

ORIGINAL ARTICLE

PREVALENCE OF HEPATITIS B VIRUS IN THE EMPLOYEES OF UNIVERSITY OF AGRICULTURE, PESHAWAR

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Objectives: To detect the presence of Hepatitis B virus (HBV) in the employees of the University of Agriculture, Peshawar. **Method:** Two hundred employees (180 males and 20 females) of the University were selected on the given criteria. Blood samples were collected and serum was separated by centrifuging the samples at 5,000 rpm for 8 minutes. The sera were transferred to eppendorf tubes and stored at -20 °C. Samples were initially screened for Hepatitis B surface Antigen (HBsAg) by Immuno Chromatographic Test (ICT). All the serum samples were used for HBV DNA extraction and subsequent two steps Polymerase Chain Reaction (PCR) assay developed at the Institute of Biotechnology and Genetic Engineering (IBGE) for the confirmation of active infection of HBV. PCR products were detected on 2% Agarose gel pre-stained with Ethidium Bromide. **Results:** Results of ICT indicated that 6 (3%) were anti HBsAg hepatitis positive, of which 5 (2.5%) were males and 1 (0.5%) was female. The PCR results confirmed that 4 (2%) were actively infected, of which 3 (1.5%) were male and 1 (0.5%) was female. The results indicated that infection with HBV was more in male than female and was more in the age group of 25–35 years. **Conclusion:** The infection with HBV in the employees of the University of Agriculture, Peshawar was 3% as determined by ICT method and 2%. It was more in male than female and was more in younger age than older age. Screening and vaccination of the employees is recommended.

Keywords: HBV, ICT, PCR, Vaccination, Screening, HBsAg

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INTRODUCTION

Hepatitis B is a viral disease caused by hepatitis B virus (HBV) and is a major health problem in Pakistan and worldwide. The disease may occur with limited or no symptoms, but often leads to jaundice, poor appetite and malaise. It is acute when lasts for less than 6 months and chronic when persists for longer time.¹

To minimize the infection of HBV, it is important to know the causative factors and the exact prevalence of the disease. Protective measures must be adapted to avoid HBV infection. The main spreading factors are coming in contact with infected blood, semen, and vaginal secretions, unprotected sexual contact, sharing needles among injection drug users, re-use of contaminated needles, sharing of razor blades and tooth brushes. Blood transfusion is a risk factor in developing countries.²

In Pakistan, HBV prevalence has been reported as 2–10% among healthy blood donors, 5–9% among health care personnel, 3.6–18.66% among the general population and 3–16% among pregnant women.^{3,4} The Nation has reported 2.4%, 2.5%, 2.4%, 1.3% and 4.3% in Pakistan, Sindh, Punjab, Khyber Pakhtunkhwa, and Baluchistan, respectively.⁵ It is reported that there is 2.8% prevalence of HBV in hospital patients of Pakistan Institute of Medical Sciences, Islamabad.⁶ It is also observed more in males (24%) than females (20.3%). Another study reported 1.8% prevalence of HBV in KPK.⁷ In Bannu, 1.93%

prevalence of HBV is reported.⁸ Most of these data are survey based and not based on laboratory investigations.

There are two valid tests used in laboratories for detection of HBV infection. One is the Immuno-Chromatographic Test (ICT) which is quicker, easy to perform and is less expensive and is used for determining the anti HBsAg positive samples. It is not necessary that all anti HBsAg positive employees will have active infection of HBV. The other one is the PCR method which is expensive, but more accurate and confirmatory method for active infection of HBV.⁹

Keeping in view the havoc created by HBV, this study was carried out to determine the prevalence of HBV infection in the employees of the University of Agriculture Peshawar and to educate them about the spreading factors and control of HBV.

MATERIAL AND METHODS

A total of 200 subjects were included from both genders in age up to 60 year from the employees of University of Agriculture, Peshawar, apparently healthy, and willing to participate in the study. Fifty subjects each were selected from the age group 25–35 years, 36–45 years, 46–55 years, and 56–60 years. Out of these 200 subjects, 180 (90%) were male and 20 (10%) were female.

Five ml blood samples from all the subjects were taken by routine method. The samples were transferred to EDTA vacutainer for biochemical analysis. Sera from all the samples were isolated

through centrifugation at 5,000 rpm for 8 minutes. The resultant serum samples were then transferred to eppendorf tubes and stored at -20 °C till analysis.

