

# Sero-prevalence and Associated Complications of Hepatitis B Virus Infected Patients of Khyber Pakhtunkhwa

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## ABSTRACT

**Background:** Hepatitis B infection is among the most significant public health challenge worldwide. The prevalence of HBV infection is common among most populations in Peshawar, including pregnant women.

**Objective:** The present study aims to estimate the prevalence and complications associated with clinical parameters and risk factors in patients with HBV.

**Methodology:** A total 150 patients were reported and subjected to Enzyme linked assay (ELISA) for HBeAg test and HBs-Ag by immunochromatographic assay (ICT). RT-PCR assay was used for detection of HBV DNA load. A study designed Performa was used for face-to-face interview to collect data from the study participants.

**Results:** According to our study, highest prevalence was found in Peshawar 34% and the second highest prevalence in Waziristan 20.2%. The results showed highest prevalence in male (54%) than female (46%) and amongst female patients, 10% were pregnant. According to patient's age, the highest prevalence was found group 26-35 years 34.2% and lowest prevalence was  $\leq 15$  years 2.6%. In our study patients were reported on the base of antigen were 94 (62.7%) of patients with HBsAg positive and 56 HBeAg positive (37.3%). Among total patients, 5.3% were co-infected with HCV and no patients were observed co-infected with HDV or HIV. In current study, the prevalence of family affected on the base of relation was found with 21.3%, amongst these 9.3% (spouse affected), 2.7% (mother affected), sibling affected patients (4%) and children affected (5.3%) were observed. The data showed patients with normal ALT 81.3% and 18.7% with elevated and the patients 9.4% and 90.6% found with elevated and normal creatinine level respectively. The normal bilirubin (93.3%), raised bilirubin (6.7%), normal ultrasound (83.7%) and 16.3% dysfunction ultrasound were reported in patients after antiviral drugs treatment. The study surveyed 64% and 36% patients reported under tenofovir and entecavir treatment with different duration and 36%. The less mortality rate of patients leading HBV infection was reported 1.3% in this study due to renal failure and diabetics.

**Conclusion:** The male are more exposed to the environment and that is why highest frequency infected as compared to the female. The main reason of high HBV prevalence was less awareness in people and highest prevalence on the base of family history in spouse because may close contact.

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

## INTRODUCTION

There are five major hepatitis-causing viruses, including HAV, HBV, HCV, HDV and HEV that cause severe contagious liver infection, including. All these viruses are different from each other based on ways of transmission, pathology and prognosis. Hepatitis B virus (HBV) is a major contagious virus that may cause acute or chronic liver infection and leads to liver fibrosis and hepatocellular carcinoma (1, 2).

According to the WHO, an expected 2 billion people with chronic hepatitis B virus infection and 5–15% of populations are chronic carriers of hepatitis B infection (3). Furthermore, hepatitis like tuberculosis is cause of 1.34 million deaths according to WHO, but higher than deaths due to HIV, 2.7 million co-infected with HBV-HIV (4). According to a study, about 20 million people in Pakistan are suffering from hepatitis B and C, annual new cases approximately 150 000 (4).

In worldwide an estimated over 240 million were found to be in chronic state (5) and 1.8 million of children under five years are living with the hepatitis B virus (6). While, chronic carrier HBV infection are more common among males compared to females, main route of

transmission of the disease is vertically transmission from infected mother to neonates (7). Additionally, hepatitis B-infected mothers with HBeAg (negative), have a risk of 10–40% of transmitting the infection to newborn baby, while infected mothers and positive for HBeAg have a 90% risk of acquiring the infection (8). In countries with high HBV occurrence, a significant proportion of people remains susceptible to HBV and is, therefore, at risk of contracting the virus during their adult age. However, most HBV transmission occurs already during childhood (9). HBV is certainly the most significant hepatocarcinogen accountable for the development of HCC, which presently orders as the sixth-most common cancer globally and the second-most common cause of cancer death (10).

