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Publisher of open access books and journals
PJMLS
Print ISSN: 2707-4471. Online ISSN: 2707-448X

Research Article	Pak-Euro Journal of Medical and Life Sciences
DOI: 10.31580/pjmls.v6i4.2567	Copyright © All rights are reserved by Corresponding Author
Vol. 6 No. 4, 2023: pp. 589-596	
www.readersinsight.net/pjmls	**Revised:** December 06, 2023 **Accepted:** December 07, 2023
Submission: September 17, 2023	**Published Online:** December 31, 2023

PREVALENCE OF HDV INFECTION AMONG HBsAg POSITIVE PATIENTS IN DISTRICT NASIRABAD BALOCHISTAN

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Abstract

Objective: *To clarify the relationship between HBV single infection and HDV-HBV dual infection in the patients of Nasirabad District of Balochistan.*

Methodology: *The 200 HBsAg positive individuals including 68% male and 32% female patients from diverse age groups were collected from the Departments of General Surgery, Bolan Medical Complex and Hospital (BMCH), Quetta and Nasirabad DHQs Hospitals in Balochistan between August 2022 and November 2022. First, the serum samples from patients whose tests for HBV using the Immunochromatographic Technology (ICT) were positive were gathered. First, the serum samples from patients whose tests for HBV using the Immunochromatographic Technology (ICT) were positive were gathered.*

Results: *It had 136 (68%) men and 64 (32%) women, with mean ages of 32.50 (for men) and 36.68 (for women) (Female). HBsAg positive samples were subjected to anti-HDV ELISA screening, Furthermore, anti-HDV antibodies were found in 6 samples (3.09% of the total HBsAg positive samples). This means that 2 out of 200 samples tested positive for both HBV and HDV infections, or 3% of the total. It was discovered that the mean values for AST, ALT, and bilirubin were, respectively, 77 IU/L, 93 IU/L, and 1.9 mg/dL.*

Conclusion: *Since subpar health standards considerably contribute to the spread of infections, healthcare standards and procedures also need to be improved. Concerned authorities should educate the public about HBV-HDV co-infection and any potential risk factors by holding seminars, workshops, and door-to-door polls.*

Keywords: *Co-infection, HBV, HDV, Prevalence, Risk factors*

INTRODUCTION

Hepatitis B virus (HBV) is a major health issue globally, impacting both national and international populations. As of my last update in January 2022, more than 290 million people worldwide are estimated to be living with chronic HBV infection. The prevalence differs across regions, with particularly high rates in sub-Saharan Africa and East Asia. Keep in mind that these numbers may have changed, and for the latest information, it's advisable to refer to current health reports. A progressive cause of liver-related illness and mortality is chronic hepatitis (1). Hepatitis B virus (HBV) and Hepatitis C virus (HCV) are two of the many hepatic viruses that are known to be the primary causes of chronic hepatitis (2). In the world, more than 350 million people have HBV infection, and at least 5% of them also have HDV (3). Co-infection and super-infection are the two methods of HDV infection. Co-infection is the outcome of severe HBV and HDV infections (4). Co-infection with HDV is recognized to worsen the severity of liver diseases in comparison to HBV infection alone. This increased disease severity represents a substantial threat to the health and well-being of individuals residing in District Nasirabad.

HBV is endemic in China and typically affects the liver Epidemics have been caused by it in regions

of Asia, including Pakistan, India, and Africa (5). The orthohepadnavirus genus and Hepadnaviridae family include HBV. DNA is present inside the nucleocapsid and is encased in a circular envelope. The HBV genome contains the DNA polymerase, which functions similarly to a reverse transcriptase, as well as core and surface proteins (6). Based on antigenic epitopes found on its envelope proteins, the virus is categorized into eight genotypes and four main serotypes (A-H). About 75% of people in Asia and the Western Pacific are chronic carriers (7). The HBV infection rate in Pakistan is about 3%. In Pakistan, the average prevalence of HBV was 2.4% in healthy individuals and 2.4% in pediatric patients (range: 1.4-11.0%). Rates were significantly higher in high-risk groupings (8). The most frequent means of HBV transmission are sex, unintentional vaccination, exposure of the eyes, nose, and mouth mucous membranes as well as contact with broken skin. Hepatitis B is primarily disseminated through improper blood transfusions and the reuse of dirty syringes (9). The presence of HDV can complicate the management and treatment of HBV, necessitating tailored and comprehensive healthcare strategies. Understanding the prevalence in this region is crucial for adapting treatment protocols to address the unique challenges posed by co-infection.

