Original Article

# Evaluation of HBeAg and HBV viral load among general population of district Bannu, Khyber Pakhtunkhwa, Pakistan

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## ABSTRACT

The main objective of this research was to evaluate the somewhat consistent association between serological and molecular characterization of hepatitis B virus patients in district Bannu. Blood samples of 563 HBsAg positive patients were collected, which were initially analyzed by an immuno-chromatographic technique (the ICT-kit method), and then further assessed by ELISA and polymerase chain reaction. Out of 563 blood samples, 303(53.81%) were positive for qPCR (viral load >200). The highest numbers of positive qPCR cases were found in the age group of 16-30 years. Out of 303 qPCR positive cases, 207(68.31%) were male, while 96(31.68%) were female patients. Out of 303 positive qPCR patients, 204 were negative for HBeAg while 99 were positive for HBeAg. HBeAg negative patients showed lower infectivity in relation to qPCR, while HBeAg positive patients showed high infectivity. Importantly, the adolescent stage of life is important in spreading and establishing the disease in general public, those having more than 100,000 IU/ml or whose HBeAg test will be positive must go for viral therapy against HBV.

**Keywords:** Hepatitis B virus, Viral load, Molecular diagnosis, Bannu

## Introduction

One of the virus families known as *Hepadnaviridae* has a notorious DNA-containing member called Hepatitis B virus (HBV) [1, 2]. It is estimated by the World Health Organization that HBV infects two billion people around the world, while three hundred seventy-eight million are chronic carriers. However four an half million new are added per year in which quarter develop liver disorder [3, 4]. Previous reports suggested that HBV has overall 2.80% prevalence in the general population of Khyber Pakhtunkhwa [5], while more than 7% only in district Bannu [5, 6]. The prime routs of cause are body fluid exchange (including sexual), congenital, skin prick by contaminated tools and contact to infected ones [7]. It is probably more fetal because of its asymptomatic nature in acute stages, however some patients may develop mild symptoms like pale skin/eye, colored urination, tiredness and gastrointestinal problems which last for few weeks. Asymptomatic infection of HBV in children may results in chronic infections [8]. Till date, serological and molecular diagnostic measurements have been adopted for HBV which evaluate its DNA quantitatively and qualitatively.

The notable markers for the confirmation of infection are antibodies and antigens of antiHBcAg, antiHBsAg, antiHBeAg

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HBeAg and HBsAg in the blood serum [9, 10] The acute infection is characterized by HBsAg surface antigen then immunoglobin M and finally antiHBc (core antigen) antibodies [11, 12]. AntiHBc antibodies may be detected after few days of HBsAg antigen appearance during incubation period. The presence of HBsAg for more than six months indicates chronic infection, while DNA levels of HBV can change and is considered to be a non-significant detection marker for chronic infection. HBeAg is the indication of elevated DNA level of HBV in blood serum and infection [13]. In order to daily serological diagnosis the total antiHBc tests are used for IgM and IgG antibodies [14].

Despite the fact that HBsAg is a common protein, it is still regarded as the hallmark for serological diagnostic tests in HBV infection. As a result, antiHBs would result in false positivity when patients have any HBV contact or simply vaccination without any HBV contact.

Additionally, this surface protein is quite heterogeneous [15]. and an investigative test using multiple-epitope protein from HBsAg would take into consideration all immune-dominant epitopes, which would result in a huge protein.

It is also a fact that the DNA region which codes for HBcAg is a conserved region for various HBV genotypes and can be used for investigative tests [14]. As in early stages of infection, HBeAg may lead to sero-positivty, which is an envelope protein of HBV. The elevated level of HBeAg presence in blood and other fluid of the body indicate rapid replication and viral infection [7].

This research was done with the main objective of determining the consistent association among serological and molecular characterization of hepatitis B virus patients of district Bannu and to determine the correlation between the viral load (HBV-DNA) and viral protein (HBeAg) present in the blood serum of hepatitis B patients.

