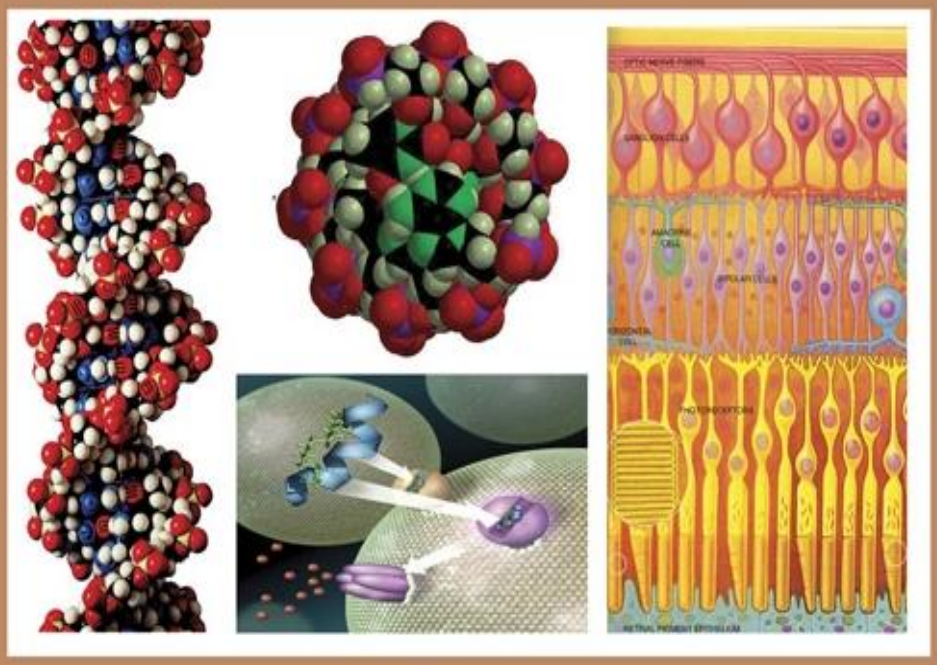
EGYPTIAN ACADEMIC JOURNAL OF

BIOLOGICAL SCIENCES

PHYSIOLOGY & MOLECULARBIOLOGY

C



ISSN
2090-0767

WWW.EAJBS.EG.NET

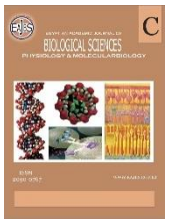
Vol. 15 No. 1 (2023)

.

Citation: *Egypt.Acad.J.Biolog.Sci. (C.Physiology and Molecular biology) Vol. 15(1) pp295-304 (2023)*
DOI: 10.21608/EAJBSC.2023.291983

Egypt. Acad. J. Biolog. Sci., 15(1):295-304 (2023)

Egyptian Academic Journal of Biological Sciences
C. Physiology & Molecular Biology
ISSN 2090-0767
www.eajbsc.journals.ekb.eg



Hepatitis B Seroprevalence and Consequences in Khyber Pakhtunkhwa Peshawar, Pakistan

Saad Alghamdi[1], Anas S. Dablool[2] and Nabeela Al-Abdullah[3]

1. Laboratory Medicine Department, Faculty of Applied Medical Sciences, Umm Al-Qura University, Makkah, Saudi Arabia
2. Department of Public Health, Health Sciences College at Al-Leith, Umm Al-Qura University, Makkah, Saudi Arabia.
3. King Abdulaziz University, Faculty of Nursing, Public Health Department, infection control unit, King Abdulaziz university hospital Jeddah, Saudi Arabia

***E-mail:** ssalghamdi@uqu.edu.sa

ARTICLE INFO

Article History
Received:21/1/2023
Accepted:21/3/2023
Available:26/3/2023

Keywords:
HBV, Elisa, ICT, virus, antiviral.

ABSTRACT

Background: Infection with the hepatitis B virus is a major global health concern. In Peshawar, HBV infection is widespread and affects all demographics, especially pregnant women. **Objective:** The purpose of this research is to determine how common HBV is, as well as the risks and complications associated with various clinical parameters and patient demographics. **Methodology:** 200 patients were reported and tested with enzyme-linked immunosorbent assay (ELISA) for HBeAg and immunochromatographic assay for HBs-Ag (ICT). For quantifying HBV DNA, an RT-PCR assay was performed. In-person interviews with study participants were conducted using the research software Performa. **Results:** In our study, a total of 200 patients were investigated for HBV infection in KP, including Peshawar, Charsadda, Kohat, Waziristan, and Mardan region. According to our study, the highest prevalence was found in Peshawar with 30% (60/200), Charsadda (10%, 20/200), Kohat (7.5%, 15/200), Mardan (20%, 40/200), Waziristan (12.5%, 25/200). Mardan had the second greatest incidence, at 20.2% (40/200). The current study indicated a prevalence of 21.3% for affected family members based on relation, including 9.3% for spouses, 2.7% for mothers, 4% for siblings, and 5.3% for children. HBV patients' ALT, bilirubin, creatinine, and ultrasonography levels indicate treatment, severity, and antiviral medicine efficacy. 200 HBV patients were monitored for these clinical indicators after entecavir and tenofovir treatment. Patients with normal ALT were 81.3% (162/200) (87.3%), 18.7% (38/200) with high, and 20/200 (10.4%) with raised creatinine. After antiviral treatment, patients had normal bilirubin 93.3%. The study assessed 64% and 36% of patients on tenofovir and entecavir for different durations. Due to renal failure and diabetes, HBV-infected patients had a 1.3% death rate. **Conclusion:** Males get infected more often than females since they're more exposed to the environment. Due to intimate contact and little awareness, HBV prevalence was highest in spouses with a family history.

