (96-100%) was added, and the mixture was subjected to vortexing for 15 seconds. Rapid centrifugation followed to expedite the separation process.
- The lysed sample was then carefully transferred to a QIAamp Mini spin column within a new 2mL collection tube. Subsequently, 500µL of AW-1 buffer was introduced, and the solution underwent centrifugation for 1 minute at 8,000rpm. The resultant filtrate, along with the collection tube, was systematically discarded.
- Following the replacement of the collection tube, 500µL of AW-2 buffer was added to the QIAamp Mini spin column. After centrifugation at 8,000 rpm for 1 minute, the resulting filtrate and collection tube were discarded.
- The collection tube was replaced once more, and 200µL of AW-2 buffer was added to the QIAamp Mini spin column. Following a 2-minute incubation at ambient temperature (15-25°C), the solution underwent centrifugation for 5 minutes at 14,000rpm. Subsequently, the filtrate and collection tube were meticulously discarded.
- The QIAamp micro spin column, housing the purified RNA, was transferred to a sterile 1.5mL Eppendorf tube. To facilitate RNA elution, 200µL of AE elution buffer was added. The resultant mixture was incubated at room temperature for 3 minutes and then centrifuged at 8,000rpm for 1 minute.

Gel Electrophoresis for RNA Quality Assessment

The quality of the extracted RNA was evaluated through a rigorous process involving 1% agarose gel electrophoresis. Additionally, quantification was performed using a NanoDrop spectrophotometer (Optizen, Mecasys, Korea), and readings were meticulously recorded at wavelengths 260/280.

This multifaceted approach ensures a comprehensive assessment of the extracted RNA, combining the visual analysis provided by gel electrophoresis with the quantitative data obtained through NanoDrop spectroscopy. The recorded readings at wavelengths 260/280 serve as a crucial indicator of the RNA's purity, laying the foundation for reliable downstream applications in molecular analysis.

Table 1. Reverse Primer for cDNA Synthesis

Name	Primer sequence	Primer length	Melting temperature (℃)	GC content (%)
HIVCR1	TGCTAGAGATTTTCCACACTGAC	23	53.5	43

Table 2: Amplification primers for the identification and confirmation of GP120 of HIV1

Name	**Primer sequence**	**Primer Length**	**Product Length**	**Melting temperature (℃)**	**GC count (%)**
HIVSPF1	ATGAAAGTGAGGGGGATCAGGAAG	24		57.4	50
HIVSPR1	TACCTCTCATGCTTGTGGTGATATTG	26	502	56.4	42
HIVSPF2	GAGGTATGGTGACAGAATATGCAC	24	597	55.7	46
HIVSPR2	CCCCTCCTGAGGAGTGACTAAA	22		56.7	55
HIVSPF3	GAGGGGATCCTGAAGTTGC	19	440	53.2	58
HIVSPR3	TCTTTTTTCTCTCTGCACCACTC	23		53.5	43

Bioinformatics Analysis:

For the assessment of HIV1 mutation frequencies, a robust bioinformatics analysis was conducted utilizing key tools, including NCBI BLAST, CLUSTAL OMEGA, and MEGA X 10.1 software. These sophisticated bioinformatics tools were employed to scrutinize and interpret the molecular sequencing data, ensuring a comprehensive exploration of potential mutations within the HIV1 genome.

