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Rate of Active Hepatitis B Virus Infection and Correlation of Hbeag and HBV DNA in Hbsag Positive Patients in Three Districts of Khyber Pakhtunkhwa

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Research Article

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ABSTRACT

Introduction and objectives: Hepatitis B along with Hepatitis C infection is the leading cause of hepatocellular carcinoma. Unlike other parts of the world, HBV prevalence and incidence is increasing day by day in Pakistan. People of KPK have very little information about HBV routes of transmission and its consequences. The purpose of this study was to determine the rate of active HBV infection (viremia) and correlation of HBeAg and HBV DNA among confirmed HBsAg positive subjects of KPK and to rule out false positivity associated with Elisa abased HBsAg screening.

Methodology: A total of 171 Blood samples from confirmed HBsAg positive patients were brought to Real time PCR laboratory from three districts, district Bunir, Peshawar and Kohat for confirmation of active HBV infection (viremia). Samples were screened for active HBV infection through Real Time PCR. Along with active HBV infection, among 45 patient's samples, correlation of HBeAg and HBV DNA was also determined in HBsAg positive subjects, using Minividas, FIA technology.

Results: The rate of active HBV infection in three districts, district Bunir, Kohat and Peshawar was 54%, 47%, 37% respectively. Moreover, in 45 patients that has been randomly selected for HBeAg and anti-HBe status in the blood of positive patients. Out of total 45 patients, 13 (29%) patients were HBeAg positive and remaining 32 (71%) were HBeAg negative. Active HBV infection among HBeAg positive and negative patients was 85% and 53%, respectively

Conclusion: It was concluded that rate of active HBV infection was variable among the different districts of KPK population, it was higher in Bunir, followed by in Kohat and least was in Peshawar. Beside this there is strong correlation in HBeAg and active HBV infection.

INTRODUCTION

Liver inflammation, referred to as hepatitis, is caused by different viruses like hepatitis A, B, C, D and E. Hepatitis B is the inflammation caused by hepatitis B virus (HBV). Hepatitis B infection sometime eliminates from the body itself, but if it persists, then it leads to some serious consequences like insufficiency of the liver tissues, cirrhosis and hepatocellular carcinoma (HCC) [1-4]. One of the most common malignant cancer is the HCC and it is increasing by an estimated 5, 60,000 new cases per year and among the men, is considered as the third most common cause of death [5]. Worldwide, hepatitis B is a great health care problem, especially in developing countries. According to an estimate, globally about one third of individuals have been infected with HBV. Hepatitis B infection is also a serious problem in Pakistan, as it has been shown by a community based study that 31% individuals had hepatitis B core antibodies (HBcAb), while hepatitis B surface antigen (HBsAg) has been found in 4.3% of the population [6-8].

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HBV belongs to the family Hepadnaviridae including several genera of the partially double stranded genome of approximately 3.2 Kilo base (KB) length. HBV has high genetic variability due to lack of proofreading ability by viral polymerases [9,10]. On the basis of this genetic variability, HBV has been classified into eight genotypes namely A, B, C, D, E, F, G and H [11-14].

In Khyber Pakhtunkhwa (KPK), one of the provinces of Pakistan, earlier studies have been conducted to find out the prevalence of hepatitis B infection, based on Enzyme Linked Immunosorbent Assay (ELISA), but no study has been carried out to determine active HBV infection among confirmed HBsAg positive patients. The people generally believe in the HBsAg screening test format as they have no idea of active infection, which is very important for the sake of treatment intake. This will exclude the probability of false negatives and false positivity, which is sometime, associated with HBV screening test formats. The aims of this study were to find out, the Rate of active HBV infection in HBsAg positive patients, false positivity associated with HBsAg screening assay and correlation of HBeAg (Hepatitis B envelope antigen), anti-HBe and HBV DNA.

METHODOLOGY

Ethical Approval

This study was approved by the Ethical Committee of the Centre of Biotechnology and Microbiology, University of Peshawar and committee of the Department of Biotechnology, Abdul Wali Khan University, Mardan.

Area Selection

This study was done in Real Time PCR laboratory, dabgarigarden, Peshawar, since January 2016 to December 2016. Three districts were selected in this study and these were, district Bunir, Kohat and Peshawar as no PCR test facilities were available in district Bunir and Kohat, that's why samples were referred to a diagnostic laboratory Peshawar. Blood samples were referred to this laboratory for confirmation of active HBV infection. These samples were referred from different clinical laboratories, hospitals and physician clinics. A total of 80, 51 and 40 samples were referred from district Bunir, Kohat and Peshawar, respectively.

Inclusion Criteria

In this study, only those patients were considered who were positive for HBsAg.

DNA Extraction

DNA was extracted from all HBsAg positive sera by following instructions of the column based extraction kit (Roboscreen Germany).