The first case of HDV was found in a person with persistent HBV infection (10). The hepatitis delta antigen (HDsAg), a single structural protein, with a nucleocapsid that measures 19 nm in diameter, together with an enveloping, the hepatitis virus is made up of spherical particles with an inner diameter of 36–43 nm (HDV), which the hepatitis B surface antigen shields (HBsAg). The only animal virus having a circular RNA genome, like those seen in plant viruses, it is also the smallest animal virus (12). Although the Mediterranean region has seen a remarkable reduction in frequency over the past ten years due to HBV vaccination initiatives and better socioeconomic conditions, HDV infection is widespread around the world (13). A lesser percentage may have been chronically infected with both HBV and HDV as a result of overlapping transmission channels (14). Patients with HBV-HCV co-infections occasionally have to deal with HDV infection three times. The viral interaction and the precise mechanisms behind this interaction are yet unknown, despite the viral influence in co-infection having been characterized (15). Clarifying the connection between HDV-HBV dual infection was the study's main objective and HBV single infection in patients from District Nasirabad Balochistan, Pakistan. Understanding the prevalence of HDV among HBsAg-positive individuals is crucial for public health planning and intervention strategies, as co-infections can exacerbate the severity of liver diseases.

MATERIALS AND METHODS

PATIENTS AND SAMPLE COLLECTION

Venous blood samples were collected from consenting participants using aseptic techniques. Trained healthcare professionals or phlebotomists performed the blood draws to ensure accuracy and minimize discomfort for participants. The 200 HBsAg positive patients from various age groups, 68% of whom were men and 32% of whom were women, participated in the current study. First, the serum samples were collected from patients whose tests for HBV using the Immunochromatographic Technology (ICT) were positive. The Nasirabad DHQs Hospitals as well as the Bolan Medical Complex Hospital (BMCH) in Quetta, Balochistan, provided the samples for collection CT and ELISA samples that were positive for HBsAg were examined, a 3rd generation ELISA assay kit from China's CDC Diagnostics was used to detect qualitative HBsAg along with all of the patient's HBV-positive sera. A 3rd generation ELISA assay kit (DSI S.r.l. Diagnostics, ITALY) was used in National Laboratories, Quetta to test ELISA for Anti-HDV Antibodies while following the instructions in the manufacturer's protocol. Each of the patients was questioned after data collection through questionnaire.

Demographic information about the study participants includes the age distributions to examine the variations in HDV prevalence over different phases of life, participants were divided into several age groups. This division made it possible to determine whether age groups were more likely to co-infect than others. The ageg roups were:18-25 years, 26-40 years, 41-60 years, 61 and above. To evaluate the ways in which participants' occupation, educational background, and socioeconomic level affect the prevalence of HDV in the community, data on these variables were gathered.

BIOCHEMICAL AND CLINICAL FACTORS

In the current investigation, a few biochemical assays were also carried out. Micro Lab 300 apparatus was used to measure the levels of bilirubin, aspartate transaminase (AST), and alanine amino transferase (ALT) (Merck).

ALANINE AMINO TRANSFERASE (ALT)

The ALT levels were assessed in accordance with the manufacturer's guidelines. In a nutshell, the kit's 1 ml of reagent was combined with 100 l of each patient's sera (DDS diagnostic system). With no sample or standard in the blank one, the standard comprised 1 ml reagent and 100 µl standard.

ASPARTATE TRANSAMINASE (AST)

The AST level was measured using a 1ml reagent comprising 100 meu l samples from each patient as well as 100 meu l standard samples. Only 1 ml of reagent in a blank control was present; there was no sample or standard. These measurements were made using a DDS diagnostic system kit. All 200 patients had their AST levels measured after 10 minutes of incubation at 37 °C.