Results

Distribution of viral load

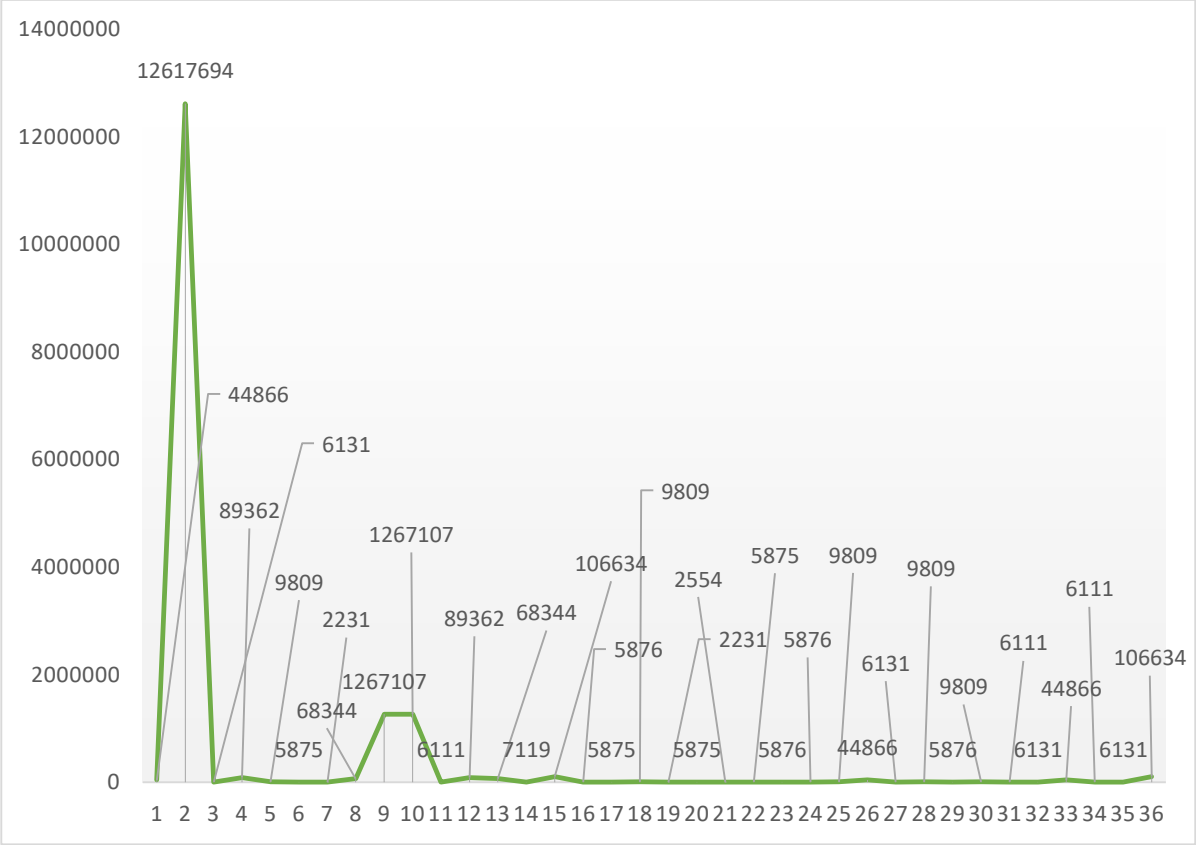

Figure 1: distribution of Viral Load of HIV-1 positive samples

Analysis of hematological parameters

Table 3: Comparison of hematological parameters between HIV Patients and Controls

Parameter	Unit	Positive Patients	Normal Patients	Reference values
HB	g/dL	11.31 ±0.63	11.84±0.57	14.0-18.0 g/dL
WBCs	10^3mm^3	6.52±2.01	7.85±0.88	4-11x10^3mm^3
PLTs		179.21±27.24	307.03±36.21	150-450x10^3mm^3

Analysis of biochemical parameters

Table 4: Incidence of Liver Function Tests (LFTs) and Renal Function Tests (RFTs) Disparities between HIV-Positive Patients and Control Subjects

Parameter	Unit	HIV-Positive	Control Group	Reference values
ALT	(U/L)	63.3 ± 12.3	28.6 ± 8.7	10 - 40 U/L
AST		52.7 ± 9.5	25.4 ± 6.8	8 - 35 U/L
UREA	mg/dL	41.1 ± 7.4	25.8 ± 6.1	15 - 45 mg/dL
Creatinine		1.7 ± 0.3	0.9 ± 0.2	0.6 - 1.2 mg/dL

Molecular characterization of HIV-1 positive samples

Table 5: Absorbance ratio A260/280 and concentration (ng/µl) of RNA in all HIV1 positive samples

A260/280	Unit	RNA
2.21	**(ng/µL)**	141.48
2.49		1322.44
1.74		1321.79
1.68		897.84
2.77		1020.54
1.71		771.67
1.38		798.19
2.51		1700.97
2.55		1242.73
2.49		913.94
2.20		2328.11
2.37		1936.47
2.03		2223.19
2.71		703.52
2.15		838.39
1.61		347.22
1.77		82.94
1.95		492.57

Figure 2: Quantification of RNA through NanoDrop

RNA Extraction and Quantification

The plasma samples were subjected to RNA extraction for HIV-1 using the QIAmp Viral RNA Mini kit from Qiagen, Germany. To assess the integrity of the extracted RNA, a portion of it was subsequently analyzed by electrophoresis on a 1% TAE agarose gel. This step is crucial for ensuring the quality and intactness of the RNA molecules, providing valuable information about the success of the extraction process and the suitability of the RNA for further downstream applications.