Polymerase Chain Reaction (PCR)

PCR was performed for each sample using real time PCR (Cepheid, USA) and Roboscreen amplification kit having internal control for each sample in order to rule out false negative results. PCR was performed in the following three steps:

- ***Mater mix preparation***

This was done by mixing the following contents of the amplification kit.

Contents	Volume for 1 sample
2x Reaction mixture (containing dNTPs)	**12.5 ul**
$MgSo_4$	1.5 ul
Primer and probe	1 ul
Taq DNA polymerase	1 ul
PCR grade water	4 ul
DNA template	5 ul
Total Volume	25 ul

- ***Plate set up***

20 ul Reaction mix was loaded in the PCR reaction tube followed by the addition of the template DNA in each tube. Unknown samples along with positive and negative controls were labeled and placed in the heating block of the PCR machine. Through software, samples and control positions were assigned.

- ***Thermal protocols***

The following thermal protocols according to kits requirements were given for the amplification of HBV DNA.

95°C	2 min
95°C	30 s
57°C	1 min (reading)
72°C	30 s Go To 2 for 40 cycles

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- ***Analysis of the results***

The results were analyzed by setting the Ct (Threshold Cycles) values for each sample. The Ct value less than 32 cycles was considered positive and more than 32 were considered as negative. The validation of each positive and negative sample was done with the help of internal controls, already extracted with samples. Sample having high florescence in the FAM channel and low or no florescence in the VIC channel was positive for HBV DNA, while sample having low or no florescence in the FAM channel was negative for HBV DNA but its internal control showing high florescence in the VIC channel and hence confirmed the validity of the result.

- ***Determination of HBeAg and anti-HBe***

A total of 45 samples were taken to determine HBeAg and anti-HBe. This was done by following instruction of the manufacturer (Bio-Murex, Minividas, France). This was based on a device having well for the sample loading. It also has signal reagents and wash buffer. Simply, 200 ul serums was loaded into the well of the device, followed by placing of the device in the block of the machine and then by assigning the test position and protocols as instructed in the manual of the manufacturer, in 30 min results were obtained.

RESULTS

The rate of active HBV infection among HBsAg positive patients in different districts of KPK was as follows:

Rate of HBV Infection In Different Districts

In district Bunir, out of 80 HBsAg positive samples, 43 (54%) were positive for HBV DNA in which 23 (53%) were male and 20 (47%) were female. In district Kohat, out of 51 positive patients, 24 (47%) patients had an active HBV infection. In these 21 (87%) were male and only 3 (12%) were female. While in district Peshawar, 12 out of 40 patients (3%) had active HBV infection in which 10 (83%) were male and only 2 (17%) were female **(Table 1)**.

Table 1. Rate of active HBV infection in different districts of Khyber PakhtunKhwa.

Names of districts	Total number HBsAg positive patients	PCR positive samples	Male	Female
Bunir	22	12 (54.54%)	6	6
Kohat	17	8 (47.08%)	7	1
Peshawar	16	6 (37.5%)	5	1
Total	55	26	18	8

Correlation of HBeAg, Anti-HBe and HBV DNA

Moreover, in 45 patients that has been randomly selected for HBeAg and anti-HBe status in the blood of positive patients. Out of total 45 patients, 13 (29%) patients were HBeAg positive and remaining 32 (71%) were HBeAg negative but anti-HBe positive. Active HBV infection among HBeAg and anti-HBe positive patients was 85% and 53% respectively **(Table 2)**.

Table 2. Correlation of HBeAg, Anti-HBe and PCR.

HBsAg positive samples	HBeAg positive samples	Anti-HBe positive samples	Anti-HBe negative samples	PCR Positive samples
29	10		10	8
		19		4

DISCUSSION

Prevalence of Hepatitis B infection varies among different geographical regions. About 2-3% of the world population has been infected with HBV and is the leading cause of the severe liver diseases like HCC [1]. In Pakistan its prevalence is 3-4% [15] and is contributing more in the mortality each year. Various factors contributing in higher infection rate of HBV. The most important is the lack of awareness regarding source of transmission and screening procedures. Differentiation of active and non-active HBV infection is the most common contradiction nowadays as they have the common serological profile. Patients having HBsAg are mostly diagnosed using PCR based technique and this trend has been emerging during the last decade [16-18]. However, in many countries and regions like western countries, US and other high or middle income countries, ELISA is still the preferred method of diagnosis. It has been shown that some patients who were negative for HBeAg, showed active HBV infection and were having HBV DNA, using PCR method and vice versa [19].