HIV co-infection has a thoughtful influence on virtually every feature of the natural history of HBV infection. The result consists of greater proportions of chronicity after acute HBV infection, greater level of HBV replication and reactivation, minimum loss, more quick development cirrhosis and HCC, greater liver associated fatality, and low therapy response than without HIV co-infection (11). HBV infection in pregnancy has serious complications, counting growing the risk of development of chronic HBV, perinatal

transmission of HBV and enhanced HBV-related liver impairment. HIV-HBV co-infection in pregnancy may change the variety of antiviral drug therapy against both viruses (12).

Co-infection with HCV is ordinarily originated in HBV widespread, as Asia, South America and sub-Saharan Africa and above 25% with HCV infected people may be co-infected with HBV. It is reported that HCV co-infection with HBV at greater danger of rising HCC, both more potent form at younger age (13).

Currently, the CHB patients are treated with two main therapies: treatment with nucleoside/nucleotide analogue (NA) or interferon- $\alpha$ , presently also uses pegylated (PegIFN $\alpha$ ) (14). Currently, there are five approved (NA), telbivudine (ADV), adefovirdipivoxil (ADV), entecavir (ETV), lamivudine (LAM) and tenofovir disoproxil fumarate (TDF) for HB treatment. This is evidence that the threat of cirrhosis and hepatocellular carcinoma (HCC) can decrease by persistent and effective suppression of HBV DNA. Therefore, long-term current treatment (NA therapies) for chronic hepatitis B is used to accomplish completely virology suppression, with loss hepatitis B surface antigen (HBs-Ag) (15).

The main objective of the study was to achieve an up-to-date prevalence and distribution of HBV infection in Peshawar city, Khyber Pakhtunkhwa, Pakistan. We also evaluate the clinical parameters, and risk factors present in active HBV infected patients. The study also evaluated their general knowledge about the complications of HBV infection.

## MATERIALS AND METHODS

This study conducted in Peshawar during the period of 2020 and clinical data of HBV patients collected from different clinics and hospitals. The data was analyzed base on prevalence according to different factors including gender, age, location, family history, antigen status, treatment and clinical profile. Serum ALT, bilirubin and creatinine were measured by standard biochemical laboratory test and HBe-Ag status (+/-) by Enzyme linked assay (ELISA) and HBs-Ag by immunochromatographic assay (ICT). RT-PCR assay was used for detection of HBV DNA level.

The primary objective of our study was to determine actual rate of HBV prevalence with clinical parameters. All serum samples from patients were tested for HBsAg by ICT (immunochromatographic test) and HBeAg using commercially available enzyme-linked immunosorbent assay (ELISA). Blood samples were collected from all tested individuals through authorized technician by using disposable syringe. Samples was centrifuged at 3000 rpm for 2 min to isolate the plasma and stored at -20°C. This technique is used to detect antigen or antibody in a sample.

The samples were screened primarily by using ICT strip to separate positive sample of HBsAg. 100ml sample was loaded under strict sterile condition in to screening strip and two bands, control band and trace band was used for indication. When color bands showed on both control and trace band indicated positive result and color on control band indicated negative result.

The samples of HBV were analyzed and confirm by using HBe-Ag kit. The Bios kit was used to confirm positive

sample of HBe-Ag. The 50ml sample was mixed with 50ml conjugate reagent, HRP enzyme (horseradish peroxidase). Both samples and conjugate was mixed at the same time and wait for 60 minutes then incubated at 37°C. Then wash was done five times with buffer solution. Second incubation did with two types of substrates, substrate A (50ml) & substrate B (50ml) was loaded and waited for 15 minutes, and enzyme generated bluish color which indicates positive sample. The stop solution used to stop reaction and which ELISA reader further analyzed.

