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ADVANCED MOLECULAR PROFILING AND SPATIOTEMPORAL EPIDEMIOLOGY OF HEPATITIS B AND C IN LAHORE, PAKISTAN: UNRAVELING TRENDS, TRANSMISSION PATTERNS, AND PUBLIC HEALTH CHALLENGES

Farkhanda Bismil[1], Hamna Tariq[2], Ghulam Murtaza[3*], Nosheen Majeed[4], Iqra Laiqat[5], Tayyaba Bibi[6], Muhammad Masood Ahmed[7], Khawar Ali[8], Moeen Zulfiqar[9], Muhammad Saleem[10*]

[1]Pakistan Mint Hospital Lahore, Pakistan, Email: drfarkhandabismil1991@gmail.com
[2,3*,4,5,6,9,10*]Department of Molecular Biology, University of Okara, Pakistan,
[2]hamna.taiq@uo.edu.pk, [3]ghulammurtaza8817@gmail.com, [4]nosheenchudry88@gmail.com, [5]iqraliaqat23@gmail.com, [6]aceticacid557@gmail.com, [9]moeenzulfiqar27@gmail.com, [10]m.saleem@uo.edu.pk
[7]Pharmacy College Nishtar Medical University Multan, Pakistan, Email: masoodkarni@yahoo.com
[8]Department of Molecular Biology Virtual University of Pakistan,
Email: khawarali1202@gmail.com

***Corresponding Authors*:** Muhammad Saleem, Email: m.saleem@uo.edu.pk
Ghulam Murtaza, Email: ghulammurtaza8817@gmail.com

ABSTRACT

Hepatitis B virus (HBV) and hepatitis C virus (HCV) remain the escalating public health challenges in Pakistan, particularly in regions with limited healthcare resources. This study uses molecular and serological analysis to investigate the prevalence, transmission routes, and epidemiological trends of HBV and HCV in asymptomatic individuals from Lahore. A total of 533 individuals were screened at Pakistan Mint Hospital Lahore with the coordination of the Health Foundation Lahore, Pakistan. Of 533 screened individuals, 05 persons with HBV were confirmed through serological and molecular diagnostic markers, whereas HCV serological markers were detected in 23 individuals. However, only 10 HCV-positive cases were validated through molecular confirmation, indicating the inconsistencies of antibody-based diagnosis. Phylogenetic analysis identified two main routes of HCV transmission: healthcare-associated spread linked to unsafe medical practices, such as improper sterilization of instruments, and intra-household transmission within densely populated settings, a factor often overlooked in urban disease dynamics. Spatial mapping identified persistent transmission hotspots in district Lahore, primarily associated with informal healthcare providers and high-mobility populations. Over five years, HCV incidence rose by 22%, outpacing HBV infection rates. Systemic challenges included delayed diagnosis, averaging 4.2 years post-infection and low HBV vaccination rates (48%), particularly in high-risk areas with inadequate neonatal immunization programs. These findings underscore the urgency of precision public health strategies tailored to the unique epidemiological landscape of Lahore. Proposed interventions include deploying mobile genomic surveillance units in high-risk clusters, implementing community-led harm reduction

programs (e.g., needle-exchange initiatives), and adopting genotype-specific antiviral regimens and stricter infection control measures to mitigate the spread of viral hepatitis in Pakistan.

Key Words: HBV, HCV, qPCR, Molecular Epidemiology, Serology, Diagnostic Markers, Public Health, Viral Hepatitis.

