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Investigation And Prevalence Of Hepatitis B Virus Genotypes In Southern And Northern Districts Of Punjab, Pakistan

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Abstract

This study aimed to evaluate the prevalence of Hepatitis B Virus (HBV) infection, analyze HBV mutations, and compare nucleotide sequences in HBsAg-positive patients across different districts of southern and northern Punjab, Pakistan. A cohort of over 100 HBsAg-non clearance (positive) subjects were chosen for this investigation. The findings revealed that both genotypes D and A were prevalent across all subgroups of HBV patients, including those with various liver diseases. Specifically, genotype D was predominant, accounting for 52% of acute cases, 28% of chronic cases, 7% of carrier cases, and 3% of cirrhosis/HCC cases. The age distribution with the percentage of HBV infection among the selected patients was as follows: the highest percentage was seen in the 21-30 years age group (24.4%), followed by the 51-60 years age group (21.1%), the 31-40 years age group (16.6%), the 41-50 years age group (14.4%), >61 years age group (11.1%), the 11-20 years age group (8.88%), and the 1-10 years age group (3.33%). Quantitative Polymerase Chain Reaction (qPCR) results indicated that 83.3% of patient samples tested positive for HBV, while 16.7% tested negative compared to the control group. The viral load analysis revealed that the majority of positive samples had viral loads ranging from 50,000 to 100,000 IU/ml (40%), followed by 100,000 to 500,000 IU/ml (29.3%), 10,000 to 50,000 IU/ml (16%), >500,000 IU/ml (8%), and the lowest proportion (6.66%) fell within the range of 1-10,000 IU/ml. Overall, genotype D (subtype D) was predominantly observed among the isolated viral agents. All samples were found to align with the NC_003977.2 reference sequence for the Hepatitis B virus (taxon: 10407), with sequence lengths ranging from a minimum of 354 base pairs to a maximum of 418 base pairs.

Keywords: *hepatocellular carcinoma (HCC), Hepadnavirideae, HBsAg, qPCR.*

Introduction

Hepatitis B virus (HBV) infection re[1]presents a widespread viral infection globally, particularly prevalent in regions such as Asia, the Pacific Islands, Africa, Southern Europe, and Latin America (Lavanchy & Kane, 2016). Chronic HBV infection prevalence within various countries ranges from 2% to 20% among the general population (Hofstraat et al., 2017). The consequences of persistent HBV infection encompass a broad spectrum of clinical presentations, including inactive carrier states, chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma (HCC) (Invernizzi et al., 2016). Notably, 15% to 40% of HBV carriers face a lifetime risk of developing cirrhosis, liver failure, or HCC (Kao, 2015). Worldwide, approximately 3.5 billion individuals have encountered HBV, with 400 million among them being chronic carriers (Bati et al., 2017). The virus can induce acute hepatitis of varying severity, persisting in 95% of children and 2–10% of adult patients, leading to chronic liver ailments, cirrhosis, hepatocellular carcinoma, and occasionally fulminant hepatitis (DHQ Hospital Mardan, Khyber Pakhtunkhwa, Pakistan, et al., 2019).

In Pakistan, 4.5 billion people are infected with chronic Hepatitis B (Butt and Ahmad., 2018). the incidence of HBV infection is on the rise, potentially attributed to insufficient

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healthcare facilities, economic disparities, and limited public awareness regarding the transmission of major communicable diseases like HBV, Hepatitis C virus, and Human Immunodeficiency Virus (Rasheed et al., 2022). HBV infection acquired early in life can progress from chronic hepatitis to cirrhosis, culminating in HCC typically after 30 to 50 years. Males exhibit a higher tendency to remain persistently infected by HBV, whereas females are more prone to transient infection and the development of anti-HBs (Iannacone & Guidotti, 2022). In males, HBV might not exert a carcinogenic effect directly. Instead, its role might involve inducing chronic liver cell damage, accompanied by inflammatory host responses and continuous liver regeneration over numerous years. This pathological sequence, particularly leading to cirrhosis, could potentially precipitate carcinogenesis without direct viral oncogenic activity. Notably, no viral oncogene, insertional mutagenesis, or viral activation of oncogenic cellular genes has been conclusively demonstrated. The expression of HBV proteins and virion release precede biochemical signs of liver disease. Additionally, significant quantities of surface antigen can persist within liver cells of apparently healthy carriers. Consequently, HBV is not inherently cytopathic. Three mechanisms are implicated in liver cell injury during HBV infections, with the primary mechanism being an HLA class I restricted cytotoxic T-cell (CTL) response targeted at HBcAg/HBeAg on HBV-infected hepatocytes (Iannacone & Guidotti, 2022). Despite Pakistan's substantial population size and high fertility rates, with an average exceeding four children per woman, comprehensive data regarding hepatitis prevalence remain surprisingly scarce. While more is understood about associated risk factors, there is a notable lack of information regarding hepatitis prevalence across the country's provinces and federally administered areas (Seerat et al., 2020; Samo et al., 2020).

Material and Methods

Study design and subjects

This cross-sectional investigation was directed during 2023–2024 from various hospitals in southern and northern districts of Punjab, including General Hospital Lahore, DHQ Hospital Sheikhupura, Nishtar Hospital Multan, Allied Hospital Faisalabad, DHQ Hospital Rajanpur, and DHQ Teaching Hospital Dera Ghazi Khan. The blood samples were taken from outdoor departments of the respective hospitals after clinical investigation from professional doctors. Previous clinical records were reported, including liver function tests and coagulation profiles.
The inclusion criteria included: (1) Age ranging from 1 to 60 years, (2) HBV DNA levels equal to or exceeding 2000 IU/mL, (3) Alanine Aminotransferase (ALT) levels equivalent to or exceeding 1 times the upper limit of the normal range, and (4) Assessment of Chronic HBV in accordance with the Pakistan Guidelines for the Treatment and Prevention of CHB virus.

The exclusion criteria comprised: (1) Previously documented allergic reactions or contraindications to Interferon-alpha (IFN-α) medications, (2) Complications with malignant tumors, (3) Epilepsy/other CNS dysfunctions, (4) Advanced cirrhosis, liver cancer, autoimmune diseases, history of mental illness, chronic obstructive pulmonary disorders, retinal diseases, thyroid diseases, etc., (5) Co-occurrence with other infectious diseases, (6) Pregnancy, preparation for pregnancy, or breastfeeding, (7) Neutrophil count prior to IFN-α treatment below 1.5×109/L and/or platelet count under 90×109/L, (8) Concurrent application of chemotherapy, traditional ayurvedic medications, or immune boosting methods (i.e., glucocorticoids, thymus peptides and pentapeptides and their method, etc.) during IFN-α treatment, (9) Incomplete treatment regimen, or (10) Loss to follow-up (Geng et al., 2024).

Blood sampling

The medical records of the subjects was retrieved and inputted into the electronic data capture software utilized in this investigation (Empower EDC, X&Y Solutions Inc., Beijing, China). This study recorded the following data: 1) Basic demographic details of

the subjects, including name, age, gender, location, and occupation. 2) Drug-related data, encompassing classification, dosage, administration method, and frequency of prescription for both Interferon-alpha (IFN-α) and Nucleos(t)ide Analogues (NUCs). 3) Lab findings prior and following sunjects underwent treatments using IFN-α medications. Parameters such as HBsAg (IU/mL), HBeAg (IU/mL), HBV DNA (IU/mL), and Alanine Aminotransferase (ALT) levels (U/L) were assessed every three months. The subjects were categorized into two cohorts: the HBsAg clearance (negative/control) group and the non-clearance (positive) group, based on whether HBsAg clearance occurred within 96 weeks of IFN-α treatment or not. Blood samples (10ml) were collected in the yellow-capped clotted vial, allowing the blood to clot correctly for 10-15 minutes. Following blood clotting, the vials underwent centrifugation at 5000 revolutions per minute (rpm) for 10 minutes, after which the serum was aseptically transferred into separate vials. The sera were distributed into 200 µl aliquots and stored at an ultra-low temperature of -70°C. These aliquots were used to extract DNA of HBV by semi-automation extraction technique using an extraction kit and amplified according to standard qPCR protocols using a Thermo Scientific DNA extraction kit (GeneJET).

