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*Full Length Research Paper*

# A study of Hepatitis B virus through RT PCR in Khyber Pakhtunkhwa

***Malala Salam Akram, Shahid Muhammad and Liaquat Faiz Ahmad**

Department of Bioinformatics and Biotechnology International Islamic University, Islamabad, Pakistan.

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**Pakistan is highly endemic for Hepatitis B virus. Many studies have shown that 32% of patients with liver diseases are suffering with hepatitis B. Objective: The present project was designed to check the prevalence of Hepatitis B in those peoples of Khyber Pakhtunkhwa who visited a specific laboratory for diagnosis. The method included a four months data of Hepatitis B positive patients from January 2011 to April 2011, diagnosed through real time polymerase chain reaction (RT PCR). For this purpose a questionnaire was prepared. Tests were conducted and data was collected from Biotech Medical Lab and Research Centre, Islamabad. The collected data was evaluated for prevalence rate, month wise prevalence, age wise prevalence, gender wise prevalence, quantitative tests range and ALT range. A comparison was also made between the results of RT PCR with that of ICT KIT. The prevalence rate was 47.06% which require a special attention. In age wise prevalence, the people more affected were in age group of 21 to 30 years. The prevalence rate was high in male as compared to female. A high number of patients (50.47%) were detected through ICT KIT, when compared to RT PCR. But RT PCR is most authentic and valuable due to its high sensitivity and specificity. The present study will provide reports and recommendations to the concerned quarter and will make awareness of Hepatitis B among patients for its elimination. Moreover, same study should be conducted in large population size to determine the other risk factors.**

**Key words:** Hepatitis B, real time polymerase chain reaction (RT PCR), prevalence, Khyber Pakhtunkhwa.

## INTRODUCTION

Hepatitis B virus (HBV) is a major worldwide cause of chronic hepatitis, cirrhosis, and Hepatocellular carcinoma, accounting for 1 million deaths annually (Alter, 1981). HBV infection is a serious global health problem, with 2 billion people infected worldwide, and 350 million suffering from chronic HBV infection. Of these, 75% are Asians (Alter, 1981; Blumberg, 1977). A condition that evolves towards liver insufficiency and hepatocellular carcinoma in approximately 15 to 40% of cases (Lok, 2002). The global prevalence of chronic HBV infection varies widely, from high (8%, e.g., Africa, Asia and the Western Pacific) to intermediate (2–7%, for example, Southern and Eastern Europe) and low (<2%, for example, Western Europe, North America and Australia) (Willis and Maddrey, 2000). Hepatitis B infection is the 10th leading cause of death worldwide, and results in 500,000 to 1.2 million deaths per year caused by chronic hepatitis, cirrhosis, and Hepatocellular carcinoma (HCC). HCC accounts for 320,000 deaths per year (Lavanchy,

*Corresponding author. E-mail: salam.malala@yahoo.com

2004).

In previous studies immunological assays are mostly found to be used, while present study was based on PCR, which is more authentic and reliable method than immunological assay. Another reason may be that in most of the studies data is collected from blood banks that represents only healthy population. So these results cannot be applied to general population for the very obvious reason. Our study represents a laboratory based population that may show an actual prevalence in Khyber Pakhtunkhwa

The global prevalence of chronic HBV infection varies widely. The prevalence of chronic HBV infection in different areas has been categorized as high (prevalence 8%), intermediate (prevalence 2–7%) or low HBV endemicity (Maddrey, 2000)**.** It has been confirmed by earlier studies that most HBV positive subjects belonged to the rural areas of low economic status (Akbar et al 1997). More than 67.5% of the Pakistani population lives in rural areas (Alam et al., 2007). It has been well documented that HBV infection is more prevalent in these low socioeconomic settings (Akbar et al., 1997).