## Materials and Methods

The 563 blood samples used in this study were collected from suspected HBsAg positive patients during the period from December 2018 to May 2019, from various localities in district Bannu, KP, Pakistan. Six ml of blood were collected from each suspected patient. 500 ul of serum was extracted from each blood sample. The initial confirmation of the infection was investigated by using immune-chromatographic technique (ICT) kits. The ICT confirmed samples were further subjected to the detection of hepatitis B envelop antigen (HbeAg) through ELISA according to the manufacturer protocols.

According to the manufacturer's instructions, DNA was extracted from the 300 ul of serum using the High Pure Viral Nucleic Acid kit. The molecular detection of HBV was carried out by using PCR. In PCR amplification, 2.5 ul of extracted DNA and 0.625µl of each primer were added to AmpliTAq Master Mix in a final volume of 25 ul. The PCR program conditions were 96°C for 5 minutes, 96°C 1 minute, 55.6°C for 45 seconds, 72°C for 1 minute, and a final extension at 72°C for 10 minutes. After PCR amplification, the products were analysed by using a 2% gel. The quantitative measurements of HBV DNA were done by real-time PCR using the Artus Light Cycler HBV DNA Kit (Qiagen, Hilden, Germany) as per kit instructions and the Light Cycler 2.0 instrument Real-Time PCR (Roche, Germany) to detect viral load.

## Results and Discussion

It was attempted to know the relationship between serological and molecular characterization and viral load (HBV-DNA) and viral protein (HBeAg) present in the serum of hepatitis B patients of Bannu district.

To achieve that goal, blood samples were collected from 563 HBV patients belonging to different localities in the district of Bannu. The ages of these patients were in the range of 6-month old infants to 84-year-old people. The serology of these patients was carefully studied through commercially available kits, and further they were molecularly characterized through quantitative PCR (qPCR) for viral load detection.

Among 563 HBV patients, 303 were positive for qPCR because their viral load was more than 200 IU/ml, and 260 patients were negative for qPCR and were undetectable through qPCR because their viral load was less than 200 IU/ml.

The high rate of infectious individuals was found in the age group 16-30, which was 284(50.44%), in which 148(52.11%) cases were positive to qPCR, while the lowest number of cases were recorded from the age group older than 60 years, which was 17(3.01%), in which 8 cases were positive to qPCR **(Table 1)**. The statistical analysis shows a positive relationship among the age groups and infection. Among 563 HBV patients, 368(65.36%) were males, in which 207(56.25%) were positive to PCR, while 195(34.63%) were females, in which 96(49.23%) were positive to qPCR. 303 HBsAg positive patients (Positive to qPCR) were divided into three groups on the basis of viral load (HBV-DNA) i-e < 20,000, >20,000, and >100,000. Among these 303 HBV patients, 124(40.92%) were found to be highly infectious individuals because their viral loads were greater than 100,000 IU/ml, while 115 (37.95%) individuals were found less infectious because their viral loads were less than 20,000 IU/ml **(Table 2)**. Age group and viral load were also found to be statistically positive. Among 303 HBsAg positive patients (positive for qPCR), 204 were HBeAg negative and 99 were HBeAg positive. Among 204 negative patients, the maximum number of cases was 95, recorded in the age group of 16-30 years, while the lowest number of HBeAg negative patients was 05, recorded in the age group exceeding 60 years.

**Table 1. HBsAg positive patients with respect to qPCR**

| Age groups | HBsAg positive | qPCR (IU/ml) | | Chi-square |
|---|---|---|---|---|
| | | >200 | < 200 | |
| 0-15 | 109 | 63 | 46 | P value= 0.037 |
| 16-30 | 284 | 148 | 136 | |
| 31-45 | 91 | 53 | 38 | |
| 46-60 | 62 | 31 | 31 | |

| | | | |
|---|---|---|---|
| > 60 | 17 | 08 | 09 |
| Total | 563 | 303 | 260 |

HBsAg: Hepatitis B Surface Antigen qPCR : Quantitative Polymerase Chain Reaction

**Table 2. HBsAg positive patients (positive to qPCR) with respect to their Age group and viral load**

| Age group | HBsAg +ve | Viral load (HBV-DNA) | | | Chi-square |
|---|---|---|---|---|---|
| | | < 20,000 | > 20,000 <100,000 | > 100,000 | |
| 0-15 | 63 | 17 | 15 | 31 | P-value =0.043 |
| 16-30 | 148 | 58 | 27 | 63 | |
| 31-45 | 53 | 27 | 15 | 11 | |
| 46-60 | 31 | 11 | 06 | 14 | |
| > 60 | 08 | 02 | 01 | 05 | |
| Total | 303 | 115 | 64 | 124 | |