BILIRUBIN LEVELS

Bilirubin levels were measured by utilizing DIALAB GmbH kit. Each study participant's 100 µl serum was obtained, along with 1 ml of the reagent, and then incubated for 10 minutes at 37 °C. Additionally utilized as a blank control was a standard control that contained 100 µl standard solutions with 1 ml of reagent and 1 ml of reagent without any sample or standard.

STATISTICAL ANALYSIS

Age and liver function tests (LFTs) were clinical variables that were provided as mean ±SD in statistical analysis. Several online statistical tools, such as the software Statistical Product and Service Solutions were used to calculate the mean and standard deviation (SD) (SPSS).

RESULTS

SCREENING OF SAMPLES FOR HBV INFECTION

Screening samples for HBV infection revealed that the ICT HBsAg screening method detected 200 patients with HBsAg positivity. Among these, the study encompassed 136 (68%) male patients with a mean age of 32.50 ± 11.36 years and 64 (32%) female patients with a mean age of 36.68 ± 12.13 years.

ELISA screening was employed to detect HBV in samples, with HBsAg ELISA utilized to confirm positive results (CDC Diagnostics, China). The assay revealed 194 cases of HBsAg positivity, while 6 samples did not yield a response. Among the positive cases, the mean age of the 130 male patients was 32.16 ± 11.27 years, and for the 64 female patients, it was 36.68 ± 12.13 years.

SCREENING FOR HDV INFECTION IN HBSAG POSITIVE CASES

After confirming HBV infection in the 194 samples, they were subsequently screened for HDV infection. Among these, six samples (3.09% of the total HBsAg-positive samples) tested positive for anti-HDV antibodies using anti-HDV ELISA. This suggests that 2 out of 200 samples, or 3% of the total, were positive for both HBV and HDV infections. The mean age of the six male patients who tested positive for HDV ELISA, with no positive cases among female patients, was 37.5 ± 12.58 years.

CLINICAL CHARACTERISTICS (LFTS) OF PATIENTS WITH SINGLE AND MULTIPLE INFECTIONS

Two cohorts were established from the pool of 200 samples: one comprised patients with singular HBV infection, and the other consisted of patients with dual infections (HBV-HDV). These cohorts underwent an assessment of blood ALT, AST, and bilirubin levels as part of liver function tests. In instances of singular infections, the average levels of AST, ALT, and bilirubin were found to be 77 IU/L, 93 IU/L, and 1.9 mg/dL, respectively. Table I presents the outcomes of this analysis.

Table I. HBV (single infection) and HBV-HDV LFT findings (Dual infection)

	Liver Function Tests (LFTs)			
---	**Mean AST**	**Mean ALT**	**Mean Bilirubin**	**Number of patients (Positive)**
Refer- Range	8- 40 IU/L	10 - 40 IU/L	0.1 - 1.2 mg/dL	---
(Single Infection) HBV	77(± 24.88)	93(± 45.83)	1.9(± 0.69)	194
HBV-HDV (Dual Infection)	126(± 66.00)	276(± 96.05)	3.25(± 0.88)	06

An examination of potential risk factors contributing to the transmission of single and dual infections revealed a variety of sources, including blood transfusions, intravenous drug use, dental procedures, sharing razors, and the use of contaminated needles for tattooing or piercing. Notably, multiple mechanisms of transmission are implicated in cases of singular HBV infection, with 18% attributed to frequent needle usage, particularly involving syringes; 22.1% associated with dental procedures; and 23.7% linked to barber practices and the sharing of contaminated razors. Additionally, significant risk factors encompass procedures and extended hospital stays (10.3%), multiple blood transfusions (12.8%), sexual transmission (2.5%), utilization of contaminated equipment for ear and nose piercing (4.1%), and intravenous drug use (6.1%).

Moreover, Fig. 1a and b provided comprehensive insights into the potential risk factors associated with both HBV single infection and HBV-HDV dual infection.

