



## MOLECULAR SCREENING OF HEPATITIS B IN YOUNG ADULTS OF DISTRICT ABBOTTABAD AND MANSEHRA

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<p><b>ARTICLE INFO</b></p> <p><b>Keywords:</b> Hepatitis B Virus (HBV), Prevalence, Young Population, Serological and Molecular Screening</p> <p><b>Corresponding Author:</b> <b>Fahim Ullah,</b> Department of Microbiology, Abbottabad University of Science and Technology, KPK, Pakistan Email: <a href="mailto:fahimwazir996@gmail.com">fahimwazir996@gmail.com</a></p> <p><b>Zubair Rehman,</b> Center for OMIC Sciences, Islamia College Peshawar, Email: <a href="mailto:Zubairkmu22@gmail.com">Zubairkmu22@gmail.com</a></p>	<p><b>ABSTRACT</b></p> <p>Human hepatitis B is the inflammation of liver cells, caused by hepatitis B virus. It is a serious liver disease that occurs worldwide among people of all ages. It is a highly infectious disease and can result in severe illness, liver damage, and cirrhosis besides increasing the risk for hepatocellular carcinoma (HCC). The disease ranges in severity from a mild illness to a serious, lifelong illness. A cross sectional study was conducted during September 2017 to March 2018 to determine the frequency distribution of active hepatitis B virus (HBV) infection in apparently healthy young residents (5-24 years) of districts Abbottabad and Mansehra (KPK), Pakistan. A total of 353 samples were collected from various schools and colleges of districts Abbottabad and Mansehra. Initially all the individuals were screened for HBV using ICT technique. ICT positive individuals were then subjected to ELISA for the detection of anti-HBV antibodies. The ELISA positive samples were then subjected to polymerase chain reaction (PCR) for further screening (detection of HBV-DNA). Out of 353 samples, 8 (2.26%) cases were found to be positive in the young population of district Mansehra, while 5 (1.41%) cases were found positive in district Abbottabad by ICT technique. By ELISA 6 (1.69%) cases were found positive in district Mansehra whereas 2 (0.56%) cases were noticed positive in district Abbottabad. Subsequent experiments, i.e., PCR showed only one case (0.699%) in district Abbottabad while 02 (0.96%) positive cases in district Mansehra. High Prevalence rate was detected both in female (2.77%) and male (2.39%) population of district Mansehra.</p>
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## INTRODUCTION

The word hepatitis is derived from ancient Greek word “hepa” meaning liver and suffix “itis” meaning inflammation (Pungpapong *et al.*, 2007). It is characterized by liver injury and subsequent inflammation of hepatocytes (But *et al.*, 2008). Hepatitis B is one of the major global public health problems among people of all ages (McMahon, 2004). HBV causes a blood born disease that is mainly transmitted through contact with infected blood, unprotected sexual activity, and from mother to child at birth. Infection can either be acute or chronic. It is a highly infectious disease and can result in severe illness, liver damage, cirrhosis and an increased risk for hepatocellular carcinoma. The disease ranges in severity from a mild illness lasting a few weeks to a serious, lifetime illness (Lewis-Ximenez *et al.*, 2002). Although many people recover and develop natural immunity to HBV after acute (initial) infection, others including infants infected at birth become chronic (lifelong) carriers. Later in life, chronic hepatitis B can lead to liver failure or liver cancer (CDC, 2010). It is estimated worldwide that there are 350 million people affected with chronic

HBV infection and each year approximately 620, 000 people die from hepatitis B associated acute and chronic liver (Wright, 2006).

Perinatal transmission is an important route of transmission for HBV. Prevention of perinatal transmission is a vital step to decrease the occurrence of human hepatitis B (Abbas *et al.*, 2003). As newborns have poor immunity, nearly all of them who acquire virus cannot clear it and become carrier. They create the bulk of carrier of hepatitis B virus and therefore cause spread in the community. The hepatitis B vaccine and HBV immune globulins (HBIG) should be given to Infants of HBV-infected mothers within 12 hours of birth. By doing this, the rate of chronic infection can be reduced from 90% to about 10% in infants (Bateyet *al.*, 2008; CDC 2007).

Pakistan is highly endemic with hepatitis B infection (Noorali *et al.*, 2008). About Nine million people infected with HBV (Hakimet *al.*, 2008) and its infection rate is on a steady increase. The reason may be the deficiency of proper health care facilities, less public awareness and poor economic status about the transmission of major infectious diseases including HBV, HCV and HIV (Alamet *al.*, 2007).