HBsAg: Hepatitis B Surface Antigen qPCR : Quantitative Polymerase Chain Reaction

Here it was found that HBeAg negative patients show low infectivity in relation to qPCR, as out of 204 patients, only 52(25.49%) have >100,000 IU/ml of viral DNA. It has also become clear that young age (16–30 years) is important in the spread and survival of the disease in a community. HBeAg negative patients' and viral load were found to be statistically correlated.

In contrast to HBeAg negative patients, the HBeAg positive patients showed a high infectious ratio in correlation to qPCR. Because it was found that out of 99 HBeAg positive patients, 72(75.7%) had > 100,000 IU/ml of viral DNA.

Here again, the maximum numbers of patients were found with a young age of 16-30 years as 42 (79.2%) out of 53 patients had >100,000 IU/ml viral DNA and it was alarming to our community **(Table 3)**. By applying the chi-square test, HBeAg positive patients and viral load were also found to be statistically positive.

**Table 3. HBeAg negative and positive (positive to qPCR) on the basis of Age with respect to qPCR**

| Age groups | HBeAg -ve | qPCR (IU/ml) | | | Chi-square | HBeAg +ve | qPCR | | | Chi-square |
|---|---|---|---|---|---|---|---|---|---|---|
| | | < 2000 | > 20000 | > 100000 | | | < 2000 | > 20000 | > 100000 | |
| 0-15 | 43 | 17 | 11 | 15 | P-value =0.006 | 20 | 4 | 4 | 12 | P-value =0.039 |
| 16-30 | 95 | 50 | 22 | 23 | | 53 | 9 | 2 | 42 | |
| 31-45 | 40 | 18 | 12 | 10 | | 13 | 2 | 1 | 10 | |
| 46-60 | 21 | 10 | 8 | 3 | | 10 | 4 | 1 | 5 | |
| > 60 | 5 | 1 | 3 | 1 | | 3 | 0 | 0 | 3 | |
| Total | 204 | 96 | 56 | 52 | | 99 | 19 | 8 | 72 | |

HBsAg: Hepatitis B Surface Antigen qPCR : Quantitative Polymerase Chain Reaction

Hepatitis B is the major health problem which affects over 350 million people worldwide [16]. Hepatitis B virus effect liver and causes liver related diseases such as cirrhosis, fibrosis and hepatocellular carcinoma [17, 18].

The report presented by the Pakistan Medical Research Council showed that in the years 2007-2008, the prevalence of HBV in the general population of Pakistan was 2.5% of the population, the greater part of which consisted of the male population [19]. Other studies reported that the prevalence of HBV was 1.3% in Khyber Pakhtunkhwa [20], while 7% in the district of Bannu [4]. AntiHBeAg, antiHBe, antiHBs, HBsAg, antiHBc, IgG, and IgM are widely used as the markers for serological diagnostic tests. The serological markers' identification allows: to recognize HBV infection patients; to explicate the natural route of chronic hepatitis B (CHB); to judge the clinical phases of infection; and to supervise antiviral therapy [21, 22] For identification of infectivity and viral replication, HBeAg and anti-HBe have been used [17]. HBV DNA tells about viral load, which is determined in order to know the viral replication [23].

Here we attempted to study the viral loads of the patients with hepatitis B and their possible co-relation with HBeAg in district Bannu. For this purpose, blood samples were taken from 563 HBsAg positive patients belonging to different localities of the district of Bannu. The ages of these patients were in the range of 06-months old infants to 84-year-old people.