DNA was extracted from 200ml of sample using DNA extraction kit (GF-1 kit). The extracted DNA was subjected to PCR for the detection of HBV. The Promotor™ HBV Hepatitis virus quantitative test kit was used for HBV DNA detection by real time-PCR and contained all the reagents and enzymes for the specific amplification of the HBV genome. ). The standard curve cut off value of HBV DNA detection for this kit is 34 to 35, below this value showed positive result and above this indicated negative result.

Data collected by the questionnaire were transferred into an Excel database and subjected to the SPSS version 20 was used for the data analysis.

## RESULTS

The study designed to collect data of HBV infected patients of KP during period 2020-2021, with clinical parameters and viral load of HBV infected patients. The population of my study was both male and female HBV patients of all ages, which analyzed for clinical parameters until negative PCR. A total 150 patients with HBV infection were reported on base of different prevalence and clinical parameters.

**Prevalence of KP HBV patients on base of location:** In our study, total 150 patients investigated for HBV infection of KP, included Peshawar, Bannu, Charat, Charsadda, Kohat, Lakki Marwat, Mardan, Swabi, Upper Dir and Waziristan. According to our study, highest prevalence was found in Peshawar with 34% (51/150), Bannu (7.3%, 11/150), Charat (10%, 15/150), Charsadda (5.3%, 8/150), Kohat (3.3%, 5/150), Lucki Marwat (6.6%, 10/150), Mardan (6%, 9/150), Swabi (3.3%, 5/150), Upper Dir (4%, 6/150). The second highest prevalence was found in Waziristan with 20.2% (30/150), shown in figure 1.

**Gender and age wise prevalence of HBV patients:** In our study, data were collected of patients having HBV infection from province of KP; higher prevalence found higher in male than female. There were 81 (54%) male and female were 69 (46%) out of 150 patients. Patients were divided into 6 groups, first group  $\leq$  15 years and last group  $\geq$  56 years, shown in table .1. The highest prevalence was found group (26-35 years) with 51 patients (34.2%) and lowest prevalence was shown group ( $\leq$  15 years) with 4 patient (2.6%). The age group (16-25 years) showed HBV infection in 42 patients (28%) and 28 (18.6%), 14 (9.3%) and 11 (7.3%) was found in age group (36-45 years), (46-55 years) and ( $\geq$  56 years) respectively, shown in table 1. The statistical data found with age mean and Std. Deviation 32.627 and 12.301 respectively.

**Prevalence of HBV patients on base HBsAg/HBeAg positivity:** The prevalence of HBV patients were reported on the base of antigen status such as HBsAg or HBeAg positivity. There were 94 (62.7%) of patients with HBsAg positive and HBeAg positive was found in 56 (37.3%)

patients. The prevalence of HBV patients with HBsAg positive is higher than HBeAg positive, shown in figure 2.

The statistical data was found significant (p.value= .003) by using chi-square test.