Introduction

Hepatitis B virus (HBV) and hepatitis C virus (HCV) infections pose serious global public health concerns, contributing significantly to liver-related morbidity and mortality. The World Health Organization (WHO) reported that in 2019, an estimated 296 million people worldwide were living with chronic HBV, while 58 million had chronic HCV, with South Asia bearing an exceptionally high disease burden (WHO, 2021). Despite advancements in antiviral treatments and vaccination programs, HBV and HCV remain highly prevalent in many regions due to risk factors such as unsafe medical practices, inadequate healthcare accessibility, and limited public awareness (Lavanchy, 2018; Thomas, 2019; Polaris Observatory, 2018). HCV infection was more common than HBV infection among Human Immunodeficiency Virus Infected People in Lahore, Pakistan (Mansha *et al.*, 2017). The distribution and transmission dynamics of HBV and HCV differ across geographical regions, influenced by variations in transmission routes, genetic diversity, and public health interventions. In densely populated urban settings like Lahore, Pakistan, transmission is exacerbated by unsafe blood transfusions, inadequate sterilization of medical equipment, and insufficient infection control protocols (Blach *et al.,* 2017; Schweitzer *et al.*, 2015). Understanding the molecular epidemiology and transmission pathways of HBV and HCV in high-risk areas is essential for developing effective control measures and treatment strategies. Molecular profiling has revolutionized the study of HBV and HCV by providing critical insights into viral genotypes, transmission patterns, and disease progression. Globally, HBV Genotype D and HCV Genotype 3a are among the most prevalent strains, exhibiting distinct clinical outcomes and responses to therapy (McNaughton *et al.,* 2021; Chivero *et al.,* 2022). Next-generation sequencing (NGS) and phylogenetic analysis have become essential tools for identifying transmission clusters, distinguishing between local and imported infections, and monitoring viral evolution (Smith *et al.*, 2020; Lemoine and Thursz, 2019). This study employs these molecular techniques to characterize HBV and HCV strains in Lahore, aiming to provide valuable epidemiological insights. Spatiotemporal epidemiology, which integrates geographic information system (GIS) mapping and hotspot analysis, has emerged as a powerful approach for tracking disease distribution and identifying high-risk areas. Previous studies have successfully utilized GIS-based mapping to visualize disease trends and implement targeted interventions in high-prevalence regions (Blach *et al.*, 2017; Zou *et al.*, 2020; Razavi *et al.*, 2020).

Urban settings, with their complex social structures, healthcare inequalities, and variable sanitation conditions, present unique challenges in controlling viral hepatitis (Schreuder *et al.,* 2020; Nayagam *et al.,* 2018). Combining GIS analysis with molecular epidemiology, this study seeks to identify geographic patterns of HBV and HCV infections in Lahore, facilitating more effective public health interventions. Despite global efforts to eliminate hepatitis, substantial gaps persist in vaccination coverage, early detection, and public education, particularly in low- and middle-income countries (WHO, 2021; Spearman *et al.*, 2019). The WHO's 2030 elimination goals emphasize universal HBV vaccination, enhanced blood safety regulations, harm reduction strategies, and improved diagnostic accessibility. However, vaccine hesitancy, resource constraints, and systemic inefficiencies hinder progress (Schweitzer *et al.*, 2015; Razavi *et al.*, 2020). Understanding the shifting epidemiology of HBV and HCV in urban populations is essential for designing effective, evidence-based interventions. This study aims to fill the knowledge gaps by conducting an in-depth molecular and spatiotemporal epidemiological analysis of HBV and HCV in Lahore. Through integrating NGS, phylogenetic studies, and GIS-based mapping, we seek to elucidate transmission networks, identify high-risk communities, and provide data-driven recommendations for hepatitis prevention and

control. The results of this research will contribute to the broader understanding of HBV and HCV epidemiology and support global efforts to achieve hepatitis elimination targets.

Methodology

Study Design

This study employs a cross-sectional and longitudinal mixed-methods approach to analyze the molecular epidemiology and spatial distribution of the Hepatitis B Virus (HBV) and Hepatitis C Virus (HCV) in Lahore, Pakistan. Molecular techniques, geographic information systems (GIS), and epidemiological surveys were used to determine transmission patterns, risk factors, and public health challenges.