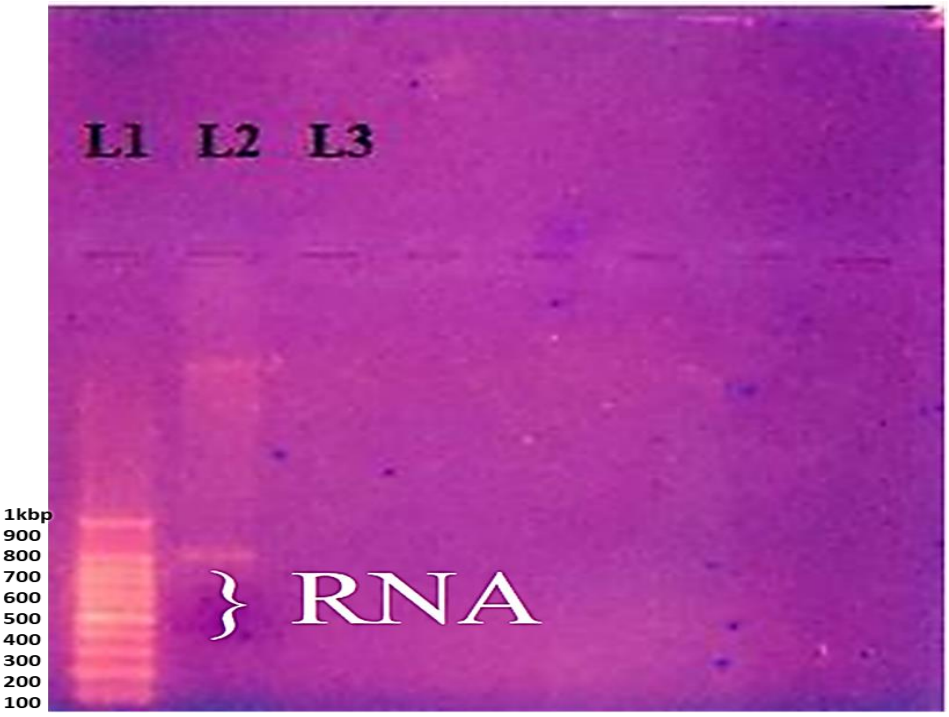

Figure 3: displays 1% TAE agarose gel electrophoresis revealing the RNA extracted from samples characterized by a high viral load. Within the figure, L1 denotes the 1kb DNA ladder, serving as a reference for size, while L2 represents the visualized RNA bands. This depiction offers a visual assessment of the quality and integrity of the RNA extracted from high viral load samples, aiding in the evaluation of the extraction process and informing subsequent analyses.