INTRODUCTION

Highly contagious liver infections can be caused by any one of five primary hepatitis-causing viruses, including HAV, HBV, HCV, HDV, and HEV. Transmission, pathophysiology, and prognosis vary greatly between the various viruses. Acute and persistent infections with the hepatitis B virus (HBV) can induce liver fibrosis and hepatocellular cancer, making HBV a major public health concern. (Burman *et al*., 2015; Flisiak *et al*. 2017). The World Health Organization estimates that 2 billion people have chronic hepatitis B virus infection and that 5-15% of the world's population is a chronic carrier of the virus (Abbas and Siddiqui, 2011). Also, 1.34 million people die each year from hepatitis-related causes, which is more than death from HIV, and 2.7 million people are co-infected with HBV-HIV (WHO, 2017). Almost 20 million individuals in Pakistan have hepatitis B or C, and roughly 150,000 people contract the virus each year, according to research. Around 240 million people were discovered to be chronic carriers (Ott *et al*., 2012), and hepatitis B is present in the bodies of an additional 1.8 million children younger than 5 years old (Razavi-Shearer and Razavi, 2018). Vertical transmission from infected mothers to their newborns is the primary mode of HBV transmission, even though chronic carrier HBV infection is more common in males than females (Tanga *et al*., 2019). In addition, mothers who are infected with hepatitis B but do not have a positive test for HBeAg have a risk of 10–40% of transmitting the infection to their newborn baby, whereas mothers who are infected and have a positive test for HBeAg have a risk of 90% of their newborn baby acquiring the infection (Khan *et al*., 2017). In regions of the world with a high prevalence of hepatitis B virus (HBV), a sizeable percentage of the population is still susceptible to HBV and, as a result, runs the risk of becoming infected with the virus during their adult years. On the other hand, the majority of HBV transmission takes place during childhood (Jha *et al*., 2012). HBV is without a doubt the single most important hepatocarcinogen that contributes to the development of HCC, which ranks as the sixth most frequent cancer in the world and the second most common cause of cancer-related deaths (Sanyal *et al*., 2010). Co-infection with HIV has a significant impact on practically every aspect of the natural history of HBV infection. This is especially true for people who also have HIV. The result is a higher rate of chronicity following acute HBV infection, a higher level of HBV replication and reactivation, minimal loss, a quicker onset of cirrhosis and HCC, a higher risk of liver-related mortality, and a poorer therapeutic response than in cases when HIV is not present (Hawkins *et al*., 2013). Infection with HBV during pregnancy can lead to several significant consequences, including an increased chance of developing chronic HBV, perinatal transfer of HBV, and an increase in the severity of liver damage caused by HBV. Co-infection during pregnancy with HIV and HBV can potentially alter the antiviral medication therapy options available to treat both viruses (Fomulu *et al*., 2013). HBV is common in Asia, South America, and sub-Saharan Africa, and 25% of HCV-infected patients are also infected with HBV. HCV co-infection with HBV increases the risk of HCC, both at younger ages and more potently (Potthoff *et al*., 2010). CHB patients are treated with nucleoside/nucleotide analog (NA) or pegylated interferon-α (PegIFNα) (Terrault *et al*., 2016). There are now five drugs that have been licensed for the treatment of Hbv. These medications include entecavir (ETV), telbivudine (ADV), adefovirdipivoxil (ADV), lamivudine (LAM), and tenofovir disoproxil fumarate (TDF). This provides evidence that the risk of developing cirrhosis and hepatocellular carcinoma (HCC) can be reduced with the consistent and efficient reduction of HBV DNA. To achieve total virology suppression and a reduction in hepatitis B surface antigen (HBs-Ag), the long-term use of current treatment (NA treatments) for chronic hepatitis B is necessary (Terrault *et al*.,

2018). The primary purpose of the research was to determine the current prevalence and distribution of HBV infection in the city of Peshawar, which is located in the province of Khyber Pakhtunkhwa in Pakistan. The analysis of the clinical characteristics and risk factors present in individuals with active HBV infection was secondary objective of this study. The tertiary objective of the study was to examine the participants' overall knowledge of the complications of HBV infection.