The present study was undertaken to determine:

1. To evaluate the currently available molecular diagnostic tests for HBV, their clinical applications.
2. To find out the prevalence of HBV infection in the people who visited to the Biotech Medical Lab and Research Centre Islamabad during the months of January-May 2011 referred by physicians or on their own wish.

3. To create awareness in the public about Hepatitis B
4. To provide data to public health department regarding the prevalence of Hepatitis B, so that the proper preventive and treatment programs / projects may be launched.

## MATERIALS AND METHODS

In present study prevalence of Hepatitis B was determined only in those people of Khyber Pakhtunkhwa who visited HMG Lab Islamic International University (Islamabad). Data of four months from January 2011 to April 2011 was collected by using a questionnaire which included information about age of patient, gender, reference of doctor, marital status, socioeconomic status and diagnostic tests etc. Many parameters were studied in present project Like Month wise Prevalence of Hepatitis B, Age wise prevalence of Hepatitis B, Gender wise Hepatitis B prevalence, Comparison of diagnostic tests, quantitative tests range, ALT range and comparison of RT PCR and ICT KIT results.

HBV DNA was detected through PCR RoboGene Hepatitis B virus (HBV) quantification kit was used. Blood (5 ml) was collected from radial vein by using disposable Syringe under strict aseptic conditions and was transferred to Effandorf tube. Serum was isolated from blood samples by keeping the samples bottles in slant position. After coagulation of blood, serum oozed out and was transferred to (10 ml) Effandorf tubes. Serum was stored at -20°C in refrigerator and was used for further analysis.

For Isolation of viral DNA from serum the extraction tube was opened and 200 µl lysis solution TLS was added. 200 µl of the sample was added and 25 µl of proteinase K was mixed vigorously by pulsed vortexing for 10 s and was incubated at 50°C for 15 min. Longer incubation times (if resulting during the preparation of a large quantity of samples) had no effect on the yield or quality of the purified DNA. It was recommended to use a shaking platform (thermo mixer, water bath or another rocking (platform) for a continuous shaking of the sample. Alternatively, sample was vertexed 3 – 4 times during incubation. After lysis the extraction tube was centrifuged shortly to remove condensate from the lid of the tube. 400 µl binding solution DBS was added to the lysed sample, and was mixed by vortexing for 10 s or by pipetting up and down 3 - 5 times. It was important that the sample and the binding solution DBS were mixed vigorously to get a homogeneous solution. The sample was applied to the spin filter located in a 2.0 ml receiver tube. The cap was closed and centrifuged at 10,000 x g (12,000 rpm) for 1 min. The receiver tube was discarded with the filtrate. The spin filter was placed into a new 2.0 ml receiver tube. The spin filter was opened and 500 µl washing solution HS was added, the cap was closed and was centrifuged at 10,000 x g (12,000 rpm) for 1 min. The receiver tube was discarded with the filtrate. The spin filter was placed into a new 2.0 ml receiver tube. The spin filter was opened and 650 µl washing solution MS was added, the cap was closed and centrifuged at 10,000 x g (12,000 rpm) for 1 min. The receiver tube was discarded with the filtrate. The spin filter was placed into a new 2.0 ml receiver tube. The spin filter was then centrifuged at max speed for 2 minutes to remove all traces of ethanol. The 2.0 ml Receiver Tube was discarded. The spin filter was placed into a 1.5 ml elution tube. The cap of the Spin Filter was opened and 60 - 100 µl elution buffer (prewarmed to 50°C) was added and incubated at room temperature for 2 min.

After that it was centrifuged at 6,000 x g (8,000 rpm) for 1 min. The solution was preserved for next process.