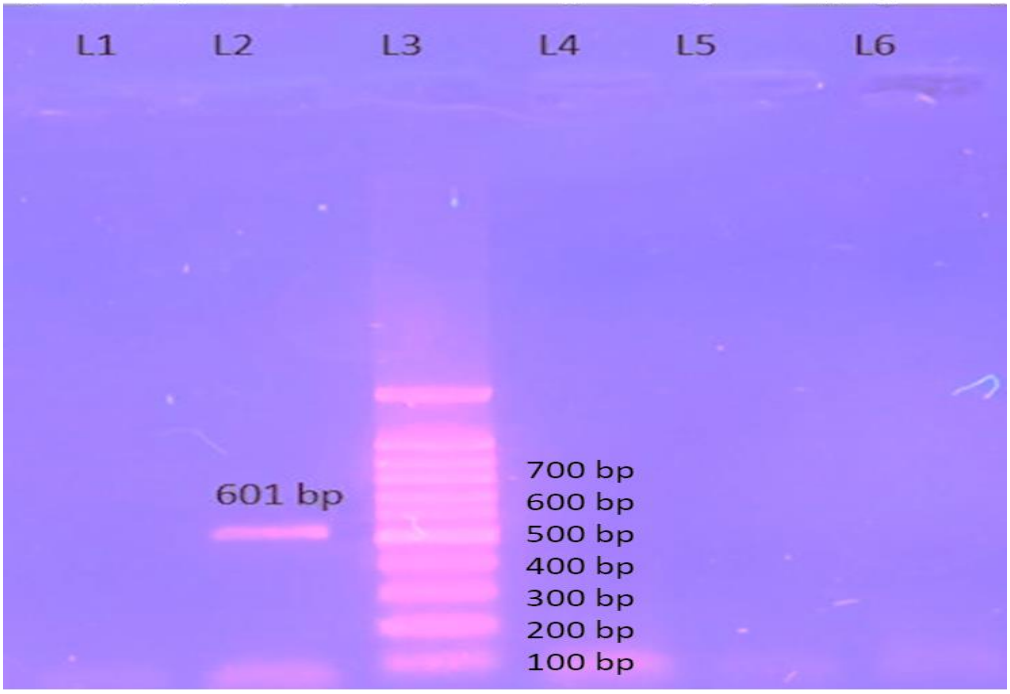

Figure 4: presents the 1.5% TBE agarose gel electrophoresis, depicting the results of the initial Fragment F1 of the surface glycoprotein of Human Immunodeficiency Virus type 1 (601 bp). The lanes are designated as follows: Lane 1 corresponds to sample number 3, Lane 2 to sample number 4, Lane 3 to the 100bp ladder, Lane 4 to sample number 10, Lane 5 to sample number 11, and Lane 6 to sample number 12. This depiction provides a visual representation of the electrophoretic separation of fragments, aiding in the analysis of the specific surface glycoprotein GP-120 in the context of different samples.

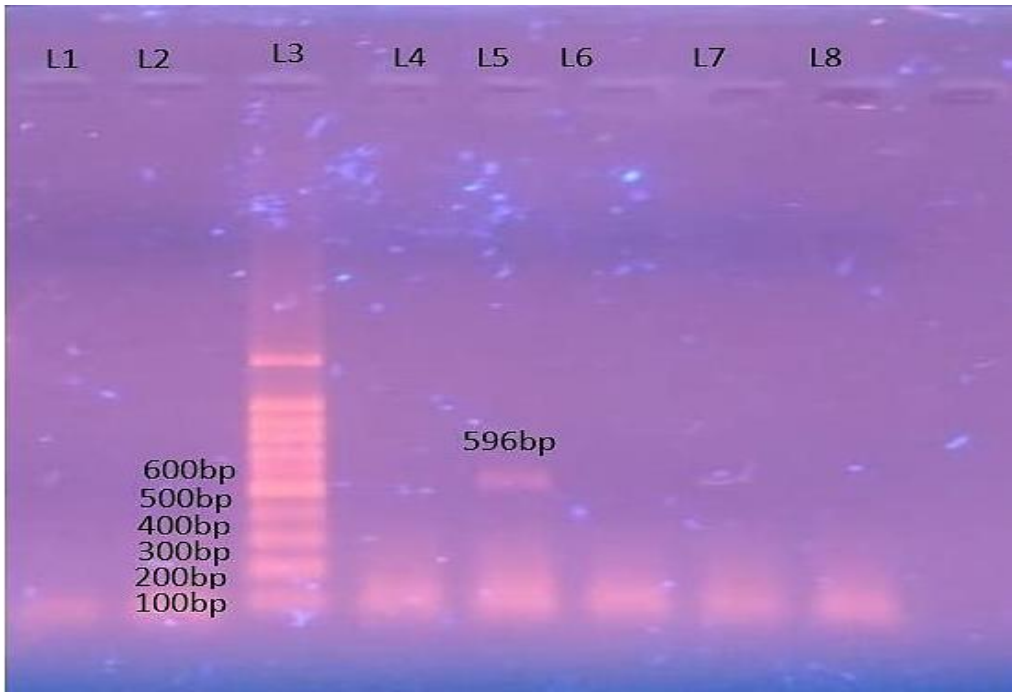

Figure 5: displays 1.5% TBE agarose gel electrophoresis, presenting the results of the second Fragment F2 of the surface glycoprotein of Human Immunodeficiency Virus type 1 (596 bp). The lanes are annotated as follows: Lane 1 corresponds to sample number 3, Lane 2 to sample number 4, Lane 3 to the 100bp ladder, Lane 4 to sample number 10, Lane 5 to sample number 11, and Lane 6 to sample number 12. This graphical representation facilitates the visual assessment of the electrophoretic separation of Fragment F2, offering insights into the specific characteristics of the surface glycoprotein GP-120 in the context of different samples.