MATERIALS AND METHODS

The clinical data of HBV patients were collected from a variety of clinics and hospitals for this study, which was carried out in Peshawar throughout 2022. The data were evaluated based on the prevalence of the condition concerning a variety of parameters, including gender, age, geography, family history, antigen status, treatment, and clinical profile. Standard biochemical laboratory tests were used to detect serum levels of ALT, bilirubin, and creatinine. The HBe-Ag status (+/-) was determined using enzyme-linked immunosorbent assay (ELISA), and HBs-Ag was determined using immunochromatographic assay (ICT). The HBV DNA level was determined through the use of an RT-PCR test. The primary purpose of our investigation was to ascertain the true prevalence rate of HBV using clinical indicators. All patient serum samples were examined for HBsAg using an immunochromatographic test (immunochromatographic test), and HBeAg using an enzyme-linked immunosorbent assay that was commercially available (ELISA). Every person who was tested had a blood sample taken from them by an authorized technician using a disposable singe. After separating the plasma from the samples by centrifuging them at 3000 rpm for two minutes, the samples were kept at a temperature of -20 ° C. To detect antigens or antibodies in a sample, this approach is utilized. The ICT strip was the primary screening tool that was used to differentiate positive samples of HBsAg from the other samples. A sample of 100 ml was put onto a screening strip in a sterile environment, and the control band and the trace band were employed to provide an indication. When color bands appeared on the control band but not the trace band, this indicated a positive outcome, whereas color appearing only on the control band indicated a negative result. By using the HBe-Ag kit, the HBV samples were tested and confirmed for their presence. It was determined that the positive sample of HBe-Ag could be confirmed using the Bios kit. The sample of 50 ml was combined with the same volume of conjugate reagent and HRP enzyme (horseradish peroxidase). After simultaneously mixing the samples and the conjugate, a waiting period of 60 minutes was followed by incubation at a temperature of 37 ℃. After that, the buffer solution was used to wash the sample five times. The second round of incubation was carried out using two distinct substrates: substrate A (50 ml) and substrate B (50 ml) were both loaded, and after 15 minutes of waiting, the enzyme produced a bluish color, which indicates that the sample was positive. The stop solution was used to cease the reaction, and the results of which were then examined by the ELISA reader. The HBV samples were tested, and confirmation was obtained by using HBDNA. DNA was isolated from 200 ml of material using a DNA extraction kit (GF-1 kit). The DNA that was collected was put through PCR so that HBV could be identified. The PromotorTM HBV Hepatitis virus quantitative test kit was utilized for HBV DNA detection by real time-PCR. This kit included all of the chemicals and enzymes necessary for the specific amplification of the HBV genome. The standard curve cut-off value for HBV DNA detection in this kit is between 34 and 35. A positive result was reported for results below this value, while a negative result was shown for results above this value. SPSS version 20 analyzed questionnaire data in an Excel database.

RESULTS

The purpose of this study is to collect data on HBV-infected patients in KP

from 2021 to 2022, including clinical parameters and viral loads of HBV-infected patients. Our study included patients diagnosed with HBV of both genders and of different ages, each of whom was examined for prognostic factors until the point at which they received negative PCR results. Based on various prevalence and clinical criteria, a total of 200 patients with HBV infection were reported.

Prevalence of KP HBV Patients On The Base Of Location:

KP (Peshawar, Charsadda, Kohat Waziristan, and Mardan) totaled 200 patients who were tested for HBV infection in our study. Our research shows that Peshawar has the highest prevalence (30%; 60/200), followed by Charsadda (10%; 20/200), Kohat (7.5%; 15/200), Mardan (20%; 40/200), and Waziristan (12.5%; 25/200). Mardan, with a prevalence of 20.2% (40/200), ranked second highest. shown in Figure 1.

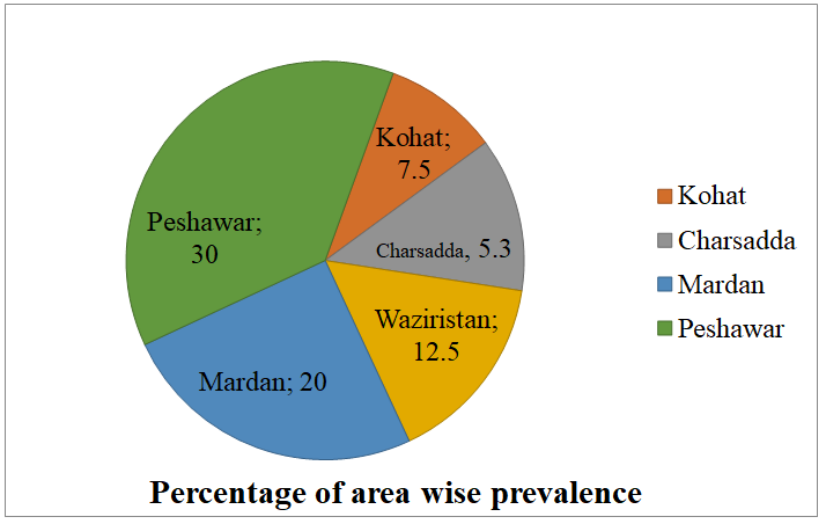


Fig.1. Location basis prevalence of HBV-infected patients.