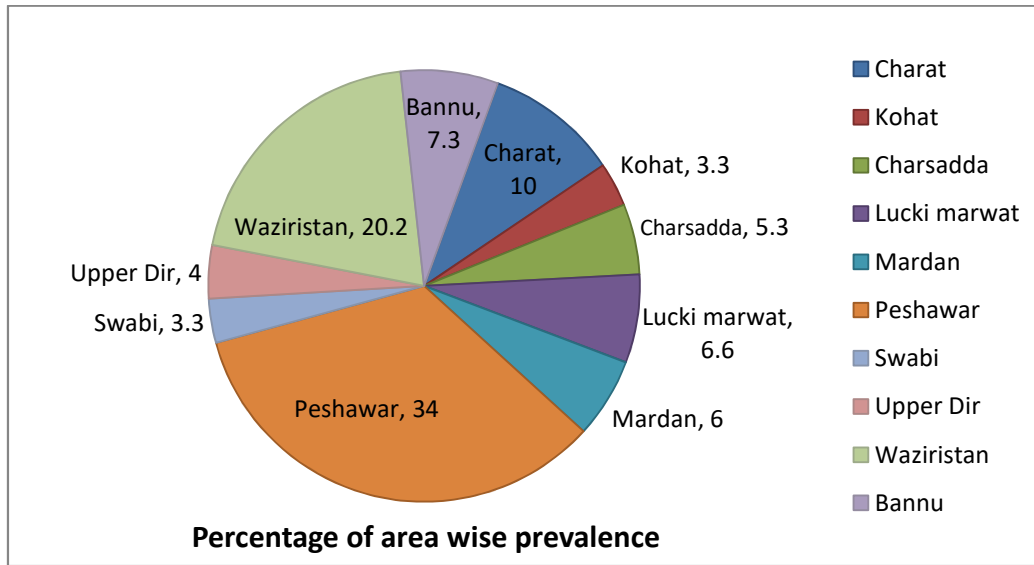


Figure 1. Location basis prevalence of HBV infected patients

Table .1: age of patients

	Frequency	Percent (%)
Valid	<= 15.00	2.6
	16.00 - 25.00	28
	26.00 - 35.00	34.2
	36.00 - 45.00	18.6
	46.00 - 55.00	9.3
	56.00+	7.3
Total	150	100.0

**Co-infection with HCV, HDV,HIV base prevalence:** A total 150 reported patients, 8 patients (5.3%) were co-infected with HCV and no patients were observed with co-infection with HDV or HIV, and remaining 142 patients (94.7%) were not infected with any co-infection, shown in figure 3. The comparison correlation between HBV with HCV and HIV confections is calculated significant (p.value=.000) by chi-square.

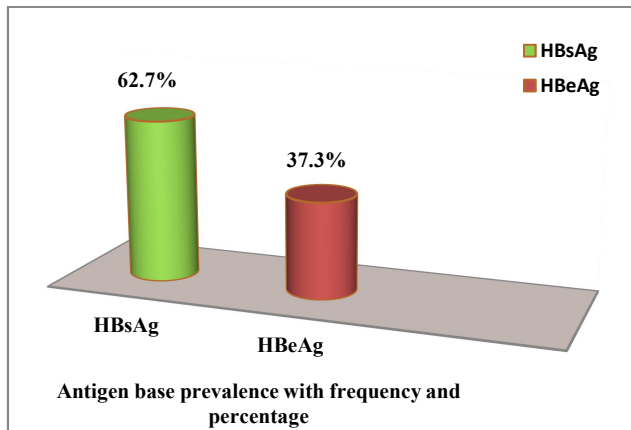


Figure 2: Prevalence of HBV patients on base of Ag-status

**Family affected base prevalence of HBV patients and their relation with patients:** The prevalence of HBV patients on the base of family affected with HBV infection also estimated. In our study, 21.3% patients were reported that their family also affected with HBV infection and 78.7% patient's family were not affected. In our study, affected family of HBV patient's prevalence observed with 21.3% patients. Among these patients, the prevalence of family affected on the base of relation was found with 9.3% (spouse affected), 2.7% (mother affected), sibling affected patients (4%) and children affected (5.3%), shown in table .2. The data showed highest prevalence found in spouse because may close contact. The statistical correlation among these data was found significantly, p.value (.000).

**Prevalence of HBV infected patients based on clinical parameters:** ALT, bilirubin, creatinine and ultrasound level are main parameters of HBV patients, which reported for treatment indication, severity of infection and efficacy of antiviral drugs. Total 150 HBV patients were observed for these clinical parameters level after antiviral drugs with such as entecavir and tenofovir. The data showed patients with normal ALT 81.3% (122/150) (81.3%) and 18.7% (28/150) with elevated and the patients 14/150 (9.4%) found with elevated creatinine level and 136/150 (90.6%) were normalized creatinine. The normal bilirubin 93.3%, raised bilirubin 6.7%, normal ultrasound 83.7% and dysfunction ultrasound 16.3% were reported in patients after antiviral drugs treatment. The statistic data was significantly calculated (p=.005).