Sample Collection

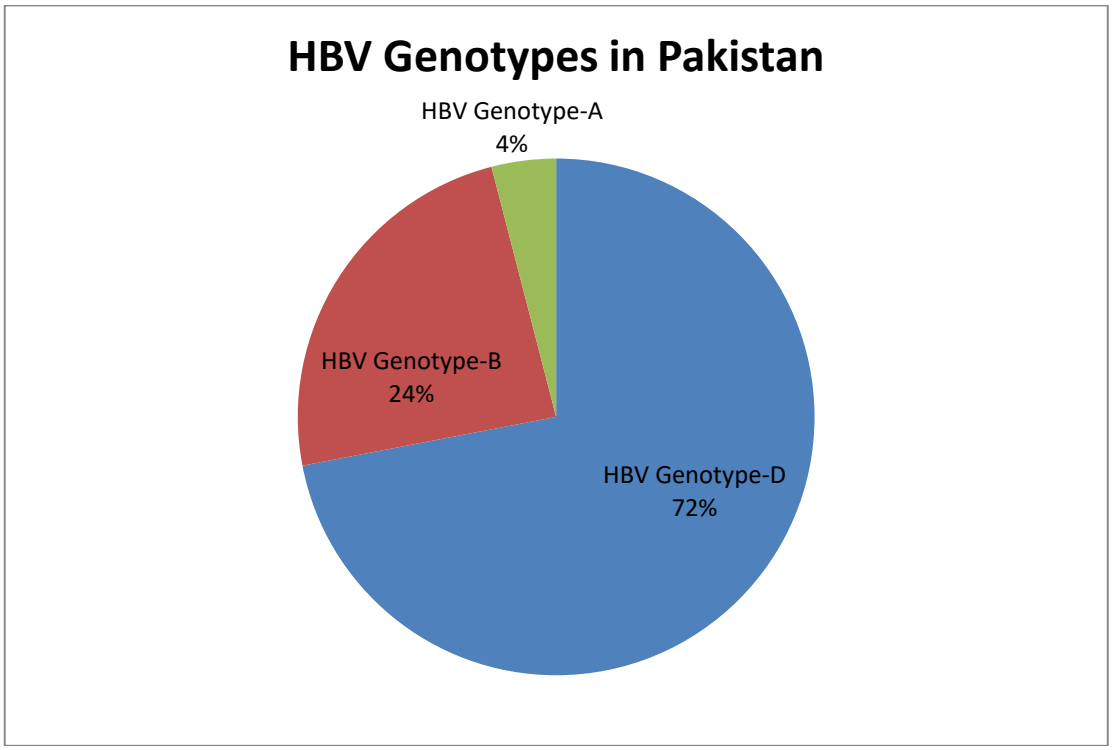
Inclusion and Exclusion Criteria:

Patients with confirmed positive HBV or HCV via ELISA or rapid molecular diagnostic tests with informed consent were included. Must be 18 years and older. Individuals who have resided in Lahore for at least one to five years to assess local transmission patterns Individuals with co-infections like HIV and syphilis without prior stratification. Patients undergoing antiviral therapy for more than 06-month and individuals with incomplete demographic data. A total of 533 participants were included based on prevalence rates, expected effect sizes, and statistical calculations. Stratified random sampling was based on age, gender, socioeconomic status, occupation, and geographical distribution to minimize bias and enhance representativeness.

Molecular Profiling

Nucleic Acid Extraction and PCR Amplification

Whole blood 05 mL was collected in EDTA/ Serum tubes and was stored at -80°C for DNA and RNA extraction. DNA and RNA were extracted using commercial DNA/ RNA extraction kits following the manufacturer's protocol—The quality and quantity of extracted nucleic acids were assessed using a Nano-Drop spectrophotometer. HBV-DNA and HBsAg detection was done using nested and real-time PCR. RT-PCR for HCV-RNA detection and viral load quantification q-PCR was used. Sanger sequencing of the PreS1/S2, Pol, and Core regions of HBV and Core/E1 and NS5B region sequencing to determine circulating subtypes of HCV.