Gender and Age-Wise Prevalence of HBV Patients:

In our study, male HBV patients from KP had a greater prevalence than females. 120 (55%) of 200 patients were male and 70 (46%) were females. Table 1, shows six patient groups, ranging from 15 years to 56 years. 70 patients (35.2%) were in the 25–40-year-old group, whereas 10 patients (5%) were in the 15-year-old group. In table 1, 55 patients (28%) in the 16-25 age group had HBV infection, 30 (15%) in the 40-45 age group, 15 (7%) in the 45-55 age group, and 20 (10%) in the >60 age group. Age mean and SD were 32.627 and 12.301, respectively.

Table 1. Age of patients

Age of patients		Frequency	Percent (%)
Valid	<= 15.00	10	5
	16.00 - 25.00	55	28
	25.00 - 40.00	70	35
	40.00 - 45.00	30	15
	45.00 - 55.00	15	7
	60.00+	20	10
	Total	200	100.0

Prevalence of HBV Patients On Base HBsAg/HBeAg Positivity:

HBsAg and HBeAg positive determined HBV prevalence. HBeAg was positive in 66 (33.3%) patients while HBsAg was positive in 134 (66.7%). Figure 2 shows that more HBV patients have HBsAg than HBeAg.

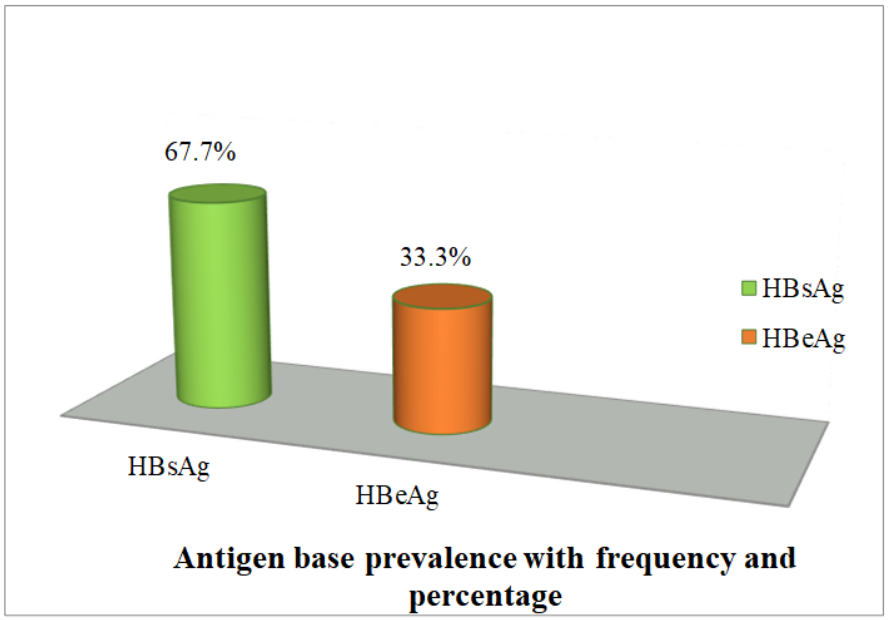


Fig. 2: Prevalence of HBV patients on the base of Ag-status.

Co-infection with HCV, HDV, HIV Base Prevalence:

Figure 3 shows that of 200 reported patients, 10 (7.3%) were co-infected with HCV, no patients were co-infected with HDV or HIV, and 192 (94.7%) were not co-infected. The association between HBV, HCV, and HIV confections is significant (p.value=.000) by chi-square.

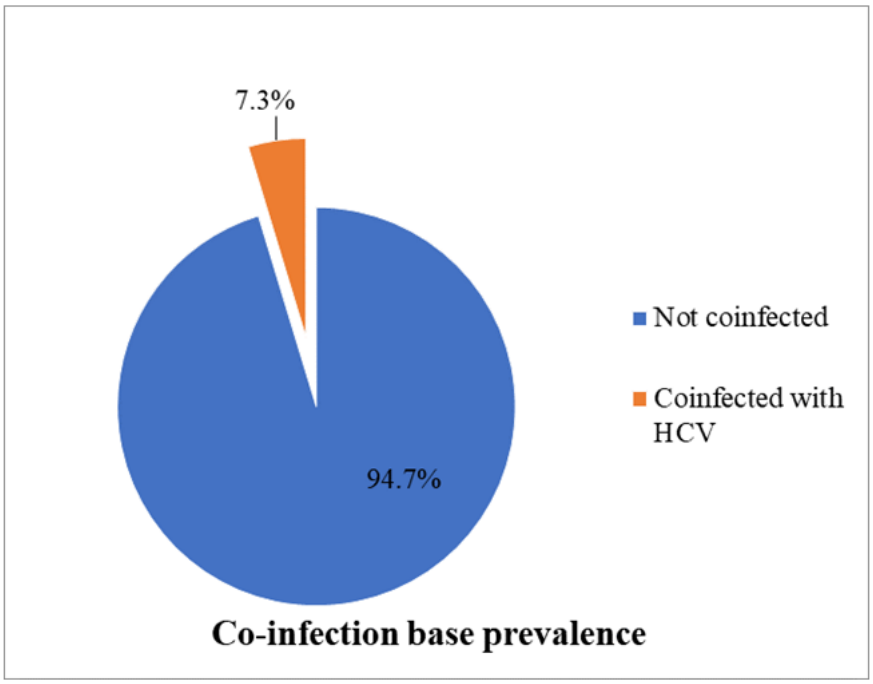


Fig. 3. Co-infection base prevalence of HBV patients.