Agarose Gel Electrophoresis/Southern Blotting

DNA extraction was done using a semi-automated method using GeneJET, a Thermo Scientific DNA extraction kit. The serum samples were taken into Eppendorf, and lysis buffer of an equal amount was added. The tubes were left in a shaking incubator for half an hour at 56°C for complete lysis of viral particles. After the incubation period, the mixture was pipetted into the spin column filters and subjected to centrifugation. Subsequently, the collection tube was replaced, and the spin filters were rinsed thoroughly. A series of washing was done with already prepared wash buffers 1 & 2. Each time, centrifugation was done after adding wash buffers. After thorough washing with wash buffers, spin filters were washed with absolute ethanol and a dry spin at maximum speed. The elution buffer was added to the spin filters, and the extracted nucleic acid was separated by centrifugation. The extracted nucleic acid was stored at 4°C in a freezer for further use. The purity of extracted DNA was initially confirmed using Agarose Gel Electrophoresis or Southern blotting followed by Boom et al. (1991). DNA was electrophoresed for 1to 2h through horizontal agarose slab gels (5 to 10 V/cm) in the buffer system described by Aaij and Borst (1972) containing1ug of Tris Borate EDTA/ml. DNA fragments were transferred to nitrocellulose filters essentially as described by Southern (1975). Purity of DNA was confirmed by the visualization of sharp bands of DNA of 1KB size.

RT-PCR Amplification

The extracted DNA was mixed with an already prepared master mix of AmpliTaq Gold Master Mix (Catalogue number: 4390939) in a recommended ratio. The YMDDR1 primers (forward & reverse) were used. The sequence of Forward YMDDR1 primer included the following nucleotide sequence (5'-CCCAACTCCTCCCAGTCCTTAA-3'), while reverse primer of YMDDR1 had sequence (5'-CCCAACTCCTCCCAGTCCTTAA-3'). 25µl of the mixture (extracted DNA and master mix) was loaded in a thermocycler and started the machine. The DNA amplification cycles involved denaturation at 95°C for 5 minutes and then for 30 seconds at the same temperature. Annealing was carried out at 45°C for 30 seconds. The final extension/ elongation, completed at 72°C in 1 minute, followed by 7 minutes for final elongation at the same temperature. The process involved 25 repeated cycles, and the final amplified DNA was held at 4°C until the product was removed from the thermocycler and stored at ultra-low temperature, i.e., -20°C.

Host Genotyping and Phylogenetic tree analysis

The gel clearing method was used to purify the PCR product, in order to send for sequencing. The protocols of FavorPrep^{TM} GEL/ PCR purification kit were followed for this purpose. The qPCR product was sent to a marketable facility provider for genotyping and sequencing. Following sequencing, BLAST was used to determine how closely our isolated strains matched up genetically with strains that had already been identified in the NCBI database. Thirty nucleotides' after carefully inspecting the analysis results for any

polymorphisms, overlaps, insertions, or deletions, sequences were chosen for phylogenetic analysis.

Statistical Analysis

SPSS 22.0 (IBM Corp., Armonk, NY, USA) and Empowerstats (X&Y Solutions, Inc.) was used for this study. The Mega 11 bioinformatics tool was used in this study to generate the phylogenetic tree of the query sequence by align with homologous sequence by using the BLAST tool. In mega 11, edit the selected homologues sequence which was retrieve through the blast, six sequence were used for generated the phylogenetic tree in mega 11 tool. The boost and causal align the cross-match sequences to generate the phylogenetic tree.

Results

From the samples of 100 patients, 75 blood samples were taken from non–clearance group (ELISA positive) and 15 blood samples were taken as clearance group (negative/control) for sequencing. All samples were processed and confirmed positive and negative from southern blotting and RT-PCR test.

The age and gender were considered as essential factor in this study. 42% of female and 58% of males were selected in this study as seen in fig 1. Subjects were divided into six different years age groups, i.e., 0-10, 11-20, 21-30, 31-40, 41-50, 51-60, and >61. It was observed that most of the subjects were belonging to the age group of 21-30 years of age (24.4%), followed by 51-60 years of age group (21.1%), 31-40 years of age group (16.6%), 41-50 years of age group (14.4%), >61 years of age (11.1%), 11-20 years of age group (8.88%) and 0-10 years ranging just (3.33%)(Table 1).

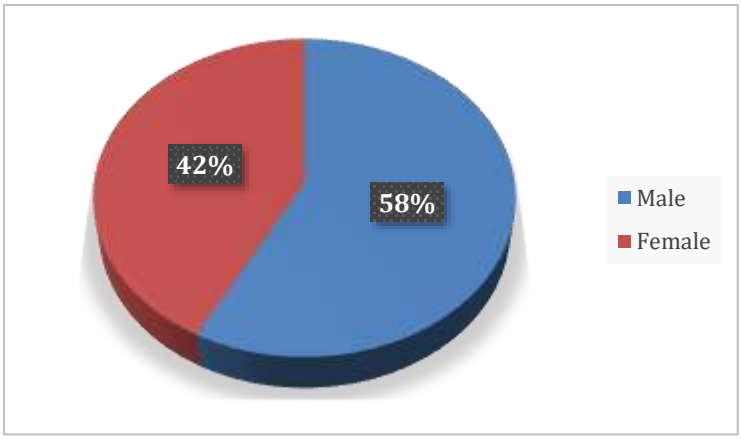


Fig. 1: Gender distribution of subjects

Table 1: Age groups distribution of subjects

Age group	No. of patients	Percentage (%)
0-10 years	03	3.33
11-20 years	08	8.88
21-30 years	22	24.4
31-40 years	15	16.6
41-50 years	13	14.4
51-60 years	19	21.1
>61 years	10	11.1
p-value	≤0.001	

Legend: p value (≤0.05) from Levene's test and Welch ANOVA from SPSS 22.0 software.

It was seen that from 100 blood samples, 75 (83.3%) samples were non-clearance/positive with RT-PCR and 15 (16.7%) were negative. The viral load of each sample given by

thermocycler was noted down and a comparative analysis was done. It was observed that most of the positive samples had viral load ranging from 50,000-100,000 IU/ml (40%), followed by 100,000-50,00,000 IU/ml (29.3%), 10,000-50,000 IU/ml (16%), >50,00,000 IU/ml (8%) and the least samples (6.66%) belonged to the viral load range 1-10,000 IU/ml as seen in table 2.

Table 2: Viral load of RT-PCR non-clearence (positive) samples

Viral load (IU/ml)	**No. of samples**	**Percentage (%)**
1-10,000	5	6.66
10,000-50,000	12	16
50,000-100,000	30	40
100,000-50,00,000	22	29.3
>50,00,000	6	8

The PCR non-clearance/positive samples (75 samples) were run on gel to confirm the amplification of required DNA fragment as seen in fig 2. After confirming the purity, the PCR products were sent for sequencing. The results gave us the genotype (subtype D) of hepatitis B isolated from patients as well as the sequenced length of samples (Table 3). It was observed that all isolated viral agents had genotype D (subtype D). It was seen that all samples were comparative to the NC_003977.2 reference sequence for Hepatitis B virus (taxon: 10407)(Fig 3). They sequence length was range from 354 base pairs minimum to 418 base pairs.

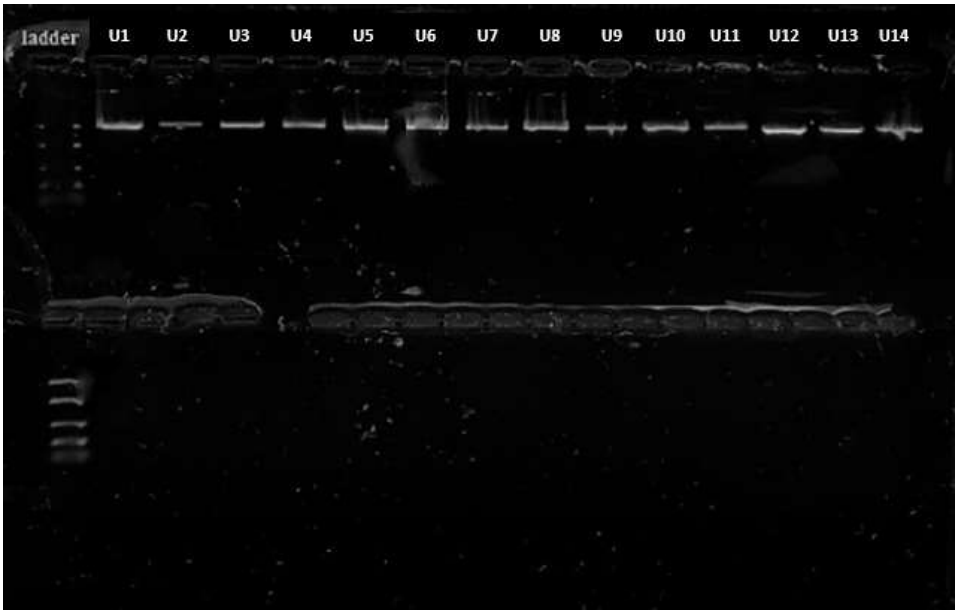


Figure 2: Band visualization under UV after gel electrophoresis (samples U1-U14)

Table 3: Genotype and sequence length of selective RT-PCR non-clearance (positive) samples

Samples	**Genotype (subtype)**	**Sequence length (bp)**
U1	D	410
U2	D	363
U3	D	410
U4	D	409
U5	D	414
U6	D	408
U7	D	394
U8	D	406
U9	D	410
U10	D	410