For quantification of HBV DNA copy number within the purified sample 5x reagent mix was prepared. 200 µl PCR grade water was added to the vial with lyophilized reagent mix (HBV_D4) and the lid was closed. Incubate the vial at 37°C for 20 min, mix by vortexin g for 3 s and centrifuge for 5 s at full speed. Preparing 1x master mix, PCR grade water (9.6 µl), 10x PCR buffer FS (2.5 µl), HBV_D4, 5x (5.0 µl), 10x ROX (2.5 µl), Taq polymerase FS (5 U/µl) (0.4 µl). It was mixed by vortexing for 3 s and centrifuged for 5 s at full speed. Sample tubes (HBV_D2_AB) and quantitation standards (HBV_D3_AB) were identified carefully and placed them onto a suitable rack (96-well reaction plate). 20 µl 1x master mix was added to sample tubes and each tube of quantitation standard. 5 µl PCR grade water was added to the desired amount of sample tubes which served as NTC and all quantitation standard tubes. 6. 5 µl of eluate was added from DNA isolation (for example, using the INSTANT Virus DNA Kit) to the respective sample tubes. Optical tape (OT_AB) was cut by using the white cutter blade contained in the box 1 according to the required size and sample and quantitation standard strips were covered carefully. Use of an appropriate applicator for fixing the tape at the tube surface of the strips was recommended. Rack was centrifuged with loaded strips at 200x g for 1 min.

The 96-well reaction plate was carefully placed into the ABI PRISM 7000/7300 SDS sample block, the plate was covered with compression pad, the cover was slided over the block and tighten the lid and PCR was switched on by pressing the main switch; subsequently start ABI PRISM SDS software. A new run was started. Active dyes were selected (ROX as passive reference dye was automatically lodged!).Sample positions and names (that is, standards with respective amounts, NTC and samples = “unknown”) were entered and fluorescence reporter dyes (“FA M” and “VIC” - data acquired on the FAM and VIC channels, respectively) and passive fluorescence dye (ROX) were selected. Cycling conditions was entered. File was saved. File name was defined which should contain date, number of run and pathway, go to main menu, select “File” menu, “Save” from the “Fil e” menu. Run

**Table 1.** Hepatitis B prevalence during January, February, March and April 2011.

| S/N | Months | No. of persons who visited HMG Lab Islamic International University Laboratory | No. of patients | Prevalence* |
|---|---|---|---|---|
| 1 | January | 121 | 69 | 0.341 |
| 2 | February | 137 | 61 | 0.302 |
| 3 | March | 128 | 47 | 0.233 |
| 4 | April | 143 | 72 | 0.356 |
| | | 529** | 249 | 1.232 |

*Prevalence was calculated as per 100,000 populations, **total Number of persons visited to HMG Lab Islamic International University (Islamabad) Laboratory from January 2011 to April 2011, ***average prevalence/month.

**Table 2.** Age wise Hepatitis B prevalence from January 2011 to April 2011.

| S/N | Age range (years) | No of patients | *Percentage % |
|---|---|---|---|
| 1 | 0-10 | 18 | 7.2 |
| 2 | 11-20 | 49 | 19.6 |
| 3 | 21-30 | 85 | 34.1 |
| 4 | 31-40 | 54 | 21.6 |
| 5 | 41-50 | 27 | 10.8 |
| 6 | 51-60 | 9 | 3.6 |
| 7 | 60 plus | 7 | 2.8 |

*Percentage was taken out of total number of patients (that is, 249)

was clicked. The process was started .After process completed the results were observed.

## RESULTS

In results it was found that out of total (529) persons only 249 were found to be positive for Hepatitis B. More positive patients were observed during April which was 72 (0.356%).

### Comparison of PCR and ICT KIT positivity ratio

The range of quantitative tests was $3.4\times10^{5}$ copies per dl. The ALT range was high in male (86.71 IU/L) as compared to female (75 IU/L) and was high in age groups of 21-30 and 31-40 years. By comparing results of the present project with other districts of Pakistan, the prevalence in Khyber Pakhtunkhwa was found to be high (47.06%).

## DISCUSSION

In the present study prevalence of Hepatitis B has been determined in those people of Khyber Pakhtunkhwa who visited Biotech Medical Laboratory and Research Center Islamabad during January-April, 2011. Many such studies have been conducted in other districts of Pakistan. But no such study has been conducted in this region.