Family Affected The Base Prevalence of HBV Patients And Their Relationship With Patients:

HBV prevalence is calculated based on family history. Our study found that 21.3% of patients had HBV-infected family members and 78.7% had not. The affected family of HBV patients was 21.3% in our study. The prevalence of family affected based on relation was 9.3% (spouse), 2.7% (mother), 4% (sibling), and 5.3% (children) among these patients, as shown in Table.2. Because of close interaction, spouses had the highest occurrence. A significant correlation was established between these data, p. value (.000).

Table 2. Relation-based prevalence of affected family.

		Frequency	Percent (%)	Cumulative Percent
Valid	Spouse affected	14	9.3	9.3
	Parent affected	4	2.7	12
	Sibling affected	6	4	16
	Children affected	8	5.3	21.3
	Not affected family	118	78.7	100.0
	Total	150	100.0	

Prevalence of HBV-Infected Patients Based On Clinical Parameters:

HBV patients' ALT, bilirubin, creatinine, and ultrasonography levels indicate treatment, severity, and antiviral medicine efficacy. 200 HBV patients were monitored for these clinical indicators after entecavir and tenofovir treatment. Patients with normal ALT were 81.3% (162/200) (87.3%), 18.7% (38/200) with high, and 20/200 (10.4%) with raised creatinine. After antiviral treatment, patients had normal bilirubin 93.3%, increased 6.7%, normal ultrasound 83.7%, and malfunction ultrasound 16.3%. (p=.005).

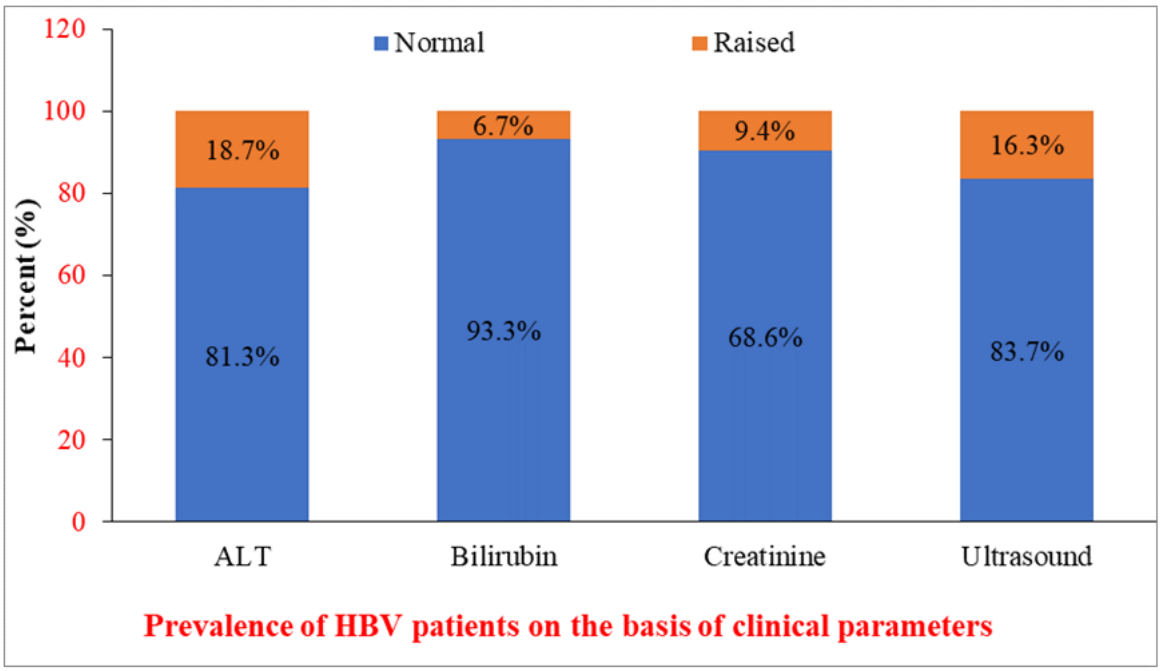


Fig. 4. Prevalence of HBV-infected patients based on clinical parameters.