**Prevalence of antiviral drugs therapy duration on base of HBsAg/HBeAg positivity:** The treatment history of HBV patients was estimated as prevalence. There were 150 patients reported in our study with two used antiviral drugs such as tenofovir and entecavir with different duration. The study showed that only entecavir and tenofovir drugs use for HBV patients in Khyber Pukhtunkhwa.

There were 96 (64%) patients reported under tenofovir treatment with different duration including 6, 12, 18, 24 and 48 months. The patients treatment with different duration were divided on basis of HBs/eAg. The 6.7% patients of HBsAg (+) used 6 months treatment and (43.3%) recovered after 12 months therapy in which HBsAg (+) were 33.3% and HBeAg (+) 10%. The HBsAg positive (10%) and HBeAg positive 6.7% were reported under tenofovir drug with 18 months therapy. Duration of 24 months tenofovir treatment was found 23.3% HBsAg positive and 3.3% HBeAg positive as shown in table. The patients (6.7%) of HBeAg positive were recovered under tenofovir drug with 48 months treatment. The study surveyed highest prevalence of tenofovir used duration found with 12 months therapy of HBV patients as mentioned in table .

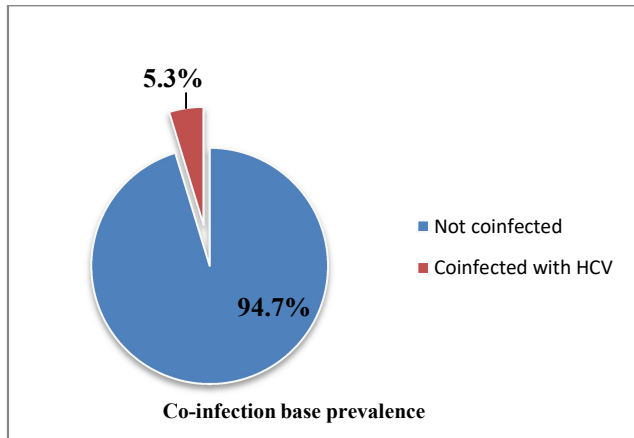


Figure 3: Co-infection base prevalence of HBV patients

Table. 2: Relation base prevalence of affected family

	Frequency	Percent (%)	Cumulative Percent
Valid	spouse affected	14	9.3
	parent affected	4	2.7
	Sibling affected	6	4
	Children affected	8	5.3
	Not affected family	118	78.7
Total	150	100.0	

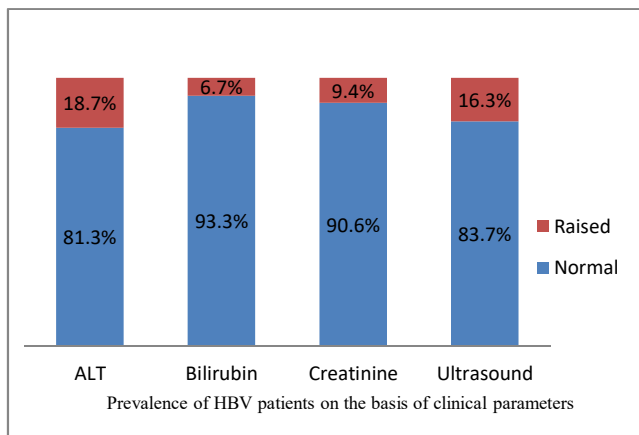


Figure 4. Prevalence of HBV infected patients based on clinical parameters

While there were total 54 (36%) patients under entecavir treatment with different duration was divided on base of positivity of HBsAg and HBeAg. Among 54 patients, duration of entecavir treatment with 6 months therapy noted with 7.1% (HBsAg positive) and (14.3%) were HBeAg positive. There were patients (21.4%) of HBsAg positive and (21.4%) of HBeAg positive under entecavir treatment with 12 months duration. The 18 months treatment of HBeAg was 14.3% and 24 months of entecavir therapy was reported 14.3% and 7.1% of HBsAg and HBeAg (+) patients respectively. The statistical p.value (.046) was found significantly.