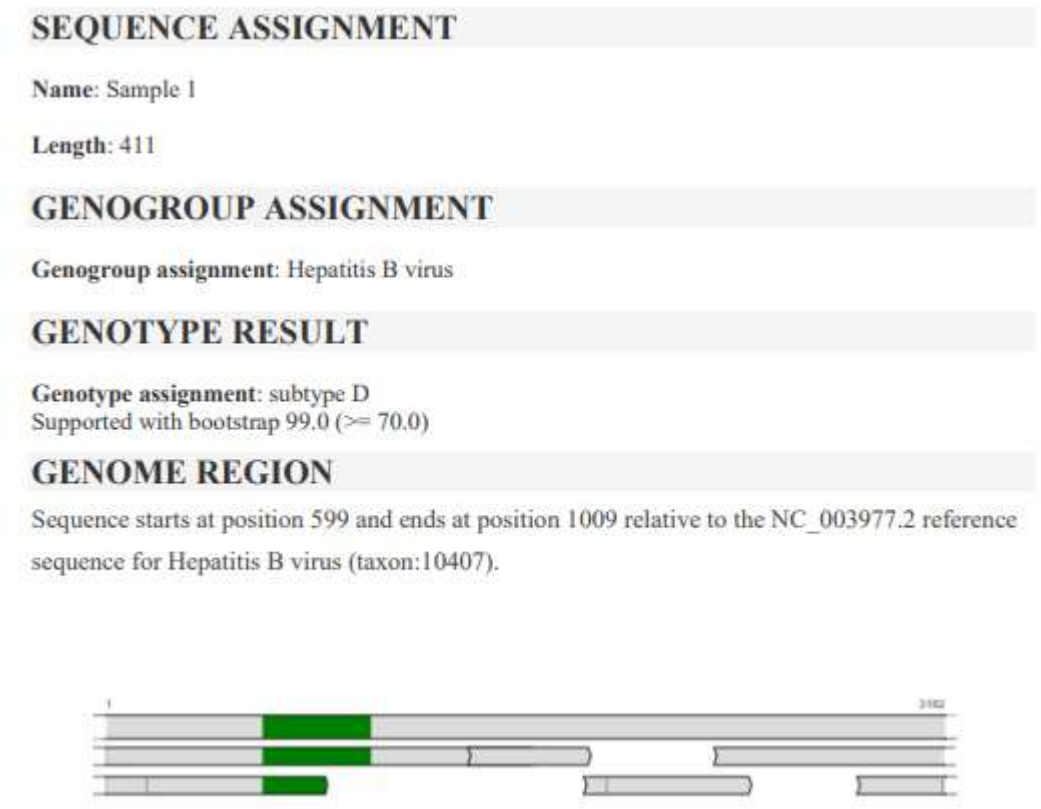


Figure 3: Sequencing result (genome region) of selective non-clearance (positive) sample

Files displayed the results of peaks between 70 and 120 nucleotides in various hues, each of which indicated a different pattern. Figure 4 traced the file analysis displayed 255–340 nucleotide sequence peaks that signify various levels. Every line search starts with a chance (+,-,d,r) description that was placed at specific time (like a BLAST), and from and to focal point, viewing the relationship on which the chances happened. Collecting type and size (in bytes) was going with data in the line before models (shown as- since no flag is set). From the start only the Explicit Congestion Notification (ECN) bit was finished by NS, and the rest of the bits were not used. The field was the IPv6 stream Id (fid) that a client can set to the OTcl content for each channel. While fid field may not be used in a re-enhancement, which may use this field for assessment purposes. The fid field was used to display stream coverage for the NAM show up in the same way. Two fields were source and goal area in focus port types. The field going showed the group course of action number of the system layer showed. The NS displayed the group course of action number of the system showed and UDP bunch movement number for evaluation purposes despite the way that UDP executions do not use schedule number. The last field displays the packs extraordinary ID.

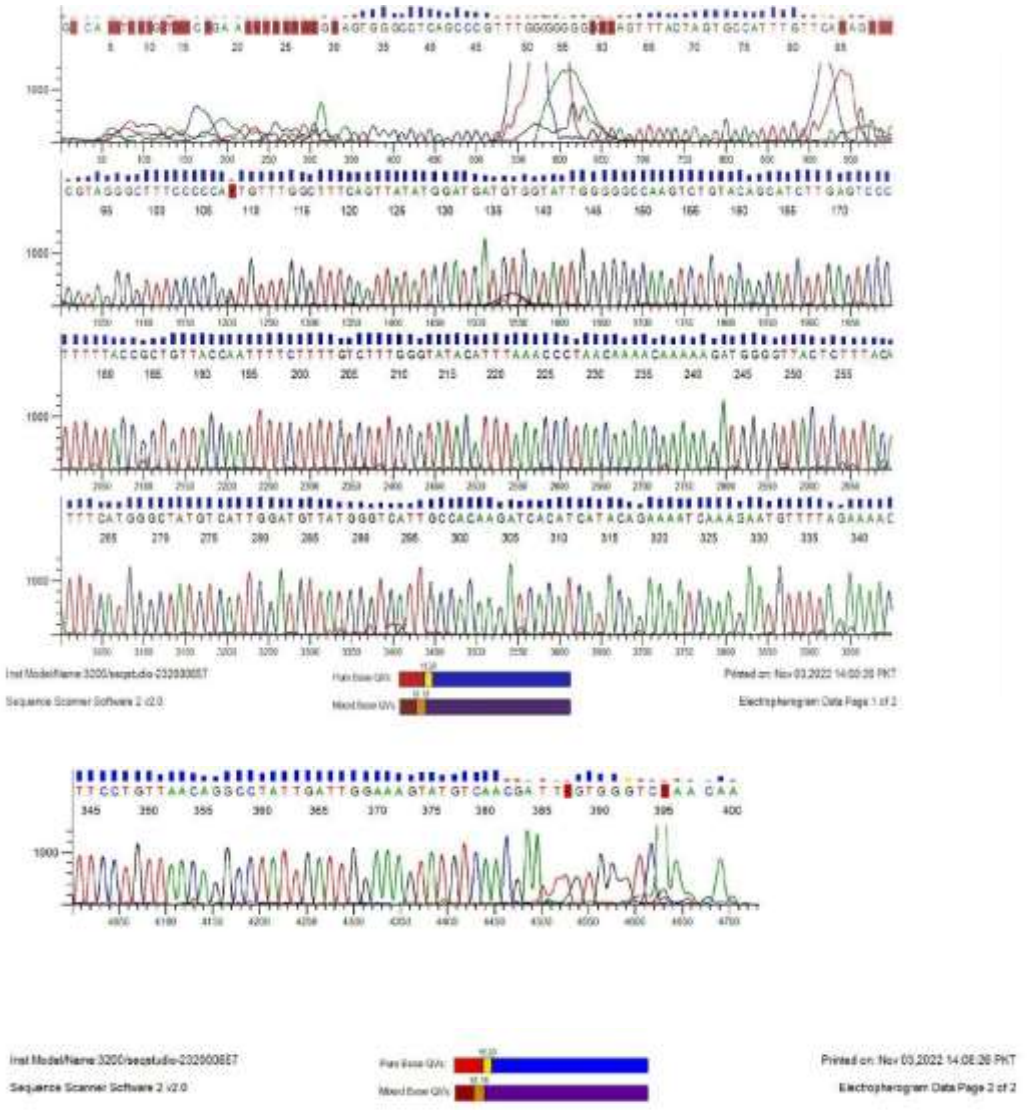
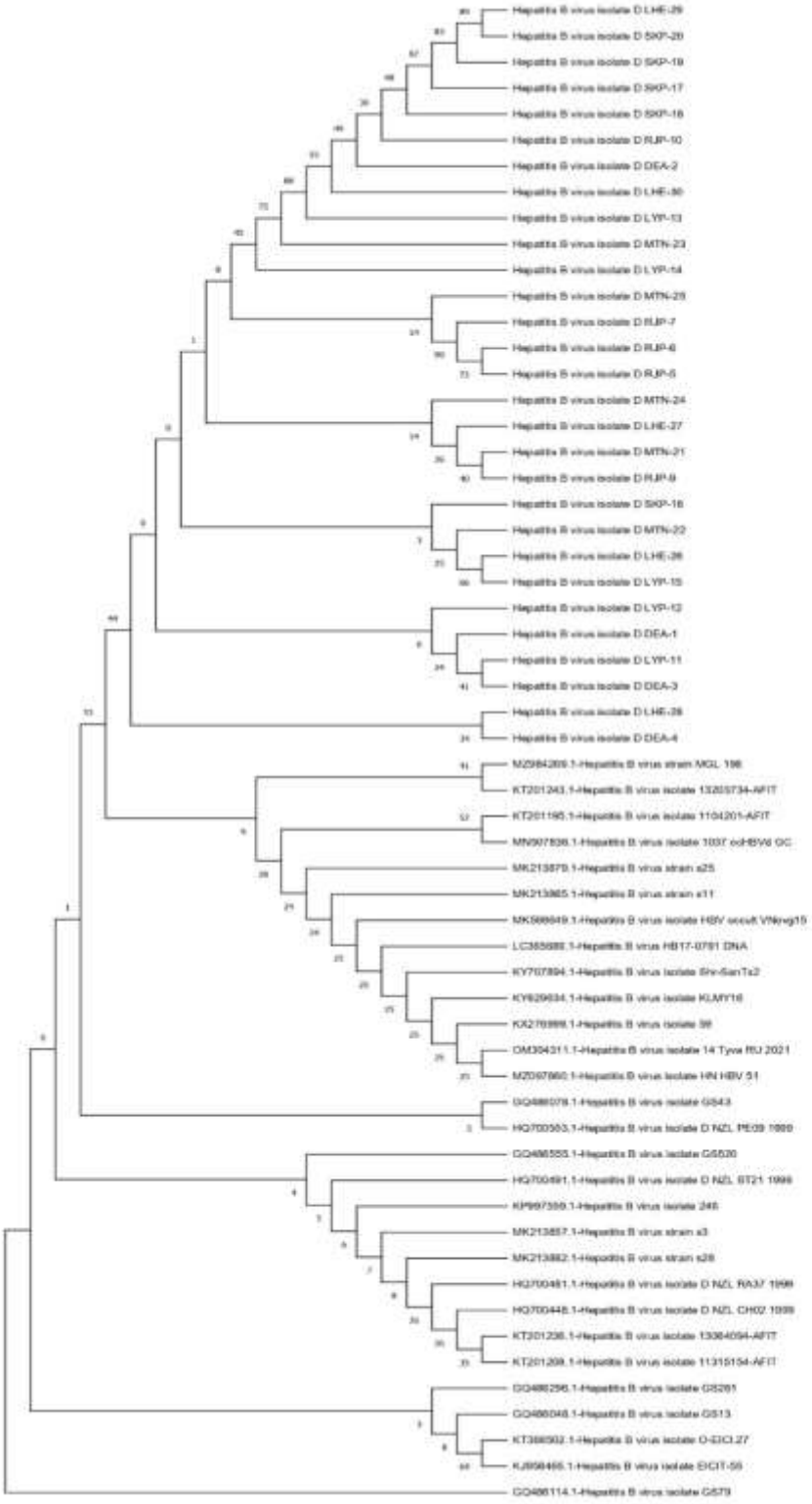