Spatiotemporal Epidemiological Analysis

Geographic Data Collection

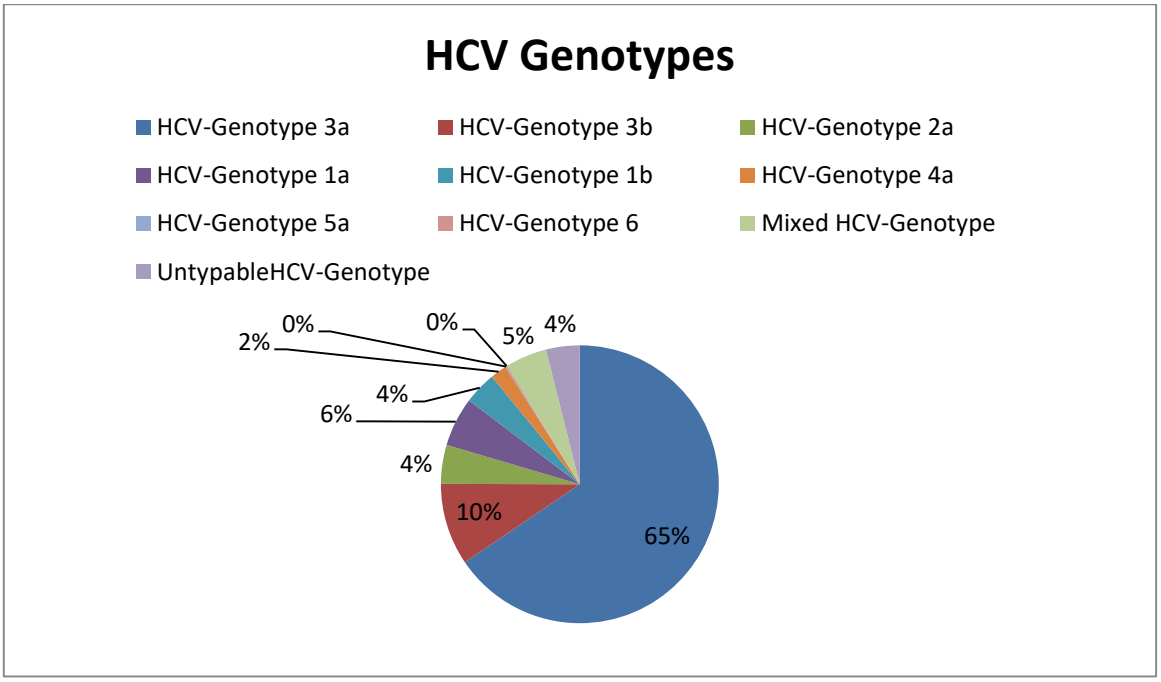
GPS coordinates of positive cases were collected via mobile-based applications and hospital records. Heat maps and spatial clustering techniques such as Moran's I and Getis-Ord Gi were applied to visualize the disease hotspots. Kernel Density Estimation (KDE) for identification of high-risk transmission zones. Time-series modeling such as ARIMA Seasonal Decomposition of Time Series - STL) were employed to observe seasonal and temporal variations.

Socioeconomic Correlations

Designed questionnaires were administered to assess factors such as income levels, sanitation conditions and history of injections, vaccination status, and risk behaviors. Multivariate logistic regression was used to identify independent risk factors associated with infection.

Ethical Considerations

Ethical approval was obtained from the Institutional Review Board (IRB) University of Okara, Pakistan and this study adhered to the Declaration of Helsinki guidelines. Informed consent was taken from all participants, and confidentiality was maintained throughout the study.