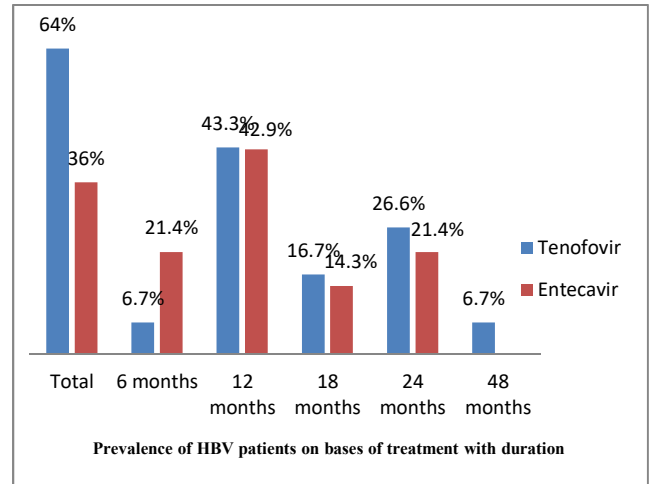


Figure 5: Prevalence of patients on bases of treatment with duration

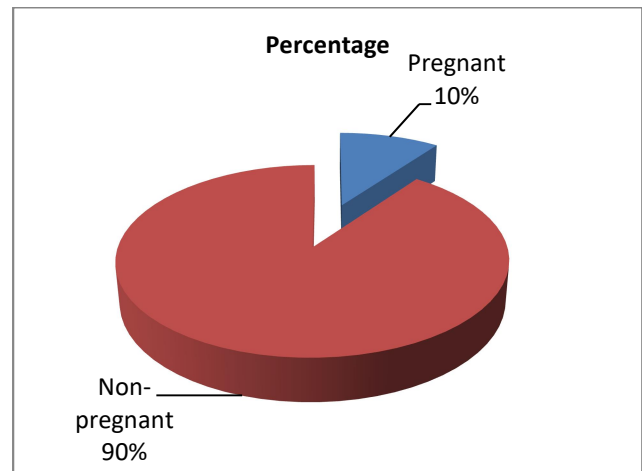


Figure 6: Female HBV patients prevalence

**Pregnancy and mortality base prevalence of HBV patients:** In our study, there were 46% (69/150) female patients with HBV infection, among female patients; 90% (62) were non-pregnant and 10% (7) were pregnant as shown in figure. All the pregnant patients were HBsAg positive and treating with antiviral drug such as entecavir or tenofovir. The less mortality rate of patients leading HBV infection was reported 1.3% in this study. The mortality rate showed in my study patient due to renal failure and diabetics.

## DISCUSSION

Hepatitis B virus (HBV) is an infectious agent that is responsible for causing inflammation of liver parenchyma cells. Almost 350 million populations are infected by chronically HBV worldwide, and result in cirrhosis and hepatocellular carcinoma in some population. Pakistan has been ranked with high threat of HBV infection in world. The study conducted to calculate rate of HBV patients in anti-HBV positive with respect to different factors.

The study has conducted that Baluchistan province has the uppermost prevalence (4.3%) and KP has the lowermost prevalence 1.3%. Upper Dir in KP showed the highest prevalence (5.0%) (16). Similarly, in our study, highest prevalence was found in Peshawar with 34% and lowest in Kohat and Swabi (3.3%). The second highest prevalence was found in Waziristan with 20.2% (30/150). In our study contrast to previous study, Upper Dir showed less prevalence and highest prevalence in Peshawar in KP because collection center of my study was Peshawar.