Figure 4: Chromatographic representation of query sequence, isolated from the non-Clearance (positive) sample

In mega 11, edit the selected homologues sequence which was retrieve through the blast, six sequence were used for generated the phylogenetic tree in mega 11 tool. The boost and causal align the cross-match sequences to generate the phylogenetic tree. The neighbour-joining was used to make the evolutionary past. The ideal tree can be seen next. The branches are accompanied with the proportion of resembling trees in which the linked taxa grouped during the bootstrap test (1000 repetitions). The tree is displayed to scale by using branch sizes that correspond to the evolutionary detachments that were used to approximate the phylogenetic tree. The reflect changes were calculated using the Highest Composite Likelihood method and are expressed as base replacements per spot. There were 58 sequences in this analysis. Non-coding codon positions 1+2+4 were comprised. For each sequence duo, all uncertain places were eliminated (pairwise deletion). The final dataset contained 421 locations altogether as seen in fig 5.



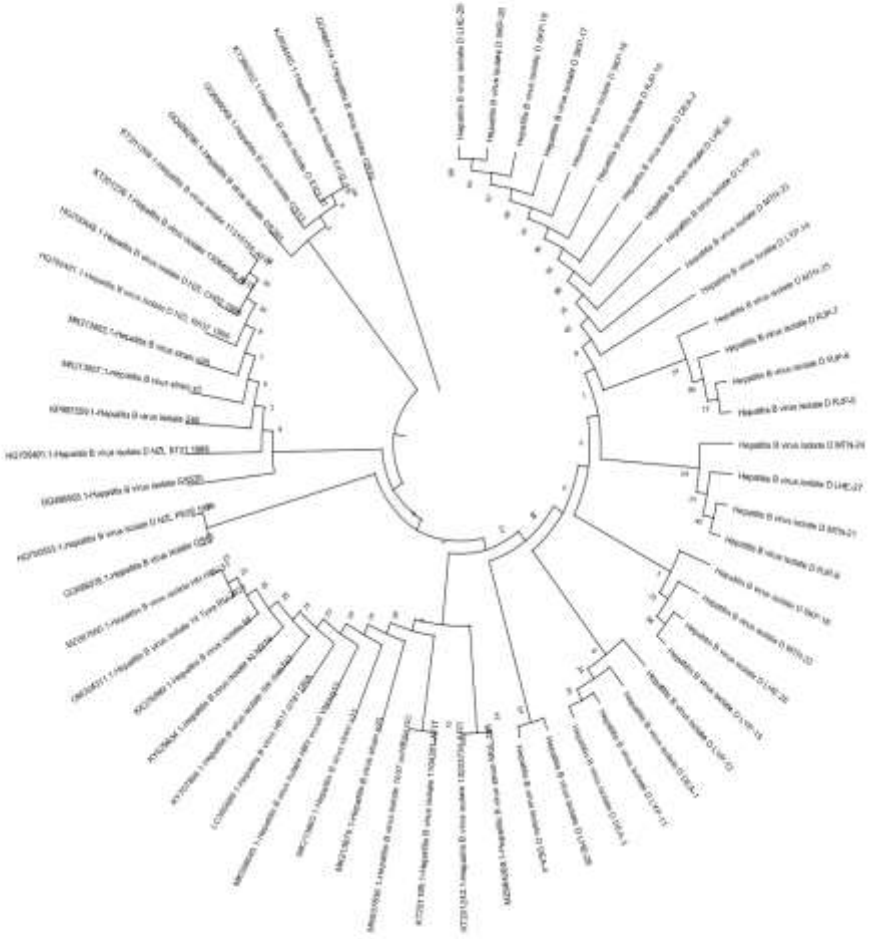


Figure 5a &b: Phylogenetic tree of query sequence, which showed alignment with Hepatitis B virus strains, genotype D strains.

Discussion

This investigation directed that the majority of the patients (72.5%) belonged to the genotype D. This specific genotype is the most prevalent genotype and has been detected worldwide. The highest frequency was seen in a region of Southern Europe, North Africa to India, as well as in West and South Africa (Baig et al., 2009; Kostaki et al., 2018). According to reports from Abbas et al. (2006), Punjab in Pakistan is one of several areas with a high HBV sero-prevalence rate, with genotype D being common (Umer & Iqbal, 2016). Nevertheless, there is presently no information on Punjab's phylogenetic relationship to other nations.

The fact that Genotype D predominates among patients found to be in contradiction to findings available at the while this investigate was in beginning in 2020. According to Idrees et al. (2004), Punjab province in Pakistan has four genotypes that were prevalent to variable degrees: A, B, C, and D. (Awan et al., 2010). In comparison to other regions in Pakistan, they informed genotype A (68%) as the main type in Sindh, genetic constitution C utmost prevalent in North West Frontier Province (N.W.F.P.) (69%), although genotypes B and C. They also reported genotype D (69%) as the predominant type in Punjab and

genotype A with less dominance in Punjab (38%), whereas genotypes B and C were published with more or less even governance in Punjab (32% and 26% respectively). The fundamental cause of this disagreement may be attributed to the fact that they did not use type precise PCR as their approach. As a result, B and C were thought to be the most common genotypes in Asia. This is because the majority of the original study on genotyping was done in Japan and China. Eventually, it was discovered that Asia is home to all seven HBV genotypes, with genotype D predominating in South East Asia (Chu & Lok, 2002). For instance, genotypes D and A are currently the most common in India (Wasitthankasem et al., 2015). Similar to this, genotype D has also been assigned to Iranian HBV isolate sequences (Khodadad et al., 2020). As a result, the genotype frequency trend in Punjab, Pakistan, is in line with findings from South East Asian countries, particularly those that border Pakistan, like Iran, Afghanistan, and India, where genotype D predominates. This aim of research is not only to emphasized the genetic connections between the neighboring nations, but it also gave researchers a chance to examine how the numerous ethnic groups that make up this country's population differ in terms of their HBV genotype distribution. The majority of the research was done at General Hospital Lahore, DHQ Hospital Sheikhupura, Nishtar Hospital Multan, Allied Hospital Faisalabad, DHQ Hospital Rajanpur, and DHQ Teaching Hospital Dera Ghazi Khan.