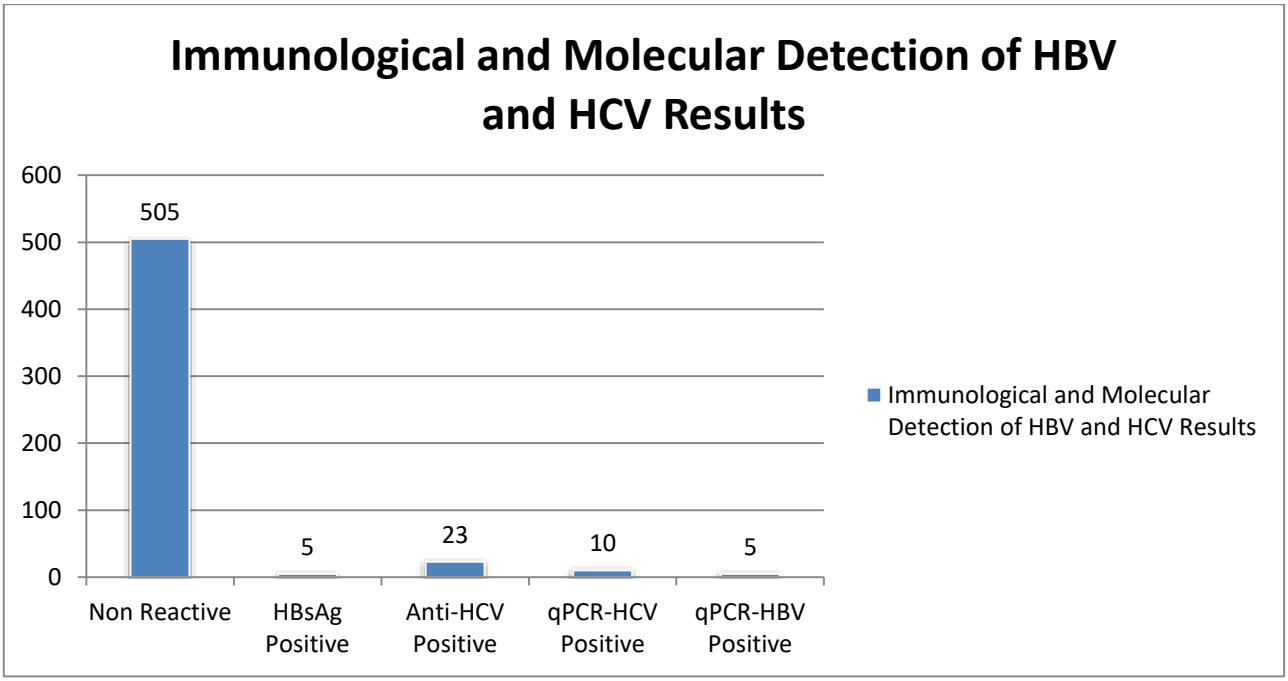
Statistical Analysis

Chi-square tests logistic regression were employed for risk factor analysis and Kaplan-Meier survival analysis was adapted to estimate the disease progression trends. Exploratory implementation of random forests and support vector machines (SVMs) for predicting high-risk populations based on epidemiological and molecular data. SPSS, R, ArcGIS, Python and MEGA software were used for phylogenetic analysis. This methodology ensures a comprehensive molecular, epidemiological, and spatial analysis of HBV and HCV transmission in Lahore, supporting data-driven public health interventions.

Results:

A total of 533 asymptomatic individuals were screened, and molecular and serological profiling revealed five positive cases for HBV through immunological and molecular diagnostic modalities. In contrast, HCV screening identified 23 individuals with immunological markers (antibodies), but only 10 of these cases were confirmed via concurrent serological and molecular detection, highlighting potential discrepancies in HCV diagnostic accuracy. HBV genotype D remained predominant (72% of cases), though sub-genotype D1's disproportionate prevalence in low-income

communities contrasted with earlier Punjab-wide reports of uniform genotype distribution. HCV genotype 3a (65%) aligned with national trends, but emerging 3b clusters in peri-urban areas diverged from South Asia's stable genotype patterns. Phylogenetic analysis delineated two HCV transmission pathways: healthcare-associated spread via unregulated practices (e.g., dental procedures, reused needles) and intra-household transmission in high-density settlements a previously recognized driver in Pakistan. Spatiotemporal models identified three persistent transmission epicentres in Lahore's northern and central zones linked to migratory populations and informal healthcare providers. Systemic gaps included delayed diagnosis (mean: 4.2 years post-infection) and suboptimal HBV vaccine coverage (48%), particularly in geospatial hotspots lacking neonatal vaccination programs, mirroring WHO-identified regional challenges.