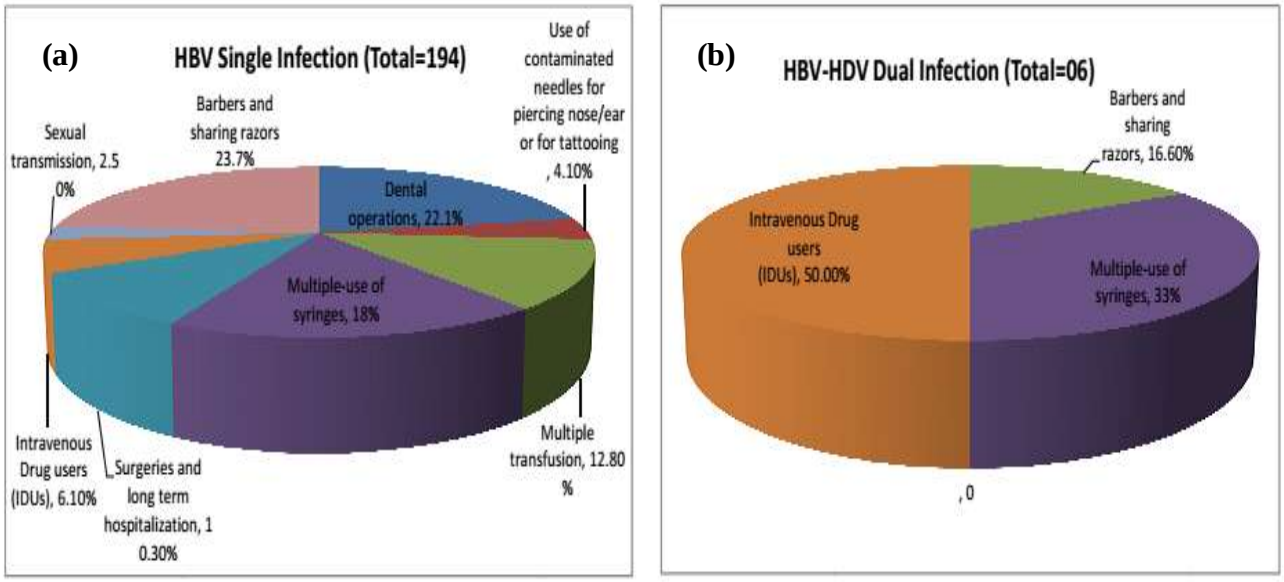

Fig. 1(a). Risk factors for the spread of infection in HBV single infections **(b).** Risk factors connected to HBV-HDV dual infection transmission

The study investigated the relationship between age groups and Liver Function Test (LFT) levels among patients, aiming to assess whether age is associated with variations in LFTs.

In the context of HBV-HDV dual infection and HBV-HDV infection, the analysis of LFT levels across different age groups revealed notable findings. Specifically, among patients with HBV single infection, elevated LFT levels were predominantly observed in the second age group (16–30 years). Furthermore, Table II illustrates that both the third age group (31-45 years) and the fourth age group (46-60 years) exhibited significantly higher LFT values.

Table II. Relationship between age groups and LFT values in HBV single infection

Age Groups	**N**	**Mean ALT**	**SD**	**Mean AST**	**SD**	**Mean Bilirubin**	**±SD**
1-15 years	05	72.32	±14.12	65.78	±23.67	1.34	±0.56
16-30 years	81	95.40	±35.65	80.67	±24.87	1.93	±0.45
31-45 years	76	90.67	±41.56	75.70	±19.78	1.87	±0.23
46-60 years	27	82.70	±31.50	71.78	±23.78	1.90	±0.54
>60 years	05	79.20	±13.76	69.80	±14.50	1.88	±0.67