Along with genotype D (72.5%), which was shown to be the most common, 11.5% of the patients had genotype A infection, and 9.3% had both type A and type D infection. In places where several genotypes are predominant, the combination of genotypes A and D is frequent. These co-circulating genotypes offer a mechanism for individual and population-wide diversity. According to Assih et al. (2018), recombinants of the genotypes A and D, B/C, and C/D have been seen in Africa, Asia, and Tibet (Li et al., 2018). Nevertheless, no recombination-related evidence was observed in the samples that were sequenced for the current investigation. The samples that contained both A and D were not sequenced because of the incredibly low HBV titre. Additionally, there are fewer recombinants between genotypes A and D because they are limited to overlapping and non-overlapping regions of the P/S open reading frames.

The evolutionary history was concluded using the neighbour-joining technique. It displays the ideal tree. The proportion of duplicate branches where the related taxa clustered in bootstrap test is included with each branch (1000 repetitions). The tree is scaled by using branch lengths that are equal to the evolution ranges used to calculate the phylogenetic tree. Using the Utmost Compound Likelihood scheme, the evolutionary detachments, which are voiced in base switches per position, were figured out. In this investigation, there were 58 different nucleotide sequences. Non-coding codon sites 1+2+4 were involved. For respectively sequence pair, all indistinct places were removed (pairwise deletion). The final dataset confined 421 sites overall. Evolutionary investigations were carried out using MEGA11.

This study involved the examination of 100 patients who tested positive for HBsAg, and it was discovered that genotype D and A were existing in all categories in 52 (58.8%) men and 38 (42.2%) women with diverse liver diseases. The prevalence of genotype D was found to be the highest among all subgroups of patients with HBV. HBV/D was positive in cases of acute (52), chronic (28), carrier (7), and cirrhosis/HCC (3). An individual who contracts an acute hepatitis B infection either achieves complete immunological elimination of the virus, conferring lifetime immunity, or contracts chronic hepatitis B. This results in the immunological tolerant, the immune cleansing phase, and the passive carrier phase with or without repetition, which are the three points of HBV infection depending on viral-host dealings. Some people who have experienced acute HBV infection may now be in the immunological tolerance phase. The carrier stage comes next, during which seroconversion of HBeAg to HBeAb takes place. Low HBV levels with normal ALT imply very minor or no hepatic damage. The inactive carrier stages could endure for a lifetime or possibly for many years. Patients in this stage of the disease may experience impulsive or

immunosuppression-induced recurrence of long-term hepatitis, characterized by raised up ALT, high level of DNA, restrained to serious liver histological action, and with or without HBeAg sero deterioration. Patients may also experience voluntary resolution of hepatitis B and development of HBsAb.

In this research it was observed both gender ratio was approximately 58.8% to 42.2%. It was observed that utmost of the affected role were belonging to the age group of 21-30 years of age (24.4%), followed by 51-60 years of age group (21.1%), 31-40 years of age group (16.6%), 41-50 years of age group (14.4%), >61 years of age (11.1%), 11-20 years of age group (8.88%) and 0-10 years ranging just (3.33%). (Table 1) Majority of patients were observed belonging to the male gender 58%. Except for autoimmune liver diseases like primary biliary cirrhosis and chronic autoimmune hepatitis, clinical research and expiry rates backing the notion that the rate of progression of HBV infections seems to be faster in men than in women. Additionally, cirrhosis is a medical condition that mainly affects men and women after menopause. Understanding the molecular processes that underlie the gender-related variations in chronic HBV infection may help develop more potent treatment options for both gender patients with liver disease. It is unclear if males and females are more or less susceptible to viral DNA integrating into their hepatocytes and causing tumour formation. In this study, from 90 patient samples, 75 (83.3%) samples were positive with PCR and 15 (16.7%) were negative (Fig. 1). The viral load of each sample given by thermocycler was noted down and a comparative analysis was done. It was observed that most of the positive samples had viral load ranging from 50,000-100,000 IU/ml (40%), followed by 100,000-50,00,000 IU/ml (29.3%), 10,000-50,000 IU/ml (16%), >50,00,000 IU/ml (8%) and the least samples (6.66%) belonged to the viral load range 1-10,000 IU/ml. It was observed in this study that almost isolated viral agents had genotype D (subtype D) and all samples were relative to the NC_003977.2 reference sequence for Hepatitis B virus (taxon: 10407). They sequence length was range from 354 base pairs minimum to 418 base pairs.

Conclusion

This investigation concluded that HBV genotype D were predominately found in the HBV-infected patients with relatively little genotypic heterogeneity of Southern and Northern Districts of Punjab in Pakistan. In addition to genotype D, the Southern and Northern Districts of Punjab also have genotype A alone and with the combination of genotypes D were detected in all categories of HBV-infected patients. This study evidences indicated that HBV genotypes can be used to predict the likelihood of developing cirrhosis, HCC, or fulminant liver diseases/acute liver failure (ALF), which may have an impact on treatment choices for these conditions in Southern and Northern Districts of Punjab. The association between genotype A and severe liver disease was also shown to be stronger, though. Mixed genotype (A/D) did not seem to have any effect on the clinical result.

References

Southern, E.M. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J.Mol.Biol., 98:503-517

Aaij, C., and P.Borst.1972.The gel electrophoresis of DNA. Biochim.Biophys.Acta ., 269:192-200.

Butt, A. S., & Ahmed, F. (2018). Management of chronic hepatitis B: knowledge and practices of physicians in Pakistan. Journal of clinical and experimental hepatology, 8(4), 342-351.

Arain, S. Q., Talpur, F. N., Channa, N. A., Ali, M. S., & Afridi, H. I. (2017). Serum lipid profile as a marker of liver impairment in hepatitis B Cirrhosis patients. Lipids in Health and Disease, 16(1), 51. https://doi.org/10.1186/s12944-017-0437-2

Assih, M., Ouattara, A. K., Diarra, B., Yonli, A. T., Compaore, T. R., Obiri-Yeboah, D., Djigma, F. W., Karou, S., & Simpore, J. (2018). Genetic diversity of hepatitis viruses in West-African countries

from 1996 to 2018. World Journal of Hepatology, 10(11), 807–821. https://doi.org/10.4254/wjh.v10.i11.807

Awan, Z., Idrees, M., Amin, I., Butt, S., Afzal, S., Akbar, H., Rehman, I., Younas, S., Shahid, M., Lal, A., Saleem, S., & Rauff, B. (2010). Pattern and molecular epidemiology of Hepatitis B virus genotypes circulating in Pakistan. Infection, Genetics and Evolution, 10(8), 1242–1246. https://doi.org/10.1016/j.meegid.2010.08.006

Baig, S., Siddiqui, A., Chakravarty, R., & Moatter, T. (2009). Hepatitis B virus subgenotypes D1 and D3 are prevalent in Pakistan. BMC Research Notes, 2(1), 1. https://doi.org/10.1186/1756-0500-2-1

Bati, N., Tekerekoglu, M., & Duman, Y. (2017). Investigation of HBV DNA in HBsAg positive patients. Medicine Science | International Medical Journal, 1. https://doi.org/10.5455/medscience.2017.06.8646

Besharat, S., Poustchi, H., Mohamadkhani, A., Katoonizadeh, A., Moradi, A., Roshandel, G., Freedman, N. D., & Malekzadeh, R. (2015). Association of Mutations in the Basal Core Promoter and Pre-core Regions of the Hepatitis B Viral Genome and Longitudinal Changes in HBV Level in HBeAg Negative Individuals: Results From a Cohort Study in Northern Iran. Hepatitis Monthly, 15(2), e23875. https://doi.org/10.5812/hepatmon.23875

Bui, T. T. T., Tran, T. T., Nghiem, M. N., Rahman, P., Tran, T. T. T., Dinh, M. N. H., Le, M. H., Nguyen, V. V. C., Thwaites, G., & Rahman, M. (2017). Molecular characterization of hepatitis B virus in Vietnam. BMC Infectious Diseases, 17(1), 601. https://doi.org/10.1186/s12879-017-2697-x

Buti, M., Gane, E., Seto, W. K., Chan, H. L. Y., Chuang, W.-L., Stepanova, T., Hui, A.-J., Lim, Y.-S., Mehta, R., Janssen, H. L. A., Acharya, S. K., Flaherty, J. F., Massetto, B., Cathcart, A. L., Kim, K., Gaggar, A., Subramanian, G. M., McHutchison, J. G., Pan, C. Q., … Marcellin, P. (2016). Tenofovir alafenamide versus tenofovir disoproxil fumarate for the treatment of patients with HBeAg-negative chronic hepatitis B virus infection: A randomised, double-blind, phase 3, non-inferiority trial. The Lancet Gastroenterology & Hepatology, 1(3), 196–206. https://doi.org/10.1016/S2468-1253(16)30107-8