In this study, the rate of active HBV infection was determined among population of the three districts of KPK. We analyzed a total of 171 samples, in which active HBV infection was 46%. District wise infection rate was variable, it was higher in district Bunir (54%) followed by in Kohat (47%) and the least was in Peshawar (37%) **(Figure 1)**. A lot of studies have shown that Pakistan is in the intermediate zone of HBV infection [6-8]. Although, this study merely emphasized on active HBV infection and not the common HBsAg Elisa based screening assays, on the basis of that, most of the studies rely and have determined the prevalence of HBV

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infection. This study was based on the most advanced, appropriate and precise technology, PCR. However the rate was below 50% that revealed the intermediate level of infection rate.

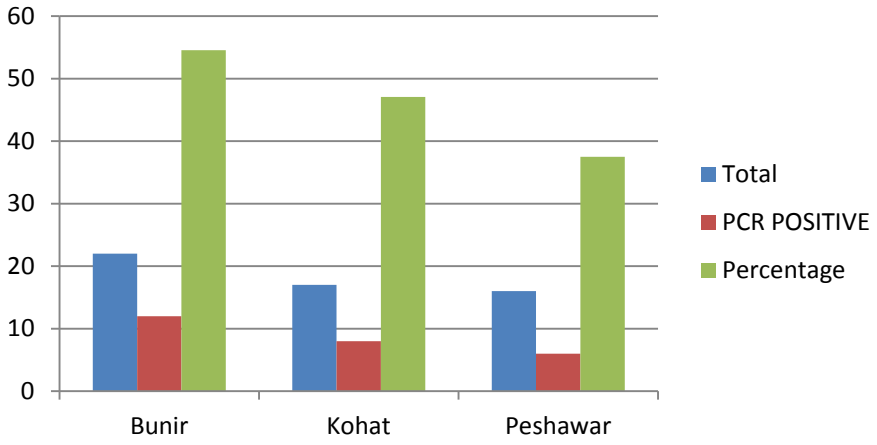

Figure 1. Histogram showing active HBV infection among the three districts of KPK.

In these districts, the HBV infection rate was never determined and we for the first time determined rate of active HBV infection. Infection rate among these was variable, it was high in district Bunir, intermediate in district Kohat and least was in Peshawar **(Table 1)**. The district Bunir is a peripheral district; away from the capital district that's why these people have low awareness regarding routes of transmission and the ways of prevention of infection. As well educated health professionals are lacking in this district and people depends for their treatment on non-health professionals, using the same used syringes for many of the people and are transmitting the hidden infection. The second important reason is the dental practices in the villages that are contributing more in the spreading of this and also other infections among the people of district Bunir. The third reason that contributes more for the active infection is the lack of information regarding proper screening procedures as these patients having HBsAg, but they are not aware of their body health status and therefore when accidently they screen themselves then the infection get exposed.

In district Kohat active HBV infection rate was 47% **(Table 1)** which was lower than Bunir and comparatively higher than Peshawar. District Kohat as compared to Bunir is well developed and people here are well educated. Comparatively, these people have sound information regarding transmission, pathogenesis and prevention of HBV infection. But, still at periphery side within the Kohat, people have fewer resources of information and also different tribes of people have migrated toward the Kohat, these may be contributing in the spreading of HBV infection.

Peshawar is the capital of the KPK; in this the infection rate was 37% **(Table 1)** which was the lowest amongst the three districts. People living here are mostly educated and are well aware of the facts of the transmission routes, consequences, treatment and prevention and that's why the infection rate was very low. However, due to some circumstances people of different castes, languages and regions have been migrated to Peshawar that may be the possible cause of transmission of HBV infection.

HBV infection was comparatively higher in the male population as compared to female **(Table 1)**. This has also been shown by the other studies [20], that males have more chances of HBV infection than the females. The reasons for more infection rate in male might be males have more chances of exposure to different routes of transmission like exposure to barbers, unhygienic foods and water intake while working, and even addiction of drugs by using same syringes.

Moreover, active HBV infection among HBeAg and anti-HBe positive patients was 85% and 53% **(Table 2).** In this study correlation of HBeAg and HBV DNA was 85% **(Table 2).** Some of the studies have a similar relationship while some have a different correlation. Like some studies have shown 100% correlation between HBeAg and HBV DNA [21], while others have shown 80-90% correlation [22]. The difference in correlation might be due to difference in geographical regions, the intensity of the viral particles/ stage of infection or might be due to spontaneous clearance.

Keeping in view the above results it is concluded that patients positive for HBeAg, would have an active HBV infection in most of the cases. Hence, it is strongly suggested that the people unable to have access for the PCR, due to unavailability of the PCR or due to socioeconomic reasons, can screen for HBeAg for the treatment intake.

CONCLUSION

It was concluded that rate of active HBV infection was variable among the different districts of KPK population and persons having HBeAg positive status will have an active HBV infection in most of the cases.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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