The study performed prevalence of HBV patients with entecavir or tenofovir treatment and assessed medical course. The collected data showed 29 males and 11 female out of total 40 patients with mean age  $57 \pm 12$  years (17). T. Kurien (2005) conducted a study to determine the prevalence of HBV infection in a southern State of India. HBsAg prevalence was 5.7% and 23.5% of having positive HBe-antigen. The highest prevalence of 32.7% reported in the 15-20 year age group (18). Similarly, in our study both male and female were included in our research investigation, there were 81 (54%) male and female were 69 (46%) out of 150 patients. The highest prevalence was found group (26-35 years) with 51 patients (34.2%) and lowest prevalence was shown group ( $\leq 15$  years) with 4 patient (2.6%). Both study showed same data with high prevalence in male than female and variant age group. The study has conducted in region of Malakand for incidence of HBV and HCV. A total 30 individuals (10.71%) were found HCV, 9.64% was HBs Ag positive, and no individuals were found having both HBV and HCV (19). Similarly, in our study total 150 HBV patients in which 62.7% of patients were HBsAg positive and 37.3% were HBeAg positive. There 5.3% patients were co-infected with HCV and remaining (95.3%) were not infected with other co-infection. The variation in our study was due to dissimilar study area or sample size.

Magaii (2021) studied of the prevalence of hepatitis B infection and co-infection with HIV in Nigeria. The result showed 12.6% were HBV co-infected with HIV and 7.2% without HIV ( $P = 0.01$ ) (20). Among pregnant women, the prevalence of HBV was higher among those with HIV. The prevalence rates of HIV-HBV co-infection in pregnancy in Africa differ from 6.3% to 25% (21). In our study, there were 46% female patients with HBV infection, among female patients; 90% (62) were non-pregnant and 10% (7) were pregnant. There were no patients found with HBV-HIV co-infection in included patients.

Similarly, in our stud total 150 HBV patients in which 62.7% of patients were HBsAg positive and 37.3% were HBeAg positive. There 5.3% patients were co-infected with HCV and remaining (95.3%) were not infected with other co-infection. The variation in our study was due to dissimilar study area or sample size.

The study conducted to compare entecavir or tenofovir with respect to liver associated mortality. The data were collected from 40 patients showed TDF was taken by 26 patients and 14 patients received ETV treatment. The Toka (2020) study reported mortality rate of 17.5% due to liver associated problem (17). Similarly, our study estimated the death rate with less frequency that is only 1.3% among all reported data. There were 96 (64%) patients reported under tenofovir treatment and 54 (36%) were taken entecavir antiviral drugs. There is contrast in our study from previous study due to associated renal or liver problems to patients. The mortality rate showed in my study patient due to renal failure and previous one was acute liver failure.

## CONCLUSION

This research study emphasizes on prevalence of HBV patients with different factors and clinical parameters after antiviral drugs treatment for chronic hepatitis B (CHB) infection. These drugs may lead to eliminate of HBV and can efficiently switch the infection with inhibiting liver damage. CHB remains a serious liver problem in Pakistan, this study present that nucleoside or nucleotide analogues are the most effective available medicine against the HBV in Pakistan. The two ideal antiviral drugs such as tenofovir and entecavir are being used in Khyber Pakhtunkhwa for treating CHB virus with efficient response rate.

The study focuses on prevalence of HBV infected patients of KP including age, gender, antigen, antiviral drugs and co-infection and affected family base wise. According to our study, male are more expose to environment than female due to which high prevalence of HBV infection found in male (54%) than female (46%). The highest prevalence was found group (26-35 years) with 51 patients (34.2%) and lowest prevalence was shown group ( $\leq 15$  years) with 4 patient (2.6%). It is concluded that young male are more expose to the environment and that is the reason they are more infected with HBV. It is likely to determine that NAs treatments are best option for co-infected patients of HBV as well as renal monitoring. The patients whose family also affected with HBV were found with 21.3%, among affected family highest percentage shown in spouse, which may close contact. The study revealed that the main reason of HBV transmission is joint family in PK and less awareness in people.

The study concludes that antiviral drugs has less significant role in the treatment of HBV patients with renal impairment. The study also demonstrates that the death of the patient due to different complication like renal impairment. This study will helps us in the prevalence of HBV infection and complication related to this infection.

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