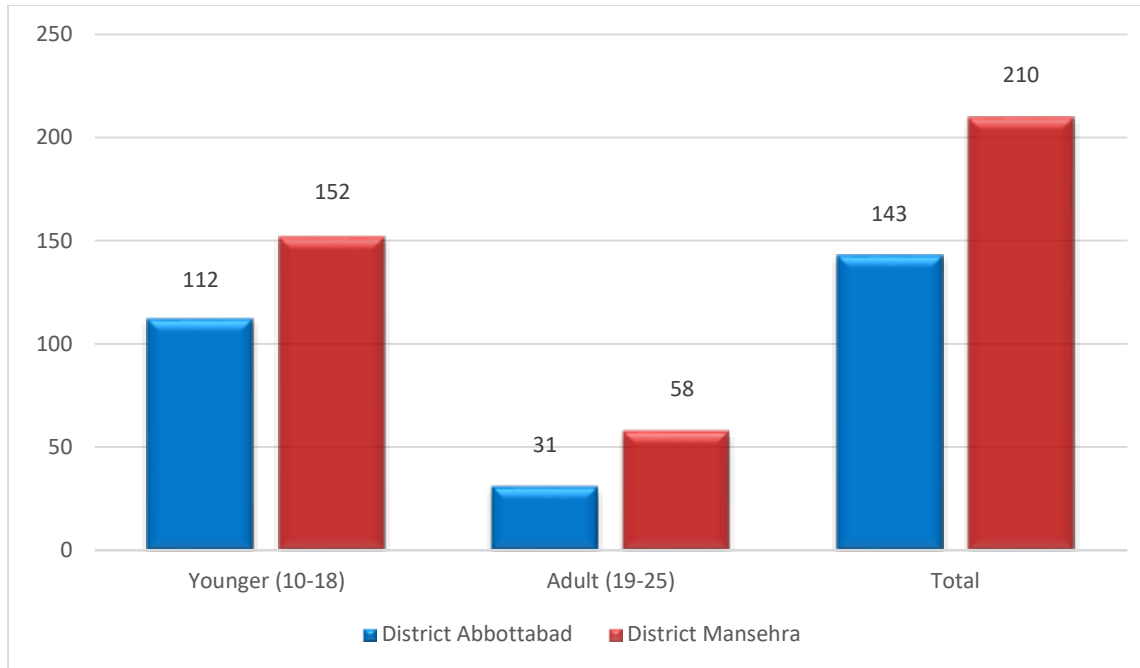
The current study was conducted to screen out the young population of district Abbottabad and Mansehra for hepatitis B viral infection by using ICT Kits, ELISA method and to analyze ELISA positive samples for the presence of viral DNA by PCR.

## **Materials and methods**

### **Study design:**

A retroactive (cross-sectional) study was conducted from September 2017 to March 2018 at the Department of Microbiology and Biomedical Science, Abbottabad University of Science and Technology, Pakistan. Young (male & female) population of all the cultural groups and races (below 24 years) belonging to district Abbottabad and Mansehra were involved in this study. In addition, the exclusion criteria were also stretched to the people having age more than 24 years of age.

A total of 353 blood samples were collected from various schools and colleges of Abbottabad and Mansehra. The data was composed of organized questionnaire. A brief clinical history was recorded after an informed written consent. The initial testing for HBV of the study participants was performed by Immunochromatographic Technique (Acon, USA) and was then confirmed by 3rd Generation ELISA Technique (EASE BN-96 TMB, Taiwan). After ELISA, HBV positive cases were further confirmed by PCR (Qualitative).



**Figure 1:** Distribution of study participants into different age groups belonging to district Abbottabad and Mansehra

## Results

A total of 353 participants (aged 5-24 years) were assessed for Hepatitis B during a period of three months. There were two age groups of the study participants: the adult group (19-24 years=89) and the young school growing children group (5-18 years=264) (Figure 1). Amongst the total participants of the study, 142 (40.22%) were females and 211 (59.78%) were males. The age limits for male participants were reported as 5 and 24 years with a mean age value of 15.54. In current study, the standard deviation in age of the male persons was found to be 5.23. On the other hand, the age limits among the female patients were reported as 5 and 24 years with a mean age value of 16.36. In current study, the standard deviation in the age of female participants were found to be 5.05 (Table 1).