Caligiuri, P., Cerruti, R., Icardi, G., & Bruzzone, B. (2016). Overview of hepatitis B virus mutations and their implications in the management of infection. World Journal of Gastroenterology, 22(1), 145–154. https://doi.org/10.3748/wjg.v22.i1.145

Chauhan, R., Lingala, S., Gadiparthi, C., Lahiri, N., Mohanty, S. R., Wu, J., Michalak, T. I., & Satapathy, S. K. (2018). Reactivation of hepatitis B after liver transplantation: Current knowledge, molecular mechanisms and implications in management. World Journal of Hepatology, 10(3), 352–370. https://doi.org/10.4254/wjh.v10.i3.352

Cheng, X., Xia, Y., Serti, E., Block, P. D., Chung, M., Chayama, K., Rehermann, B., & Liang, T. J. (2017). Hepatitis B virus evades innate immunity of hepatocytes but activates cytokine production by macrophages. Hepatology, 66(6), 1779–1793. https://doi.org/10.1002/hep.29348

Chu, C.-J., & Lok, A. S. F. (2002). Clinical significance of hepatitis B virus genotypes: Clinical significance of hepatitis B virus genotypes. Hepatology, 35(5), 1274–1276. https://doi.org/10.1053/jhep.2002.33161

Croagh, C. M., Desmond, P. V., & Bell, S. J. (2015). Genotypes and viral variants in chronic hepatitis B: A review of epidemiology and clinical relevance. World Journal of Hepatology, 7(3), 289–303. https://doi.org/10.4254/wjh.v7.i3.289

de Almeida Pondé, R. A. (2021). Dynamic profile of the HBeAg-anti-HBe system in acute and chronic hepatitis B virus infection: A clinical-laboratory approach. Molecular Biology Reports, 48(1), 843–854. https://doi.org/10.1007/s11033-020-06056-4

DHQ Hospital Mardan, Khyber Paktoonkhwa, Pakistan., Khan, M. A., Waqar, M., PCR Section, Genome Centre for Molecular Based Diagnostics & Research Lahore, Pakistan., Rehman, Z., PCR Section, Genome Centre for Molecular Based Diagnostics & Research Lahore, Pakistan., Idrees, M., Centre for Applied Molecular Biology, University of the Punjab, Lahore, Pakistan., Wasim, M., Department of Medicine, Khyber Medical Collage Peshawar Khyber Paktoonkhwa, Pakistan, Asghar, A., Department of Zoology, University of Education, Lahore, Pakistan., Shahrukh, Q., & Department of Zoology, Kohat University of Science and Technology, Kohat, Khyber Pakhtoonkhwa, Pakistan. (2019). Hepatitis B Virus (HBV) a Severe Health Problem in Mardan, Khyber Pakhtunkhwa: A Molecular Based Study. BioScientific Review, 01(01), 25–32. https://doi.org/10.32350/BSR.0101.03

Ebrahimi, F., Ahangar Oskoee, M., Bannazadeh Baghi, H., Sadeghi, J., Ahangarzadeh Rezaei, M., Gholizadeh, O., Mohammadzadeh, N., Dashti, A., & Poortahmasebi, V. (2022). Phylogenetic and Immune Epitope Analysis of Hepatitis B Surface Antigen in Chronic Carriers from Tabriz,

Northwest of Iran. ImmunoAnalysis, 2, 7. https://doi.org/10.34172/ia.2022.07

Fabrizi, F., Dixit, V., Messa, P., & Martin, P. (2015). Transmission of Hepatitis B Virus in Dialysis Units: A Systematic Review of Reports on Outbreaks. The International Journal of Artificial Organs, 38(1), 1–7. https://doi.org/10.5301/ijao.5000376

Gasteiger, G., Ataide, M., & Kastenmüller, W. (2016). Lymph node—An organ for T-cell activation and pathogen defense. Immunological Reviews, 271(1), 200–220. https://doi.org/10.1111/imr.12399

Gish, R. G. (2020). We Are All Africans: A Highly Personal Migratory View of the History of Hepatitis B. Clinical Liver Disease, 16(Suppl 1), 24–33. https://doi.org/10.1002/cld.989

Hofstraat, S. H. I., Falla, A. M., Duffell, E. F., Hahné, S. J. M., Amato-Gauci, A. J., Veldhuijzen, I. K., & Tavoschi, L. (2017). Current prevalence of chronic hepatitis B and C virus infection in the general population, blood donors and pregnant women in the EU/EEA: A systematic review. Epidemiology and Infection, 145(14), 2873–2885. https://doi.org/10.1017/S0950268817001947

Hu, J., Lin, Y.-Y., Chen, P.-J., Watashi, K., & Wakita, T. (2019). Cell and Animal Models for Studying Hepatitis B Virus Infection and Drug Development. Gastroenterology, 156(2), 338–354. https://doi.org/10.1053/j.gastro.2018.06.093

Hu, J., & Liu, K. (2017). Complete and Incomplete Hepatitis B Virus Particles: Formation, Function, and Application. Viruses, 9(3), 56. https://doi.org/10.3390/v9030056

Hu, X. F., & Chan, H. M. (2018). Factors associated with the blood and urinary selenium concentrations in the Canadian population: Results of the Canadian Health Measures Survey (2007–2011). International Journal of Hygiene and Environmental Health, 221(7), 1023–1031. https://doi.org/10.1016/j.ijheh.2018.07.001

Iannacone, M., & Guidotti, L. G. (2022). Immunobiology and pathogenesis of hepatitis B virus infection. Nature Reviews Immunology, 22(1), 19–32. https://doi.org/10.1038/s41577-021-00549-4

İnan, N., & Tabak, F. (2015). Hepatitis B Virus: Biology and Life Cycle. Viral Hepatit Dergisi, 21(1), 1–7. https://doi.org/10.4274/vhd.36036

Invernizzi, F., Viganò, M., Grossi, G., & Lampertico, P. (2016). The prognosis and management of inactive HBV carriers. Liver International, 36, 100–104. https://doi.org/10.1111/liv.13006

Jayaraman, T., Lee, Y., Chan, W., & Mahadeva, S. (2020). Epidemiological differences of common liver conditions between Asia and the West. JGH Open, 4(3), 332–339. https://doi.org/10.1002/jgh3.12275

Jiang, B., Himmelsbach, K., Ren, H., Boller, K., & Hildt, E. (2016). Subviral Hepatitis B Virus Filaments, like Infectious Viral Particles, Are Released via Multivesicular Bodies. Journal of Virology, 90(7), 3330–3341. https://doi.org/10.1128/JVI.03109-15

Kao, J.-H. (2015). Hepatitis B vaccination and prevention of hepatocellular carcinoma. Best Practice & Research Clinical Gastroenterology, 29(6), 907–917. https://doi.org/10.1016/j.bpg.2015.09.011

Karimova, M., Beschorner, N., Dammermann, W., Chemnitz, J., Indenbirken, D., Bockmann, J.-H., Grundhoff, A., Lüth, S., Buchholz, F., Wiesch, J. S. zur, & Hauber, J. (2015). CRISPR/Cas9 nickase-mediated disruption of hepatitis B virus open reading frame S and X. Scientific Reports, 5(1), 13734. https://doi.org/10.1038/srep13734

Keane, E., Funk, A. L., & Shimakawa, Y. (2016). Systematic review with meta-analysis: The risk of mother-to-child transmission of hepatitis B virus infection in sub-Saharan Africa. Alimentary Pharmacology & Therapeutics, 44(10), 1005–1017. https://doi.org/10.1111/apt.13795

Khodadad, N., Seyedian, S. S., Moattari, A., Biparva Haghighi, S., Pirmoradi, R., Abbasi, S., & Makvandi, M. (2020). In silico functional and structural characterization of hepatitis B virus PreS/S-gene in Iranian patients infected with chronic hepatitis B virus genotype D. Heliyon, 6(7), e04332. https://doi.org/10.1016/j.heliyon.2020.e04332

Khuroo, M. S. (2019). A Review of Acute Viral Hepatitides Including Hepatitis E. In R. Ozaras & J. E. Arends (Eds.), Viral Hepatitis: Acute Hepatitis (pp. 77–107). Springer International Publishing. https://doi.org/10.1007/978-3-030-03535-8_6

Kim, G.-A., Han, S., Choi, G. H., Choi, J., & Lim, Y.-S. (2020). Moderate levels of serum hepatitis B virus DNA are associated with the highest risk of hepatocellular carcinoma in chronic hepatitis B patients. Alimentary Pharmacology & Therapeutics, 51(11), 1169–1179. https://doi.org/10.1111/apt.15725

Kmet Lunaček, N., Poljak, M., & Matičič, M. (2018). Distribution of hepatitis B virus genotypes in Europe and clinical implications: A review. Acta Dermatovenerologica Alpina Pannonica et Adriatica, 27(3). https://doi.org/10.15570/actaapa.2018.28