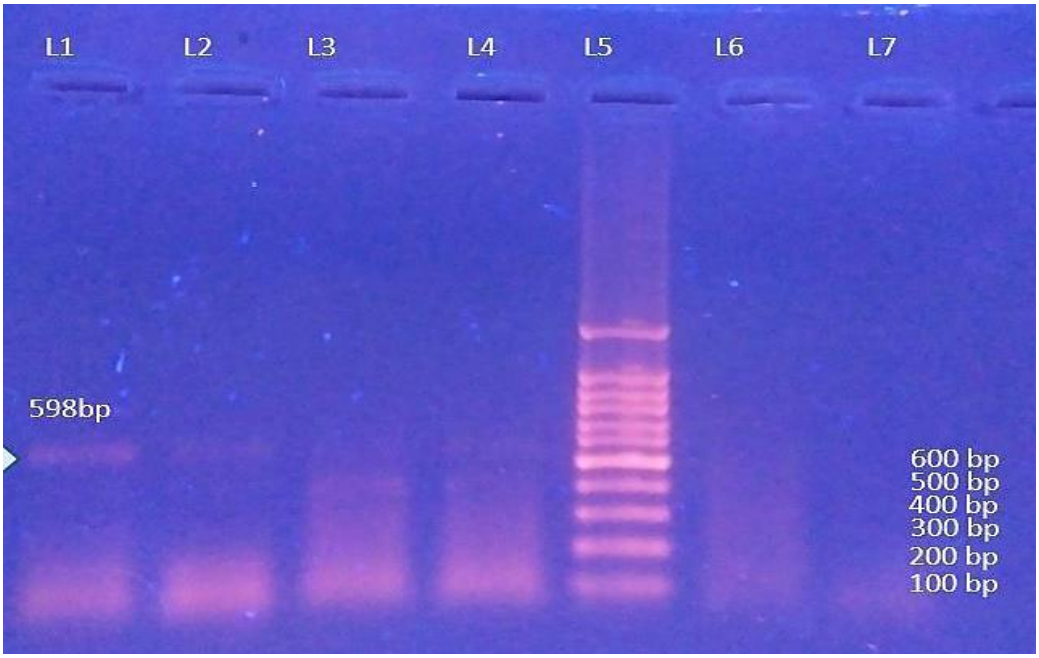

Figure 6: illustrates 1.5% TBE agarose gel electrophoresis, presenting the results of the third Fragment F3 of the surface glycoprotein of Human Immunodeficiency Virus type 1 (598 bps). The lanes are designated as follows: Lane 1 corresponds to sample number 3, Lane 2 to sample number 4, Lane 3 to the 100bp ladder, Lane 4 to sample number 10, Lane 5 to sample number 11, and Lane 6 to sample number 12. This graphical representation serves to visually convey the electrophoretic separation of Fragment F3, contributing insights into the characteristics of the surface glycoprotein GP-120 across various samples.

Discussion

The identification of the Human Immunodeficiency Virus (HIV) in 1983 by eminent scientists Francoise Barre-Sinoussi and Luc Montagnier marked a pivotal moment in virology. Initially designated as "lymphadenopathy-associated virus" (LAV), HIV's remarkable contagiousness and its role in precipitating Acquired Immunodeficiency Syndrome (AIDS) garnered significant attention (13).

Among the structural genes Env, Pol, and Gag the Env gene, due to its rapid mutation rate, has remained relatively underexplored in Pakistan. Sequences from Gag, Pol, and Env genes, sourced from databases at Los Alamos and Stanford University, underwent phylogenetic analysis to elucidate genetic diversity. The predominant subtype in Pakistan was identified as subtype A, with no reported mutations in the Env gene (14). Notably, a unique recombinant form, URF_DG, closely related to the UK variant, was also documented (15).