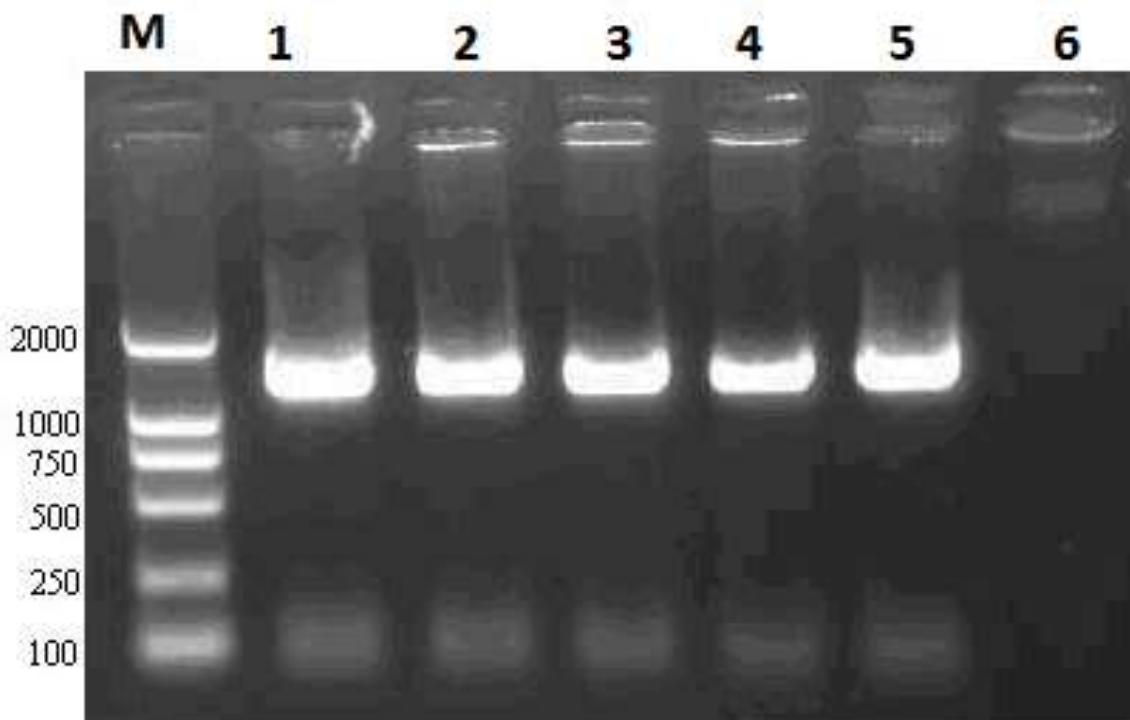
**Table 1:** Age statistics of male participants of the study

	Male	Female
Number of values	211	142
Minimum	5.000	5.000
25% Percentile	10.00	12.75
Median	16.00	17.00
75% Percentile	20.00	21.00
Maximum	24.00	24.00
Mean	15.54	16.34
Std. Deviation	5.296	5.005
Std. Error of Mean	0.3646	0.4200
Lower 95% CI of mean	14.82	15.51
Upper 95% CI of mean	16.25	17.17

Blood samples of all the participants were collected, and the initial screening of the study members were performed by ICT (Immunochromatographic technique). By ICT, 13 (3.68%) cases were found positive for Hepatitis B infection among 353 cases. Out of 13 positive samples, 5 (1.41%) cases were found positive in Abbottabad whereas 8 (2.26%) cases were found to be positive in the young population of Mansehra. Moreover, ICT positive cases of HBV were then confirmed by ELISA techniques. By ELISA method, 08 (2.26%) cases were reported positive for Hepatitis B surface antigen (HBsAg) among all tested subjects including 2 (0.56%) cases were positive in district Abbottabad whereas 6 (1.69%) cases from district Mansehra (Table 2).

**Table 2:** Distribution of HBV positive cases by ICT method ELISA method PCR

Study area	Total No. of sample	ICT method		ELISA method		PCR	
		Positive	Negative	Positive	Negative	Positive	Negative
Abbottabad	143	05	138	02	141	01	142
Mansehra	210	08	202	06	204	02	208
<b>Total</b>	353	13	340	08	345	03	350



**Figure 2:** Gel picture showing band size of different HBV DNA samples

HBV positive cases reported by ELISA technique were then further confirmed by PCR (Qualitative) to detect the active (acute) infection of HBV. Total of 08 cases which were originally reactive on ELISA were therefore subjected to PCR. By PCR technique only one case (0.699%) was detected from district Abbottabad while 02 (0.96%) cases were found positive from district Mansehra. It was found that only 0.85% of the originally ELISA positive cases showed HBV amplification (Table 2: Figure 2). As for as the sex specific prevalence of HBV was concerned in

Abbottabad and Mansehra, a comparatively high prevalence was observed both in male and female of young, aged population of district Mansehra (Table 3).