Koffas, A., Petersen, J., & Kennedy, P. T. (2020). Reasons to consider early treatment in chronic

hepatitis B patients. Antiviral Research, 177, 104783. https://doi.org/10.1016/j.antiviral.2020.104783

Kostaki, E.-G., Karamitros, T., Stefanou, G., Mamais, I., Angelis, K., Hatzakis, A., Kramvis, A., & Paraskevis, D. (2018). Unravelling the history of hepatitis B virus genotypes A and D infection using a full-genome phylogenetic and phylogeographic approach. ELife, 7, e36709. https://doi.org/10.7554/eLife.36709

Lavanchy, D., & Kane, M. (2016). Global Epidemiology of Hepatitis B Virus Infection. In Y.-F. Liaw & F. Zoulim (Eds.), Hepatitis B Virus in Human Diseases (pp. 187–203). Springer International Publishing. https://doi.org/10.1007/978-3-319-22330-8_9

Lazarevic, I., Banko, A., Miljanovic, D., & Cupic, M. (2020). Biological features of hepatitis B virus strains associated with fulminant hepatitis. Future Virology, 15(7), 455–469. https://doi.org/10.2217/fvl-2020-0011

Le, T., Vu, T., Mai, H., Nguyen, L., Truong, N., Hoang, C., Nguyen, S., Nguyen, C., Nguyen, B., Tran, T., Tran, B., Latkin, C., Ho, C., & Ho, R. (2019). Social Determinants of Stigma and Discrimination in Vietnamese Patients with Chronic Hepatitis B. International Journal of Environmental Research and Public Health, 16(3), 398. https://doi.org/10.3390/ijerph16030398

Li, H., She, Q., Liu, Y., Ding, Y., Shi, S., Li, J., Wu, H., & Wang, Z. (2018). Clinical implication and viral mutation in basal core promoter/pre-core of hepatitis B virus C/D recombinant. Hepatology International, 12(5), 447–455. https://doi.org/10.1007/s12072-018-9885-7

Li, W., & Urban, S. (2016). Entry of hepatitis B and hepatitis D virus into hepatocytes: Basic insights and clinical implications. Journal of Hepatology, 64(1), S32–S40. https://doi.org/10.1016/j.jhep.2016.02.011

Liang, Y., Teng, W., Chen, C., Sun, C., Teng, R., Huang, Y., Liang, K., Chen, Y., Lin, C., Su, C., Tao, M., & Wu, J. (2021). Clinical Implications of HBV PreS/S Mutations and the Effects of PreS2 Deletion on Mitochondria, Liver Fibrosis, and Cancer Development. Hepatology, 74(2), 641–655. https://doi.org/10.1002/hep.31789

Lin, C.-L., & Kao, J.-H. (2017). Natural history of acute and chronic hepatitis B: The role of HBV genotypes and mutants. Best Practice & Research Clinical Gastroenterology, 31(3), 249–255. https://doi.org/10.1016/j.bpg.2017.04.010

Lin, C.-L., & Kao, J.-H. (2018). Review article: The prevention of hepatitis B-related hepatocellular carcinoma. Alimentary Pharmacology & Therapeutics, 48(1), 5–14. https://doi.org/10.1111/apt.14683

Liu, C.-J., & Chen, P.-J. (2020). Elimination of Hepatitis B in Highly Endemic Settings: Lessons Learned in Taiwan and Challenges Ahead. Viruses, 12(8), 815. https://doi.org/10.3390/v12080815

Liu, F., Campagna, M., Qi, Y., Zhao, X., Guo, F., Xu, C., Li, S., Li, W., Block, T. M., Chang, J., & Guo, J.-T. (2013). Alpha-Interferon Suppresses Hepadnavirus Transcription by Altering Epigenetic Modification of cccDNA Minichromosomes. PLoS Pathogens, 9(9), e1003613. https://doi.org/10.1371/journal.ppat.1003613

Liu, J., Wang, J., Yan, X., Xue, R., Zhan, J., Jiang, S., Geng, Y., Liu, Y., Mao, M., Xia, J., Yin, S., Tong, X., Chen, Y., Ding, W., Huang, R., & Wu, C. (2022). Presence of Liver Inflammation in Asian Patients With Chronic Hepatitis B With Normal ALT and Detectable HBV DNA in Absence of Liver Fibrosis. Hepatology Communications, 6(4), 855–866. https://doi.org/10.1002/hep4.1859

Liu, Z., Zhang, Y., Xu, M., Li, X., & Zhang, Z. (2021). Distribution of hepatitis B virus genotypes and subgenotypes: A meta-analysis. Medicine, 100(50), e27941. https://doi.org/10.1097/MD.0000000000027941

Lok, A. S., Zoulim, F., Dusheiko, G., & Ghany, M. G. (2017). Hepatitis B cure: From discovery to regulatory approval. Journal of Hepatology, 67(4), 847–861. https://doi.org/10.1016/j.jhep.2017.05.008

Lozano, R., Fullman, N., Abate, D., Abay, S. M., Abbafati, C., Abbasi, N., Abbastabar, H., Abd-Allah, F., Abdela, J., Abdelalim, A., Abdel-Rahman, O., Abdi, A., Abdollahpour, I., Abdulkader, R. S., Abebe, N. D., Abebe, Z., Abejie, A. N., Abera, S. F., Abil, O. Z., … Murray, C. J. L. (2018). Measuring progress from 1990 to 2017 and projecting attainment to 2030 of the health-related Sustainable Development Goals for 195 countries and territories: A systematic analysis for the Global Burden of Disease Study 2017. The Lancet, 392(10159), 2091–2138. https://doi.org/10.1016/S0140-6736(18)32281-5

Malhotra, R., Soin, D., Grover, P., Galhotra, S., Khutan, H., & Kaur, N. (2016). Hepatitis B virus and hepatitis C virus co-infection in hemodialysis patients: A retrospective study from a tertiary care hospital of North India. Journal of Natural Science, Biology, and Medicine, 7(1), 72–74. https://doi.org/10.4103/0976-9668.175076

Nayagam, S., Thursz, M., Sicuri, E., Conteh, L., Wiktor, S., Low-Beer, D., & Hallett, T. B. (2016).

Requirements for global elimination of hepatitis B: A modelling study. The Lancet Infectious Diseases, 16(12), 1399–1408. https://doi.org/10.1016/S1473-3099(16)30204-3

Ni, Y.-H., Chang, M.-H., Jan, C.-F., Hsu, H.-Y., Chen, H.-L., Wu, J.-F., & Chen, D.-S. (2016). Continuing Decrease in Hepatitis B Virus Infection 30 Years After Initiation of Infant Vaccination Program in Taiwan. Clinical Gastroenterology and Hepatology, 14(9), 1324–1330. https://doi.org/10.1016/j.cgh.2016.04.030

Ofori-Asenso, R., & Agyeman, A. A. (2016). Hepatitis B in Ghana: A systematic review & meta-analysis of prevalence studies (1995-2015). BMC Infectious Diseases, 16(1), 130. https://doi.org/10.1186/s12879-016-1467-5

Omata, M., Cheng, A.-L., Kokudo, N., Kudo, M., Lee, J. M., Jia, J., Tateishi, R., Han, K.-H., Chawla, Y. K., Shiina, S., Jafri, W., Payawal, D. A., Ohki, T., Ogasawara, S., Chen, P.-J., Lesmana, C. R. A., Lesmana, L. A., Gani, R. A., Obi, S., … Sarin, S. K. (2017). Asia–Pacific clinical practice guidelines on the management of hepatocellular carcinoma: A 2017 update. Hepatology International, 11(4), 317–370. https://doi.org/10.1007/s12072-017-9799-9

Ozakyol, A. (2017). Global Epidemiology of Hepatocellular Carcinoma (HCC Epidemiology). Journal of Gastrointestinal Cancer, 48(3), 238–240. https://doi.org/10.1007/s12029-017-9959-0

Ozaras, R., Inanc Balkan, I., Yemisen, M., & Tabak, F. (2015). Epidemiology of HBV subgenotypes D. Clinics and Research in Hepatology and Gastroenterology, 39(1), 28–37. https://doi.org/10.1016/j.clinre.2014.06.005

Rasheed, H., Khawar, M. B., Habiba, U., Aman, S., Shah, S. S., Afzal, A., Hamid, S. E., Abbasi, M. H., Sheikh, N., Rafiq, M., Azam, F., Mehmood, R., & Afzal, N. (2022). Variations in Peripheral Hematological Parameters as a Diagnostic Biomarker of HBV Infection. Asian Journal of Health Sciences, 8(2), 45–45. https://doi.org/10.15419/ajhs.v8i2.516

Rezaei, N., Asadi-Lari, M., Sheidaei, A., Gohari, K., Parsaeian, M., Khademioureh, S., Maghsoudlu, M., Amini Kafiabad, S., Zadsar, M., Motevalian, S. A., Delavari, F., Abedini, S., & FarzadFar, F. (2020). Epidemiology of Hepatitis B in Iran from 2000 to 2016: A Systematic Review and Meta-Regression Analysis. Archives of Iranian Medicine, 23(3), 189–196.