A crucial finding in this study pertains to a significant mutation in Sequence 2 Lys231Glu at Glycosite 230 of GP120 in the Env gene. This mutation correlates with heightened resistance to neutralization interface contact between GP120 and Gp41 and constitutes a statistically significant sequence alteration observed in 1 out of 5 patient post-VRC01 infusion. The T455E mutation in subtype B strain YU2 was noted to decrease mAb B12 binding to less than 25% of wild-type binding (16).

The Env gene has emerged as a central target for the initial class of antiretroviral drugs against HIV-1. Global investigations have revealed a spontaneous CD4-independent entry phenotype, suggesting the potential for direct viral entry through co-receptor attachment to GP120. Dumonoceaux et al. (2020) reported a correlation between this phenotype and seven mutations in various regions of GP120 within the Env gene, including C2, C3, and the V3 loop.

Within the purview of this research, two specific mutations Ser440Tyr and Pro120Arg in Sequence 1 and Sequence 3, respectively, were identified in the co-receptor binding site just outside the V3 loop. These mutations may significantly impact the binding or attachment of HIV-1 to host CD4 cells. Sequence 3 exhibited an additional mutation, Glu321Thr, within the V3 loop, potentially influencing the viral entry process within the co-receptor-specific R5/X4 site.

Compensatory mutations in the Env gene were identified, conferring broad escape from defects in virus replication and facilitating highly efficient cell-to-cell transmission (17). Additionally, a study reported the production of defective mutants through recombination in the Env region, promoting the phylogenetic evolution of the virus and increasing the diversity of the virus population. The role of defective genes may transition from being considered "junk" to assuming functional significance (18).

Conclusion

In conclusion, our study has provided a comprehensive insight into the clinical and biochemical profiles of HIV-positive individuals, elucidating potential correlations with viral load and genetic variations in the surface glycoprotein GP-120 of Human Immunodeficiency Virus type 1 (HIV-1). The hematological analysis revealed significant deviations in HB, WBCs, and PLTs among HIV-positive patients compared to the control group, indicating potential hematological alterations associated with HIV infection.

Our findings highlight the systemic effects of HIV on multiple organ systems, necessitating vigilant monitoring and management. Furthermore, our genetic analysis, represented through agarose gel electrophoresis, provided a visual representation of RNA quality and the electrophoretic separation of Fragments F1, F2, and F3 of GP-120. The identification of distinct characteristics and variations in GP-120 across different samples enhances our understanding of viral genetic diversity, which is crucial for tracking the evolution of the virus and potential implications for treatment strategies.

Our study emphasizes the need for ongoing research to further delineate the intricate interplay between hematological, biochemical, and genetic factors in HIV infection. The insights gained from this study may inform future therapeutic interventions and aid in the development of more targeted and effective strategies for managing HIV-positive individuals.

Acknowledgment:
We would like to express my sincere gratitude to ***Dr. Hasnain*** from Punjab AIDS control Program, and ***Zunair Akmal*** from PKLI&RC for their invaluable support and contributions to this research. Their guidance, expertise, and encouragement played a pivotal role in the successful completion of this paper. I am truly thankful for their time and commitment.

Conflict of Interest:
The authors declare that there is no conflict of interest regarding the publication of this paper.

Authors Contributions
Aqib Munir: Data collection, Data curation, Editing, Writing the first draft, Supervision; **Fatima Ali**: Study design, Data curation, Data analysis, Editing & Proofreading; **Aamna Shah**: Data analysis, Editing; **Maham Nasir & Muhammad Umer**: Data analysis, Editing. **Tasneem Noor Mohammad & Hafeezullah Wazir Ali**: Study design, Data analysis; **Ateeq Ur Rehman**: Data collection, Data curation. All co-authors contribute equally.