Prevalence of Antiviral Drugs Therapy Duration on the base of HBsAg/HBeAg Positivity:

HBV treatment history prevalence was assessed. Our study included 150 individuals using tenofovir and entecavir for varying durations. The study found that Khyber Pukhtunkhwa HBV patients utilize solely entecavir and tenofovir. 96 (64%) patients received tenofovir for 6, 12, 18, 24, or 48 months. HBs/eAg determined patient therapy duration. At 12 months, 33.3% of HBsAg (+) patients and 10% of HBeAg (+) patients recovered. Tenofovir therapy for 18 months showed 10% HBsAg positive and 6.7% HBeAg positive. 23.3% of tenofovir-treated patients had HBsAg and 3.3% had HBeAg. With 48 months of tenofovir treatment, 6.7% of HBeAg-positive patients recovered. The study demonstrated the highest proportion of tenofovir use duration with 12-month HBV therapy. HBsAg and HBeAg positive split 54 (36%) entecavir-treated patients into distinct durations. 7.1% (HBsAg positive) and 14.3% (HBeAg positive) of 54 patients received entecavir for 6 months. Entecavir-treated HBsAg- and HBeAg-positive patients were 21.4% and 21.4%, respectively. HBsAg and HBeAg (+) individuals treated with entecavir for 18 and 24 months had 14.3% and 7.1%, respectively. A significant p.value (.046) was detected.

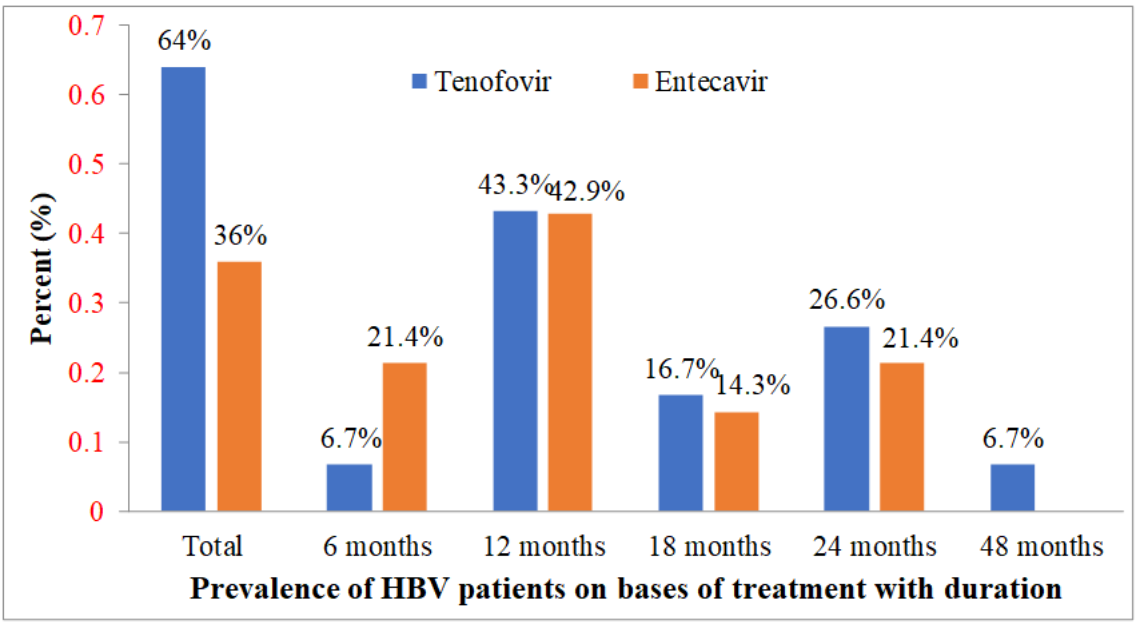


Fig. 5: Prevalence of patients on the bases of treatment with duration.

Pregnancy and Mortality Base Prevalence of HBV Patients:

Our study found 46% (69/200) female patients with HBV infection, 90% (62) of whom were non-pregnant and 10% (7) were pregnant. All pregnant patients were HBsAg positive and using entecavir or tenofovir. In this study, 1.3% of HBV-infected patients died. Our study patient were died from renal failure and diabetes.

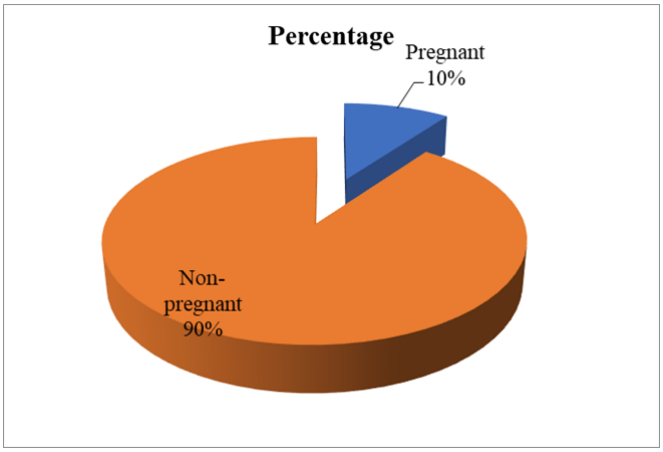


Fig. 6. Female HBV patient prevalence.