Ringelhan, M., O'Connor, T., Protzer, U., & Heikenwalder, M. (2015). The direct and indirect roles of HBV in liver cancer: Prospective markers for HCC screening and potential therapeutic targets: Direct and indirect roles of HBV in liver cancer. The Journal of Pathology, 235(2), 355–367. https://doi.org/10.1002/path.4434

Sagnelli, E., Macera, M., Russo, A., Coppola, N., & Sagnelli, C. (2020). Epidemiological and etiological variations in hepatocellular carcinoma. Infection, 48(1), 7–17. https://doi.org/10.1007/s15010-019-01345-y

Samo, A. A., Laghari, Z. A., Baig, N. M., & Khoso, G. M. (2020). Prevalence and Risk Factors Associated with Hepatitis B and C in Nawabshah, Sindh, Pakistan. The American Journal of Tropical Medicine and Hygiene, 104(3), 1101–1105. https://doi.org/10.4269/ajtmh.20-1228

Schweitzer, A., Horn, J., Mikolajczyk, R. T., Krause, G., & Ott, J. J. (2015). Estimations of worldwide prevalence of chronic hepatitis B virus infection: A systematic review of data published between 1965 and 2013. The Lancet, 386(10003), 1546–1555. https://doi.org/10.1016/S0140-6736(15)61412-X

Seeger, C., & Mason, W. S. (2015). Molecular biology of hepatitis B virus infection. Virology, 479–480, 672–686. https://doi.org/10.1016/j.virol.2015.02.031

Seerat, I., Mushtaq, H., Rafiq, M., & Nadir, A. (2020). Frequency and Associated Risk Factors of Hepatitis B Virus and Hepatitis C Virus Infections in Children at a Hepatitis Prevention and Treatment Clinic in Lahore, Pakistan. Cureus. https://doi.org/10.7759/cureus.7926

Seitz, S., Habjanič, J., Schütz, A. K., & Bartenschlager, R. (2020). The Hepatitis B Virus Envelope Proteins: Molecular Gymnastics Throughout the Viral Life Cycle. Annual Review of Virology, 7(1), 263–288. https://doi.org/10.1146/annurev-virology-092818-015508

Seto, W.-K., Lo, Y.-R., Pawlotsky, J.-M., & Yuen, M.-F. (2018). Chronic hepatitis B virus infection. The Lancet, 392(10161), 2313–2324. https://doi.org/10.1016/S0140-6736(18)31865-8

Sharma, S., Carballo, M., Feld, J. J., & Janssen, H. L. A. (2015). Immigration and viral hepatitis. Journal of Hepatology, 63(2), 515–522. https://doi.org/10.1016/j.jhep.2015.04.026

Tan, A. T., & Schreiber, S. (2020). Adoptive T-cell therapy for HBV-associated HCC and HBV infection. Antiviral Research, 176, 104748. https://doi.org/10.1016/j.antiviral.2020.104748

Tout, I., Loureiro, D., Mansouri, A., Soumelis, V., Boyer, N., & Asselah, T. (2020). Hepatitis B surface antigen seroclearance: Immune mechanisms, clinical impact, importance for drug development. Journal of Hepatology, 73(2), 409–422. https://doi.org/10.1016/j.jhep.2020.04.013

Twagirumugabe, T. (2018). Epidemiology, molecular characterization of hepatitis viruses in

Rwanda and implication on liver disease. Göteborgs universitet.

Umer, M., & Iqbal, M. (2016). Hepatitis C virus prevalence and genotype distribution in Pakistan: Comprehensive review of recent data. World Journal of Gastroenterology, 22(4), 1684–1700. https://doi.org/10.3748/wjg.v22.i4.1684

Vedio, A., Liu, E. Z. H., Lee, A. C. K., & Salway, S. (2017). Improving access to health care for chronic hepatitis B among migrant Chinese populations: A systematic mixed methods review of barriers and enablers. Journal of Viral Hepatitis, 24(7), 526–540. https://doi.org/10.1111/jvh.12673

Velkov, S., Ott, J., Protzer, U., & Michler, T. (2018). The Global Hepatitis B Virus Genotype Distribution Approximated from Available Genotyping Data. Genes, 9(10), 495. https://doi.org/10.3390/genes9100495

Venkatakrishnan, B., & Zlotnick, A. (2016). The Structural Biology of Hepatitis B Virus: Form and Function. Annual Review of Virology, 3(1), 429–451. https://doi.org/10.1146/annurev-virology-110615-042238

Vyas, A. K., Jindal, A., Hissar, S., Ramakrishna, G., & Trehanpati, N. (2017). Immune balance in Hepatitis B Infection: Present and Future Therapies. Scandinavian Journal of Immunology, 86(1), 4–14. https://doi.org/10.1111/sji.12553

Wai, S. N. (2019). Analysis of Hepatitis B in novel primary hepatocyte cultures. https://doi.org/10.25560/93879

Walsh, R., Hammond, R., Yuen, L., Deerain, J., O'Donnell, T., Leary, T., Cloherty, G., Gaggar, A., Kitrinos, K., Subramanian, M., Wong, D., & Locarnini, S. (2019). Predicting HBsAg clearance in genotype A chronic hepatitis B using HBsAg epitope profiling: A biomarker for functional cure. Liver International, 39(11), 2066–2076. https://doi.org/10.1111/liv.14207

Wang, H., Naghavi, M., Allen, C., Barber, R. M., Bhutta, Z. A., Carter, A., Casey, D. C., Charlson, F. J., Chen, A. Z., Coates, M. M., Coggeshall, M., Dandona, L., Dicker, D. J., Erskine, H. E., Ferrari, A. J., Fitzmaurice, C., Foreman, K., Forouzanfar, M. H., Fraser, M. S., … Murray, C. J. L. (2016). Global, regional, and national life expectancy, all-cause mortality, and cause-specific mortality for 249 causes of death, 1980–2015: A systematic analysis for the Global Burden of Disease Study 2015. The Lancet, 388(10053), 1459–1544. https://doi.org/10.1016/S0140-6736(16)31012-1

Wang, W., Lempp, F. A., Schlund, F., Walter, L., Decker, C. C., Zhang, Z., Ni, Y., & Urban, S. (2021). Assembly and infection efficacy of hepatitis B virus surface protein exchanges in 8 hepatitis D virus genotype isolates. Journal of Hepatology, 75(2), 311–323. https://doi.org/10.1016/j.jhep.2021.03.025

Wasitthankasem, R., Vongpunsawad, S., Siripon, N., Suya, C., Chulothok, P., Chaiear, K., Rujirojindakul, P., Kanjana, S., Theamboonlers, A., Tangkijvanich, P., & Poovorawan, Y. (2015). Genotypic Distribution of Hepatitis C Virus in Thailand and Southeast Asia. PLOS ONE, 10(5), e0126764. https://doi.org/10.1371/journal.pone.0126764

Wei, L., & Ploss, A. (2021). Mechanism of Hepatitis B Virus cccDNA Formation. Viruses, 13(8), 1463. https://doi.org/10.3390/v13081463

Wong, G. L.-H. (2018). Management of chronic hepatitis B patients in immunetolerant phase: What latest guidelines recommend. Clinical and Molecular Hepatology, 24(2), 108–113. https://doi.org/10.3350/cmh.2017.0068

Yang, J. D., Hainaut, P., Gores, G. J., Amadou, A., Plymoth, A., & Roberts, L. R. (2019). A global view of hepatocellular carcinoma: Trends, risk, prevention and management. Nature Reviews Gastroenterology & Hepatology, 16(10), 589–604. https://doi.org/10.1038/s41575-019-0186-y

Yi, P., Chen, R., Huang, Y., Zhou, R.-R., & Fan, X.-G. (2016). Management of mother-to-child transmission of hepatitis B virus: Propositions and challenges. Journal of Clinical Virology, 77, 32–39. https://doi.org/10.1016/j.jcv.2016.02.003

Yuen, M.-F., Chen, D.-S., Dusheiko, G. M., Janssen, H. L. A., Lau, D. T. Y., Locarnini, S. A., Peters, M. G., & Lai, C.-L. (2018). Hepatitis B virus infection. Nature Reviews Disease Primers, 4(1), 18035. https://doi.org/10.1038/nrdp.2018.35

Zampino, R., Boemio, A., Sagnelli, C., Alessio, L., Adinolfi, L. E., Sagnelli, E., & Coppola, N. (2015). Hepatitis B virus burden in developing countries. World Journal of Gastroenterology, 21(42), 11941–11953. https://doi.org/10.3748/wjg.v21.i42.11941