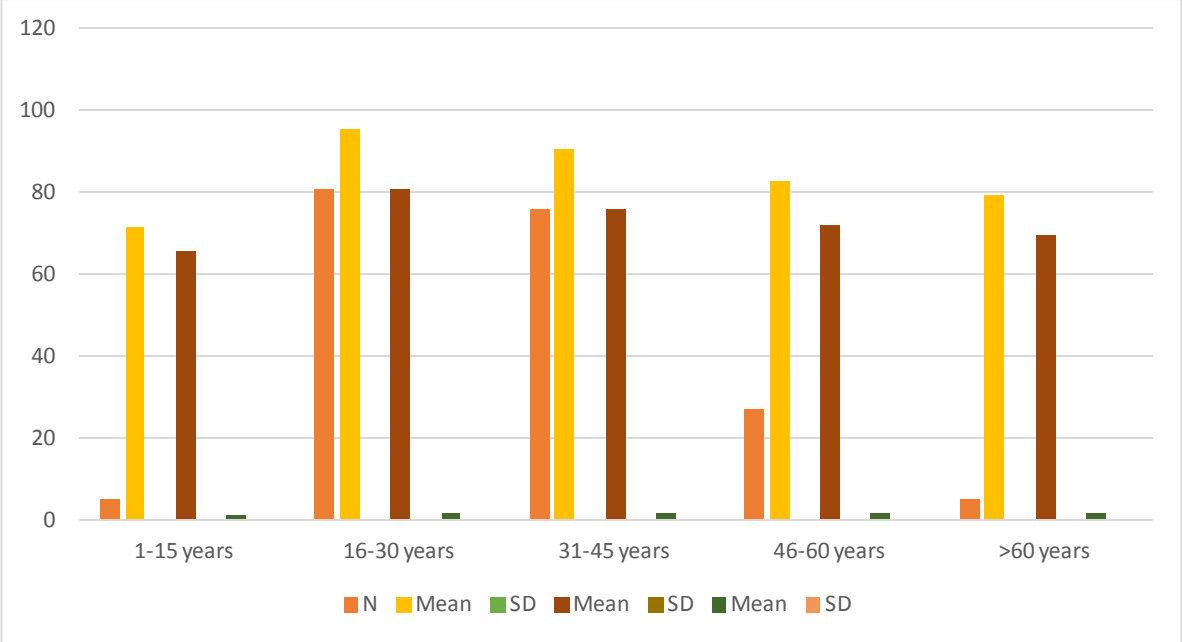

Fig. 2. Relationship between age groups and LFT values in HBV single infection

Potential co-transmission pathways in co-infection: Data from the participants was divided into 10 groups and compared to the routes of transmission between patients who were co-infected with HBV-HDV and those who were co-infected with HBV alone in order to establish a relationship between the two infections and the route of transmission. After a detailed review of the data, it was discovered that intravenous drug use (IDU), where 20% (3/12) of the patients had HBV and HDV co-infections, was the most common method of transmission linked with co-infection. With 5.4% (2/35) of the co-infected individuals, multiple syringe use was the other significant related route of transmission for the co-infection, according to these findings. As shown in Table 3, sharing barbers and exchanging contaminated razors with barbers (each with 2.12% of co-infected patients) might be regarded as linked modes of transmission for the co-infection.

Table III. Co-potential infection's linked route of transmission

Transmission Path	Non Co-Infected	Co-Infected	No. of patients (200)
Sharing Razors Among Barbers	46 (97.8 %)	01 (2.12 %)	47
Dental Procedures	43 (100 %)	00	43
Uses of used Needles For Piercing purpose	08 (100 %)	00	08
Transfusion In Many Times	25 (100 %)	00	25
Again and again Usage of same Syringes	35 (94.59 %)	02 (5.40 %)	37
Long-Term Hospitalization And Surgeries	20 (100 %)	00	20
Users Of I.V Drugs (Idus)	12 (80%)	03 (20 %)	15
Sexual Interaction	05 (100 %)	00	05

DISCUSSION

The chronic hepatitis, primarily instigated by Hepatitis B Virus (HBV) and Hepatitis C Virus (HCV), poses significant public health concerns globally due to its association with various liver-related disorders (16). Complications stemming from chronic hepatitis include cirrhosis, liver failure, and hepatocellular carcinoma (HCC), resulting in approximately 100,000 deaths annually worldwide. Concurrent infections with multiple viruses exacerbate treatment challenges and increase morbidity and mortality rates (17).

Recent research has shown a paucity of information concerning HBV-HDV dual infection in Pakistan (18). This study aimed to ascertain the prevalence of HDV infection among HBsAg-positive patients in District Nasirabad, Balochistan, Pakistan, correlate dual infection with liver function indicators such as ALT, AST, and serum bilirubin levels, analyze the prevalence of HDV infection across various age groups and genders, and delineate potential risk factors associated with the transmission of HBV single and HBV-HDV dual infections. The investigation involved testing 200 HBsAg-positive serum samples, comprising 68% men and 32% women, for HBV-HDV dual infection. Among these samples, 194 (97%) tested positive for HBV

infection via ELISA, with 6 (3%) exhibiting dual infection. The mean ages of male and female patients with HBV infection were 32.50 and 36.68 years, respectively.