**Table 3:** Sex specific distribution of the study participants belonging to districts Abbottabad and Mansehra.

Study Area	Male		Female	
	Total Number	Positive Cases	Total Number	Positive Cases
n=353				
Abbottabad	92	02	51	00
Mansehra	168	04	42	02
Total	260	06	93	02

### Discussion

Hepatitis B is one of the major infectious diseases of human being posing severe global public health problems. Globally, Out of the two billion people who has been infected with hepatitis B virus (HBV), more than 350 million have chronic liver infections (Andre, 2000). In Pakistan, studies conducted on different sections of population have presented variable degree of frequency in different risk groups (Abbas *et al.*, 2003; Hamid, 2003). According to an estimate, there are about nine (9) million hepatitis B Virus carriers all over the Pakistan (Akhtar *et al.*, 2004). In present study a total of 353 young female and male individuals were screened for HBV, from different schools and colleges of Abbottabad and Mansehra. The information concerning age, spousal status, residential area, history of minor/major surgical operation, dental procedures, skin tattooing, history of jaundice, blood transfusions, therapeutic injections, etc. were taken. The age range of the study members was between 5 – 24 years. Initial screening of the study applicants was done by ICT method. Out of 353 samples, 5 (1.41%) cases were found positive in district Abbottabad while 8 (2.26%) cases were found positive for HBV in the young population of district Mansehra,. The frequency rate is relatively high in District Mansehra as compared to Abbottabad. This may be due to lack of awareness about the disease and poor medical facilitation in district Mansehra than district Abbottabad. These results are much similar to another study conducted by Khan *et al.*, (2007).

Moreover, ICT positive cases of HBV were then tested by ELISA method. Out of 353 samples, 2 (0.56%) cases were positive for HBV in district Abbottabad whereas 6 (1.69%) cases were found to be positive for HBV in the young population of district Mansehra. Our ELISA results are in great agreement to some other studies carried out by (Choudhary *et al.*, 2005; Alam *et al.*, 2007). HBV positive cases of ELISA were then further confirmed by PCR (Qualitative) to detect the active (acute) infection of HBV. Among 8 positive cases by ELISA, only one case was detected in district Abbottabad while 02 were found positive in district Mansehra by PCR.

Of the 353 samples tested during the stated period, 08 (2.26%) samples were finally confirmed positive for hepatitis B surface antigen. The Results revealed that the incidence rate of hepatitis B surface antigen in young adults, belonging to district Mansehra was reported as 1.69% including; 0.56% cases of female and 1.13% cases of male population were infected. On the other hand; 0.56% cases were reported positive for HBV in district Abbottabad including 0.56% male and 0.00% female. The maximum cases by positive HBsAg were noted in participants belonging to district Mansehra i.e., 1.69%. High prevalence rate of Hepatitis B in district Mansehra as compared to Abbottabad was also reported by Abbas *et al.*, (2003).

As for as the sex specific prevalence of Abbottabad and Mansehra is concerned, there were high prevalence observed both in male (2.39%) and female (2.77%) of the young population of district Mansehra, whereas an overall low prevalence rate was observed in district Abbottabad i.e (2.16%). Higher HBV Infection rate in males than female may be due to their social responsibility like being working outsides their homes. Other possible reason can be common visiting of barber shops and

also participation of male in blood transfusion practices, while women are frequently involved in house hold activities based on the social, cultural and religious fondness and inspirations. These results are much similar to other studies conducted by Zuberi, (1996) and Qureshi and Bengali, (2003).

### **Conclusion**

The current study exposes the gender-wise prevalence and risk factors related with HBV between different age groups of young population belonging to Abbottabad and Mansehra districts, Pakistan. Male were more commonly exposed to the risk factors for HBV infection than to female population of the selected study area. Similarly, the adult age group between (19-25) have high rate of infection as compared to that of the young age group. This high prevalence was due to the lack of awareness about numerous risk factors convoluted in HBV transmission among most of the defendants. These risks can be reduced by broad measures both in public and private sector to confirm the necessity for ruling and control of the transfusion practices in Pakistan. In order to stop HBV infection in our country; government must take hostile steps towards the awareness programs which involve both the media and public sectors administrations. Information should also be provided to the public; that hepatitis B infection can be prevented via vaccine. Massive awareness and vaccination programs are required to decrease the future burden of HBV from Pakistani population.

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