The serology of these patients was carefully studied through commercially available kits and further characterized molecularly through quantitative PCR (qPCR) for the detection of viral load. Among 563HBsAg patients, 303 were positive to qPCR (Viral load >200) while 260 were negative to qPCR (viral load <200), showing that in HBsAg patients (positive to qPCR) the viral load was not detected as viral load below 200 cannot be detected through PCR.

In the current study, patients were divided into five age groups, i-e., 0-15, 16-30, 31-45, 46-60, and older than 60 years of age. Here, it was observed that the infectivity rate of hepatitis B became lower gradually with increasing age. The highest number of cases was reported in the age group of 16-30 years, which was 284, followed by the 0-15 year age group, which was 109. Similarly, 91 in the age group of 31–45 years, 62 in the age group of 46–60 years, while the lowest number of patients was reported in the age group above 60 years, which was 17. The patients in the 16-30 age groups were well exposed to the risk factors and had not developed immunity against hepatitis B. People older than 60 years showed low infectivity because most of them had developed active immunity against hepatitis B.

Out of 563 samples, 368 (65.36%) were males while 195 (34.63%) were females, which showed that there was a higher infectivity rate of hepatitis B in males than in females. It is due to the fact that the males were more closely exposed to the risk factors of hepatitis B than the females, i.e., barber shops, drug

abuse through syringes, and the men working in health care centers, etc. Out of 303 positive samples of qPCR, 207 were males while 96 were females, which also showed the high infectivity rate in males.

On the basis of viral load (HBV DNA), positive patients with hepatitis were also studied. The patients who had a viral load of above 100,000 IU/ml were 40.92%, followed by the patients whose viral load was below 20,000 IU/ml were 37.95%, while 21.12% of cases were recorded of those patients whose viral load was in between 20,000 IU/ml and 100,000 IU/ml. It shows that the viral load increases rapidly when someone gets the infection, which means that the virus replicates itself rapidly within the infected person's cells.

Here, HBeAg patients with respect to qPCR were also studied. HBeAg indicates that either the virus is in an active state or not. If the virus is in an active state, then it is an alarming condition for the patient because it means that the virus is going to damage the liver cells. At this stage, the virus can also be transmitted from one person to another through blood transfusion, organ transplantation, and other risk factors.

In the current study, it was reported that among 303 HBsAg positive patients (positive for qPCR), 204 were HBeAg negative and 99 were HBeAg positive. Among 204 negative patients, the maximum number of cases was 95, recorded in the age group of 16-30 years, while the lowest number of HBeAg negative patients was 05, recorded in the age group exceeding 60 years.

Here it was found that HBeAg negative patients show low infectivity with respect to qPCR because, out of 204 patients, only 52 (25.49%) have >100,000 IU/ml of viral DNA. Here it also become cleared that young age (16-30 years) is important in distribution and widespread of disease in the society.

In comparison to HBeAg negative patients, positive patients with HBeAg showed an elevated transmittable ratio in respect to qPCR. Because it was found that out of 99 patients with HBeAg, 72 (75.7%) have > 100,000 IU/ml of viral DNA.

Here again, the highest numbers of patients were found in the age group of 16-30 years, because here, out of 53 patients, 42 had >100,000 IU/ml viral DNA, and it was alarming for the general public. The key objective of the present study was to find a reliable correlation between viral load (HBV-DNA) and HBeAg protein present in the blood serum of patients with hepatitis B. It was confirmed that HBeAg has a direct correlation with viral load because about 75% of HBeAg positive patients have a viral load of more than 100,000 IU/ml. It means that in HBeAg positive patients, the virus can replicate itself rapidly and damage the liver cells.

## Conclusion

HBeAg negative patients showed low infectivity with respect to qPCR, while HBeAg positive patients showed a high infectious ratio with respect to qPCR. In the current study we We found no correlation between Hepatitis B and season (months) in the current study. It means that hepatitis B can infect anyone at any time and has no seasonality. Moreover, people in the age group of 16-30 years are important in the transmission of disease in society.

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