Previous studies have indicated an increased prevalence of HBV and HDV infections with advancing age, often attributed to medical injections (19). Additionally, biochemical assays such as ALT, AST, and bilirubin levels were employed to assess liver function. Elevated levels of these enzymes typically signify liver damage. In patients with HBV infection alone, mean AST, ALT, and bilirubin values were reported as 77 ± 24.88 IU/L, 93 ± 45.83 IU/L, and 1.9 ± 0.69 mg/dL, respectively. Similarly, concurrent HBV-HDV infection resulted in mean ALT and AST levels of 93 ± 97 U/L, as reported by Chauhan *et al.* (2018) (20). Notably, mean serum ALT enzyme values for chronic hepatitis patients caused by HBV, HCV, and HDV were 121 ± 99 U/L, 110 ± 63 U/L, and 144 ± 90 U/L, respectively, while AST enzyme levels were 94 ± 96 U/L, 86 ± 73 U/L, and 110 ± 63 U/L, respectively.

Comparisons with existing research play a crucial role in contextualizing the study findings. Discrepancies in prevalence rates between District Nasirabad and other regions or countries may necessitate further investigation into potential contributing factors, such as differences in healthcare infrastructure, immunization coverage, or socioeconomic status. Moreover, insights gained from the study can enhance understanding of HDV prevalence among HBsAg-positive individuals globally, shedding light on regional variations and prompting further research into underlying determinants.

The study also explored various potential risk factors associated with HBV and HDV transmission, including barber practices, sharing razors, blood transfusions, repeated needle use, intravenous drug use, and sexual transmission. Notably, barbershops/sharing razors (23.7%) and dental procedures (22.1%) were identified as primary transmission pathways for HBV infection alone, alongside other factors such as prolonged hospitalization and piercing with infected jewelry.

However, the study has certain limitations, including the possibility of sampling bias and the cross-sectional design's inability to establish causal relationships or monitor temporal changes in HDV prevalence. Moreover, reliance on self-reported data may introduce recall bias or underreporting of demographic and risk factor information.

CONCLUSION

In Pakistan, the co-infection of HDV in HBV patients presents a significant concern. Recent research indicates that the highest occurrence of HBV single infections is observed among individuals aged 16 to 30 and 31 to 45 years. Conversely, individuals within the age brackets of 16 to 30, 31 to 45, and 46 to 60 demonstrate equal vulnerability to HBV-HDV dual infection. It is imperative to disseminate these study findings to policymakers and healthcare professionals to advocate for the routine inclusion of HDV screening in the prognosis of liver diseases.

Acknowledgements:

The authors extend their gratitude to the medical staff at Bolan Medical Complex in Quetta for their invaluable assistance with clinical diagnosis. It is important to note that while their contributions were instrumental in this study, they were not directly involved in the planning, analysis, interpretation, or writing of the article.

References:

1. Marcellin P, Kutala BK. Liver diseases: A major, neglected global public health problem requiring urgent actions and large-scale screening. Liver International. 2018;38:2
2. Ashhab AA, Rodin H, Campos M, Abu-Sulb A, Hall JA, Powell J, Debes JD. Response to hepatitis B virus vaccination in individuals with chronic hepatitis C virus infection. PLoS One. 2020;15(8):e0237398.
3. Lazarus JV, Al-Rifai A, Sanai FM, Alghamdi AS, Sharara AI, Saad MF, van Selm L, Alqahtani SA. Hepatitis delta virus infection prevalence, diagnosis and treatment in the Middle East: A scoping review. Liver International. 2023;43:116-23.