DISCUSSION

Hepatitis B virus (HBV) causes liver parenchyma cell inflammation. Over 350 million people are chronically infected with HBV, which can cause cirrhosis and hepatocellular cancer. Pakistan has a high HBV infection risk. HBV patients who are anti-HBV positive were calculated in the study. The survey found that Baluchistan had the highest prevalence (5.3%) and KP the lowest (1.3%). The highest occurrence (20%) was Mardan KP (Saeeda *et al*., 2008). In our study, Peshawar had the greatest prevalence (30%), and Kohat and Charsada had the lowest (7.3%). 12.5% (25/200) was the second highest incidence in Waziristan. In our study, Peshawar had the greatest prevalence in KP, whereas Kohat had the lowest. The study examined HBV patients on entecavir or tenofovir and their medical course. 29 males and 11 females out of 40 patients had a mean age of 57 ±12 years (Toka *et al*., 2020). T. Kurien (2005) examined HBV prevalence in southern India. 5.7% had HBsAg and 23.5% HBe-antigen. 32.7% of 15–20-year-olds had the highest prevalence (Kurien *et al*., 2005). In our study, 120 (54%) male and 80 (46%) female patients were involved. 51 patients (34.2%) were identified in the 26–35-year-old group, while 4 patients (2.6%) were found in the 15-year-old group. Both studies showed male prevalence was higher than female prevalence and varied age groups. HBV and HCV prevalence was studied in Malakand. There was a total of 40 people who tested positive for HCV (11.71%), while 8.64% of them tested positive for HBs Ag. Nobody tested positive for both HBV and HCV (Kalim *et al*., 2017). Similarly, out of a total of 200 HBV patients in our study, 62.7% of those patients tested positive for HBsAg, and 37.3% of those patients tested positive for HBeAg. There were 5.3% of patients who also had HCV infection, however, the rest of the patients (95.3% overall) did not have any additional co-infections. The different study areas or sample sizes contributed to the differences we found in our research. The frequency of hepatitis B infection and co-infection with HIV in Nigeria was investigated by Magaii *et al*. (2021). According to the findings, 12.6% of patients had HBV infection in addition to HIV, while 7.2% did not have HIV infection (P = 0.01). (20). It was found that those pregnant women who were HIV positive had a greater frequency of HBV. There is a wide range of co-infection rates between HIV and HBV in pregnant women across Africa, from 6.3% to 25% (Spearman *et al*., 2017). In our research, we found that 46% of female patients had HBV infection; of these patients, 90% (62) were not pregnant and 10% (7) were expecting a child. In the group of patients who were examined, neither HBV nor HIV co-infection was discovered in any of them. In our study of 200 HBV patients, 62.7% were HBsAg positive and 37.3%

were HBeAg positive. 5.3% were co-infected with HCV, while 95.3% were not. Our study's variation was attributable to the study area or sample size differences. The study compared entecavir and tenofovir for liver-related mortality. 26 individuals took TDF and 14 took ETV, according to 40 patient data. In the Toka *et al*. (2020) study, liver-related mortality was 17.5%. our analysis calculated the death rate with a lower frequency of 1.3%. 54 (36%) patients took entecavir and 96 (64%) took tenofovir. Our study differs from others due to patients' renal or hepatic issues. My research patient's mortality rate was attributable to renal failure and acute liver failure.

Conclusion

The investigation examined the prevalence of HBV and its clinical characteristics after antiviral medication treatment for chronic hepatitis B (CHB). These medications can cure HBV and prevent liver damage. Nucleoside or nucleotide analogs are the most effective HBV treatments in Pakistan, where CHB is a significant liver disease. Tenofovir and entecavir are effective CHB virus treatments in Khyber Pakhtunkhwa. The study examined KP patients with HBV infection by age, gender, antigen, antiviral medications, co-infection, and affected family. Our study indicated that HBV infection is higher in men (55%) than in women (46%). The highest prevalence was (26-40 years) with 70 patients (34.2%) and the lowest prevalence was (≤ 15 years) with 10 patients (5.6%). The more activity and exposure to the surroundings makes young guys more susceptible to HBV. HBV-co-infected patients may benefit from NAs therapy and renal monitoring. The spouse had the greatest percentage of HBV-positive family members at 21.3%. Joint families in KP and low awareness are the main causes of HBV transmission, according to the study. The patients with renal disorders are not only difficult to treat with antiviral medications that may also cause complications. HBV prevalence and complications will be determined through this investigation

REFERENCES

Abbas, Z., & Siddiqui, A. R. (2011). Management of hepatitis B in developing countries. *World Journal of Hepatology*, *3*(12), 292.

Burman, B. E., Bacchetti, P., Ayala, C. E., Gelman, N., Melgar, J., & Khalili, M. (2015). Liver inflammation is a risk factor for prediabetes in at-risk latinos with and without hepatitis C infection. *Liver International*, *35*(1), 101-107.