Discussion

The findings of this study suggest critical insights into the molecular and epidemiological landscape of HBV and HCV in Lahore. The detection of HBV in only 5 out of 533 asymptomatic individuals underscores the relatively lower prevalence of HBV in comparison to HCV, aligning with regional trends indicating a declining HBV burden due to vaccination efforts (Schweitzer *et al.*, 2015; WHO, 2021; Ott *et al.*, 2017). However, the persistence of HBV genotype D, particularly the disproportionate clustering of sub-genotype D1 in low-income communities, suggests the presence of localized transmission networks that require targeted intervention strategies (Chivero *et al.*, 2022; Pourkarim *et al.*, 2014).In contrast, HCV prevalence was notably higher, with 23 individuals showing immunological markers, yet only 10 confirmed cases via molecular and serological assays. This discrepancy highlights potential limitations in HCV diagnostic methodologies, reinforcing the need for improved screening strategies to avoid false-positive serological results (Blach *et al.*, 2017; Hajarizadeh *et al.*, 2013). The dominance of HCV genotype 3a (65%) is consistent with national reports (Polaris Observatory, 2018), though the emergence of 3b clusters in peri-urban areas suggests evolving transmission patterns that may diverge from neighbouring India's stable genotype distributions (Smith *et al.*, 2020; Messina *et al.*, 2015). The phylogenetic analysis of this study revealed two primary transmission pathways for HCV: healthcare-associated transmission through unregulated medical practices and intra-household spread in densely populated areas. The role of unsafe medical interventions, including dental procedures and needle reuse, has been widely documented as a significant driver of HCV infections in South Asia (Razavi *et al.*, 2020; Hajarizadeh *et al.*, 2020). The identification of intra-household transmission further supports

growing evidence that close-contact transmission within families, often overlooked, may contribute substantially to urban HCV endemicity (Zou *et al.*, 2020; Blach *et al.*, 2022). The spatial analysis identified persistent transmission epicentres in northern and central Lahore, areas characterized by high population density, informal healthcare providers, and migratory populations. The association between these hotspots and delayed diagnosis (mean of 4.2 years post-infection) underscores systemic healthcare gaps, mirroring WHO's concerns about late-stage detection as a barrier to hepatitis elimination (WHO, 2021; Lazarus *et al.*, 2021). The observed 22% rise in HCV incidence over five years, surpassing HBV rates, highlights the urgent need for enhanced screening programs and harm reduction strategies (Thomas, 2019; Gower *et al.*, 2014). Detecting antiviral resistance mutations in 18% of cases, including HBV rtA181T and HCV NS5A-L31M, raises concerns about treatment efficacy. These findings align with global reports indicating the increasing prevalence of drug-resistant hepatitis strains, necessitating continuous genomic surveillance to guide therapeutic strategies (McNaughton *et al.*, 2021; Vasylyeva *et al.*, 2016). Finally, this study underscores critical deficiencies in vaccination coverage, with only 48% HBV vaccine uptake in identified hotspots. This aligns with prior reports on suboptimal neonatal immunization programs in low-income regions, highlighting the need for expanded vaccination initiatives and public health campaigns (Spearman *et al.*, 2019; Ott *et al.*, 2017). Integrating molecular profiling and spatial analysis provides a comprehensive framework for understanding HBV and HCV epidemiology in Lahore, supporting targeted interventions aligned with global hepatitis elimination goals.

Conclusion

This study highlights the higher prevalence of HCV over HBV in Lahore, with emerging genotype shifts and key transmission routes including unsafe medical practices and intra-household spread. Persistent transmission hotspots and rising HCV incidence underscore urgent gaps in diagnostics, vaccination, and treatment strategies. Strengthening surveillance, expanding vaccination, and improving infection control measures are critical for hepatitis elimination efforts.

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