4. Baskiran A, Akbulut S, Sahin TT, Koc C, Karakas S, Ince V, Yurdaydin C, Yilmaz S. Effect of HBV-HDV co-infection on HBV-HCC co-recurrence in patients undergoing living donor liver transplantation. Hepatology International. 2020;14(5):869-80.
5. Asrani SK, Devarbhavi H, Eaton J, Kamath PS. Burden of liver diseases in the world. Journal of hepatology. 2019 ;70(1):151-71.
6. Mehmood S, Raza H, Abid F, Saeed N, Rehman HM, Javed S, Khan MS. National prevalence rate of hepatitis B and C in Pakistan and its risk factors. Journal of Public Health. 2020;28:751-64.
7. Al-Sadeq DW, Taleb SA, Zaied RE, Fahad SM, Smatti MK, Rizeq BR, Al Thani AA, Yassine HM, Nasrallah GK. Hepatitis B virus molecular epidemiology, host-virus interaction, coinfection, and laboratory diagnosis in the MENA region: An update. Pathogens. 2019;8(2):63.
8. Mehmood S, Raza H, Abid F, Saeed N, Rehman HM, Javed S, Khan MS. National prevalence rate of hepatitis B and C in Pakistan and its risk factors. Journal of Public Health. 2020;28:751-64.
9. Samo AA, Laghari ZA, Baig NM, Khoso GM. Prevalence and risk factors associated with hepatitis B and C in Nawabshah, Sindh, Pakistan. The American journal of tropical medicine and hygiene. 2021;104(3):1101.
10. Aguilera A, Trastoy R, Rodríguez-Calviño J, Manso T, de Mendoza C, Soriano V. Prevalence and incidence of hepatitis delta in patients with chronic hepatitis B in Spain. European Journal of Gastroenterology & Hepatology. 2018;30(9):1060-2.
11. Seitz S, Habjanič J, Schütz AK, Bartenschlager R. The hepatitis B virus envelope proteins: molecular gymnastics throughout the viral life cycle. Annual review of virology. 2020;7:263-88.
12. Cai Z, Fan Y, Zhang Z, Lu C, Zhu Z, Jiang T, Shan T, Peng Y. VirusCircBase: a database of virus circular RNAs. Briefings in Bioinformatics. 2021;22(2):2182-90.
13. Jefferies M, Rauff B, Rashid H, Lam T, Rafiq S. Update on global epidemiology of viral hepatitis and preventive strategies. World journal of clinical cases. 2018;6(13):589.
14. Revill PA, Tu T, Netter HJ, Yuen LK, Locarnini SA, Littlejohn M. The evolution and clinical impact of hepatitis B virus genome diversity. Nature Reviews Gastroenterology & Hepatology. 2020;17(10):618-34.
15. Morozov, V. A., & Lagaye, S. (2018). Hepatitis C virus: Morphogenesis, infection and therapy. World Bartolomé FJ, Moraleda G, Castillo I, Martinez MG, Porres JC, Carreño V. Presence of HBV-DNA in peripheral blood mononuclear cells from anti-HIV symptomless carriers. Journal of hepatology. 1990;10(2):186-90.
16. Liu H, Shi Y, Hayden JC, Ryan PM, Rahmani J, Yu G. Tenofovir treatment has lower risk of hepatocellular carcinoma than entecavir treatment in patients with chronic hepatitis B: a systematic review and meta-analysis. Liver Cancer. 2020;9(4):468-76.
17. Weldemhret L. Epidemiology and challenges of HBV/HIV Co-infection amongst HIV-infected patients in endemic areas. HIV/AIDS-Research and Palliative Care. 2021:485-90.
18. Aftab M, Amin I, Idrees M, Ali A, Rafique S, Naz S. Molecular epidemiology of hepatitis delta and hepatitis B viruses circulating in two major provinces (East and North-West) of Pakistan. Infection, Genetics and Evolution. 2018;64:65-9.
19. Seto WK, Lo YR, Pawlotsky JM, Yuen MF. Chronic hepatitis B virus infection. The Lancet. 2018;392(10161):2313-24.
20. Chauhan R, Lingala S, Gadiparthi C, Lahiri N, Mohanty SR, Wu J, Michalak TI, Satapathy SK. Reactivation of hepatitis B after liver transplantation: Current knowledge, molecular mechanisms and implications in management. World journal of hepatology. 2018;10(3):352.