Total enrolled persons were 529 in which 249 (47.06%) were found to be positive for Hepatitis B during January-April, 2011, (Table 1) which has been found to be high from other studies been conducted in other regions of Pakistan. The present study showed deviation from Arshad et al. (2005) found 8.3% among afghan refugees in Baluchistan, Kakepoto et al. (1996) found 2.28% prevalence of Hepatitis B among blood donor at Agha Khan university Hospital, Aamir et al., 2000 have found that 19% were positive for Hepatitis B in periurban community of Karachi, Anwar et al. (2002) found 23% positive patients for Hepatitis B at Shaikh Zayed hospital and Jinnah hospital Lahore. The main reason for this deviation may be due to difference in diagnostic methods used. (Table 2) Present study age wise prevalence was also determined by making seven different age groups and a comparison was made between these age groups. In which the age group 21-30 years was found to be more infected and age group of 60 plus was found to be less infected in the people visited to selected laboratory, (Table 3) The present finding is in agreement with the study of Nafees et al. (2009) who found that prevalence was high in age group of 20-29 years and was less in age group of 10-29 years. Similarly the study of Saeed (2011) is also in agreement with present study He found that age group of 17-26 was more infected, while study of Zahid et al. (2011) showed deviation from present study who found that 41-60 years age group is more infected and

**Table 3.** Age wise genders distribution of patients.

| S/N | Age range (years) | Female* (%) | Male** (%) |
|---|---|---|---|
| 1 | 0-10 | 9.40 | 5.30 |
| 2 | 11-20 | 13.67 | 25.0 |
| 3 | 21-30 | 30.7 | 37.12 |
| 4 | 31-40 | 28.2 | 15.90 |
| 5 | 41-50 | 10.25 | 11.36 |
| 6 | 51-60 | 3.41 | 3.78 |
| 7 | 60 plus | 4.27 | 1.51 |

*Female are 117 out of 249 (46.98%), **male 132 are out of 249 (53.01%).

100 plus age group is less infected. These variations may be due to different selection criteria and difference in social and host factors, therefore it is difficult to compare our results with their studies. Gender wise prevalence was also included in the present study. In which male were found to be more (53%) infected by HBV as compared to female (47%).The present study is not in agreement with the study of Zobia et al. (2005) conducted a study at Government Health College, Lahore and found that female were more (56%) infected as compared to male (44%). This deviation may be due to less participation of male in their study. While mostly, studies showed agreement with the present project like in the study of Muhammad et al. (2007) male were found to be more (73.92%) infected as compared to female (26.07%). Similarly the finding of Sarwar et al. (2007) is also in agreement, who found that male were more (2.0%) infected as compared to female (1.6%) in students of Lahore Medical And Dental College, Lahore. In the present study, RT PCR and ICT KIT positivity ratio was also compared in the persons who visited Biotech Medical Laboratory and Research Center Islamabad. ICT KIT positivity ratio was found to be 50.47% and positivity ratio of PCR was 47.06%. But PCR is found to be a more accurate method than ICT KIT.

## Conclusion

It has been concluded from the present project that in Khyber Pakhtunkhwa, Pakistan, Hepatitis B is an emerging disease. PCR test was used for the diagnosis of Hepatitis B in persons who visited Biotech Medical Lab and Research Centre, Islamabad. This is a reliable test for detection of HBV DNA. A high prevalence rate was found in subjects that were entertained in present study, there is need to check prevalence in general population of Khyber Pakhtunkhwa to sort out the exact number of patients, also it has been found that male were at high risk as compared to female, the 21-30 age group was highly affected by Hepatitis B virus. The results of the present data were also compared with the data of other districts. Comparison of RT PCR and ICT KIT was also undertaken in the present study, in which positivity ratio of RT PCR was low as compared to ICT KIT.

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