Flisiak, R., Pogorzelska, J., & Flisiak-Jackiewicz, M. (2017). Hepatitis C: efficacy and safety in real life. *Liver International*, *37*, 26-32.

Fomulu, N. J., Morfaw, F. L., Torimiro, J. N., Nana, P., Koh, M. V., & William, T. (2013). Prevalence, correlates and pattern of Hepatitis B among antenatal clinic attenders in Yaounde-Cameroon: is perinatal transmission of HBV neglected in Cameroon? *BMC Pregnancy and Childbirth*, *13*(1), 1-10.

Hawkins, C., Christian, B., Ye, J., Nagu, T., Aris, E., Chalamilla, G., ... & Fawzi, W. (2013). Prevalence of hepatitis B co-infection and response to antiretroviral therapy among HIV-infected patients in Tanzania. *Aids*, *27*(6), 919-927.

Jha, A. K., Chadha, S., Bhalla, P., & Saini, S. (2012). Hepatitis B infection in microbiology laboratory workers: prevalence, vaccination, and immunity status. *Hepatitis research and treatment*, *2012*, 1-5.

Kalim, M., Imran, M., Hussain, F., Khan, I. U., Habib, N., Iqbal, M. N., & Ashraf, A. (2017). Detection of HBV and HCV by ICT and ELISA Method in Different Areas of District Malakand. *PSM*

Microbiology, *2*(1), 5-8.

Khan, T., Jung, I. H., Khan, A., & Zaman, G. (2017). Classification and sensitivity analysis of the transmission dynamic of hepatitis B. *Theoretical Biology and Medical Modelling*, *14*(1), 1-17.

Kurien, T., Thyagarajan, S. P., Jeyaseelan, L., Peedicayil, A., Rajendran, P., Sivaram, S., ... & STD Study Group. (2005). Community prevalence of hepatitis B infection & modes of transmission in Tamil Nadu, India. *Indian Journal of Medical Research*, *121*(5), 670.

Magaji, F. A., Okolo, M. O., Yiltok, E. S., Golit, W., Anzaku, S. A., Ogwuche, J., ... & Cohn, S. E. (2021). Prevalence of hepatitis B virus infection in pregnant women with and without HIV in Jos, Nigeria. *International Journal of Infectious Diseases*, *104*, 276-281.

Ott, J. J., Stevens, G. A., Groeger, J., & Wiersma, S. T. (2012). Global epidemiology of hepatitis B virus infection: new estimates of age specific HBsAg seroprevalence and endemicity. *Vaccine*, *30*(12), 2212-2219.

Potthoff, A., Manns, M. P., & Wedemeyer, H. (2010). Treatment of HBV/HCV coinfection. *Expert opinion on pharmacotherapy*, *11*(6), 919-928.

Razavi-Shearer, D., & Razavi, H. (2018). Global prevalence of hepatitis B virus infection and prevention of mother-to-child transmission–Authors' reply. *The Lancet Gastroenterology & Hepatology* , *3*(9), 599.

Saeeda B., Siddiqui A. A., Chakravarty R., Moatter T., Unnissa T., & Nazr-ul-Hasnain. (2008). Phylogenetic analysis of Hepatitis B virus in Pakistan. *Journal of the College of Physicians and Surgeons Pakistan*, *18*, 688-94.

Sanyal, A. J., Yoon, S. K., & Lencioni, R. (2010). The etiology of hepatocellular carcinoma and consequences for treatment. *The oncologist*, *15*, 14-22.

Spearman, C. W., Afihene, M., Ally, R., Apica, B., Awuku, Y., Cunha, L., ... & Sonderup, M. W. (2017). Hepatitis B in sub-Saharan Africa: strategies to achieve the 2030 elimination targets. *The lancet gastroenterology & hepatology*, *2*(12), 900-909.

Tanga, A. T., Teshome, M. A., Hiko, D., Fikru, C., & Jilo, G. K. (2019). Sero-prevalence of hepatitis B virus and associated factors among pregnant women in Gambella hospital, Southwestern Ethiopia: facility based cross-sectional study. *BMC infectious diseases*, *19*(1), 1-7.

Terrault N. A., Bzowej N. H., Chang K. M., Hwang J. P., Jonas M. M., Murad M. H., *et al* (2016). AASLD guidelines for treatment of chronic hepatitis B. *Hepatology*, *63*, 261–283.

Terrault N. A., Lok A. S., McMahon B. J., Chang K. M., Hwang J. P., & Jonas M. M. (2018). Update on prevention, diagnosis, and treatment of chronic hepatitis B: *AASLD 2018 Hepatitis B guidance. Hepatology*, *67*, 1560-1599.

Toka, B., Koksal, A. S., İskender, G., Çakmak, E., Üsküdar, O., Sezikli, M., ... & Eminler, A. T. (2020). HBV flare associated with immunosuppressive treatments: it is still dangerous in the third-generation antivirals era. *Antiviral Therapy*, *25*(3), 121-129.

World Health Organization. (2017). *Global hepatitis report 2017*. World Health Organization.