References
1. Anjum A, Rehman Au, Siddique H, Rabaan AA, Alhumaid S, Garout M, et al. Evaluation of Hematological, Biochemical Profiles and Molecular Detection of Envelope Gene (gp-41) in Human Immunodeficiency Virus (HIV) among Newly Diagnosed Patients. Medicina. 2022;59(1):93.
2. Vizcarra P, Pérez-Elías MJ, Quereda C, Moreno A, Vivancos MJ, Dronda F, et al. Description of COVID-19 in HIV-infected individuals: a single-centre, prospective cohort. The lancet HIV. 2020;7(8):e554-e64.
3. Del Amo J, Polo R, Moreno S, Díaz A, Martínez E, Arribas JR, et al. Incidence and severity of COVID-19 in HIV-positive persons receiving antiretroviral therapy: a cohort study. Annals of internal medicine. 2020;173(7):536-41.
4. Hayes RJ, Donnell D, Floyd S, Mandla N, Bwalya J, Sabapathy K, et al. Effect of universal testing and treatment on HIV incidence—HPTN 071 (PopART). New England Journal of Medicine. 2019;381(3):207-18.
5. Ssentongo P, Heilbrunn ES, Ssentongo AE, Advani S, Chinchilli VM, Nunez JJ, et al. Epidemiology and outcomes of COVID-19 in HIV-infected individuals: a systematic review and meta-analysis. Scientific reports. 2021;11(1):6283.
6. Fleming J, Mathews WC, Rutstein RM, Aberg J, Somboonwit C, Cheever LW, et al. Low-level viremia and virologic failure in persons with HIV infection treated with antiretroviral therapy. Aids. 2019;33(13):2005-12.
7. Al-Mughales JA. Development and Validation of a Three-Parameter Scoring System for Monitoring HIV/AIDS Patients in Low-Resource Settings Using Hematological Parameters. HIV/AIDS-Research and Palliative Care. 2023:599-610.
8. Coco-Bassey SB, Asemota EA, Okoroiwu HU, Etura JE, Efiong EE, Inyang IJ, et al. Glutathione, glutathione peroxidase and some hematological parameters of HIV-seropositive subjects attending clinic in University of Calabar teaching hospital, Calabar, Nigeria. BMC Infectious Diseases. 2019;19(1):1-10.
9. Aziz N, Quint JJ, Breen EC, Oishi J, Jamieson BD, Martinez-Maza O, et al. 30-year longitudinal study of hematological parameters of HIV-1 negative men participating in Los Angeles multicenter AIDS cohort study (MACS). Laboratory medicine. 2019;50(1):64-72.
10. Metry A, Bhaskar J. A study on correlation between blood parameters and CD4 count in people living with HIV/AIDS.
11. Thongsahuan S, Fonghoi L, Kaewfai S, Srinoun K. Precision and accuracy of the Mindray BC-5000Vet hematology analyzer for canine and feline blood. Veterinary Clinical Pathology. 2020;49(2):207-16.

12. Kumar V, Banala AK, Garcia EG, Cao J, Keck TM, Bonifazi A, et al. SUPPORTING DATA EXPERIMENTAL METHODS 1. Chemistry.
13. Patel P, Rose CE, Collins PY, Nuche-Berenguer B, Sahasrabuddhe VV, Peprah E, et al. Noncommunicable diseases among HIV-infected persons in low-income and middle-income countries: a systematic review and meta-analysis. AIDS (London, England). 2018;32(Suppl 1):S5.
14. Wang H, Wolock TM, Carter A, Nguyen G, Kyu HH, Gakidou E, et al. Estimates of global, regional, and national incidence, prevalence, and mortality of HIV, 1980–2015: the Global Burden of Disease Study 2015. The lancet HIV. 2016;3(8):e361-e87.
15. Control CfD, HIV/AIDS. CfIDDo, HIV/AIDS. NCfIDDo. HIV/AIDS Surveillance: US Department of Health and Human Services, Public Health Service, Centers …; 1990.
16. Grinspoon SK, Fitch KV, Zanni MV, Fichtenbaum CJ, Umbleja T, Aberg JA, et al. Pitavastatin to prevent cardiovascular disease in HIV infection. New England Journal of Medicine. 2023;389(8):687-99.
17. Yeo JY, Goh G-R, Su CT-T, Gan SK-E. The determination of HIV-1 RT mutation rate, its possible allosteric effects, and its implications on drug resistance. Viruses. 2020;12(3):297.
18. Domingo E, García-Crespo C, Lobo-Vega R, Perales C. Mutation rates, mutation frequencies, and proofreading-repair activities in RNA virus genetics. Viruses. 2021;13(9):1882.