The serum samples were screened for anti HBsAg through Immuno-Chromatographic Test (ICT) at the Institute of Biotechnology and Genetic Engineering (IBGE). Screening was carried out using ICT devices (Abbot Laboratories, Chicago, USA.). Ten µL of serum was dropped into a strip well and one or two drops of buffer were added. Appearance of a coloured band was interpreted as anti HBsAg positive.

DNA extractions were carried out through DNA Extraction Kit (Promega, Madison, Wisconsin, USA), following the manufacturer protocol. The extracted DNA of all the samples were checked in the 2 step Polymerase Chain Reaction (PCR) assay developed at IBGE for active infection confirmation of HBV. For increasing the accuracy, positive and negative controls were used.

PCR products were detected on 2% agarose gel pre-stained with Ethidium Bromide at 110 V for 30 minutes. A volume of 10 µL amplified DNA PCR product was subjected to electrophoresis in horizontal gel with Tris-borate buffer (45 mM Tris-borate and 1 mM EDTA). The gel stained with Ethidium Bromide (0.5 µg per ml), was exposed to ultraviolet light to visualize the amplified PCR product and photographed. 1000 base pair (bp) ladders (Gene Ruler, Fermentas) were used as DNA size marker.

RESULTS

The presence of anti HBsAg and HBV active infection as determined by ICT and PCR methods are given in Table-1. Primarily the sera of the 200 registered employees were screened for anti HBsAg by the ICT technique. Out of the 200 employees, 6 (3% of 200) were positive for anti HBsAg. Out of these 6 positive subjects by ICT, 5 (2.5% of 200) were male subjects and one (0.5% of 200) was female.

The sera of the same 200 registered employees were tested by PCR for confirmation of HBV active infection. Now out of the same 200 employees, 4 (2% of 200) were confirmed for HBV active infection. These 4 individuals were those who were already detected as anti HBsAg positive by ICT method. Out of these 4 who were having HBV active infection, 3 (1.5% of 200) were male subjects and one (0.5% of 200) was female subject. The gel photograph of the PCR product is shown in Figure-1

Presence of HBV in various age groups is given in Table-2. The highest prevalence of HBV was observed in the youngest age group (25–35 years) which was 6% by ICT and 4% by PCR. The prevalence of HBV in the age group 46–55 years was higher (4%) than the age group of 36–45 years (2%) by ICT procedure but

was the same (2%) in both the age groups by PCR method. In the oldest age group (56–60 years), HBV was not detected by both ICT and PCR procedures.

Table-1: Presence of HBV determined by ICT and PCR methods

Gender	Employees	ICT Positive	PCR Positive
Male	180	5 (2.5%)	3 (1.5%)
Female	20	1 (0.5%)	1 (0.5%)
Total	200	6 (3.0%)	4 (2.0%)

Percentages are calculated on the basis of total 200 registered employees

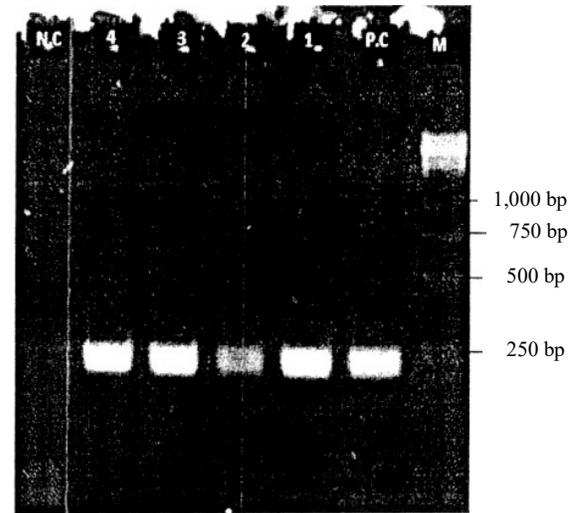


Figure-1: Gel photograph of HBV PCR amplified products

Lane N.C shows Negative Control, lane M is KB marker, Lane 2: 1000 bp DNA Size marker, Lane 1, 2, 3 and 4: Positive samples. Lane P.C: Positive control

Table-2: Prevalence of HBV in age groups

Age Group	Total Employees	ICT Positive	PCR Positive
25–35	50	3 (6%)	2 (4%)
36–45	50	1 (2%)	1 (2%)
46–55	50	2 (4%)	1 (2%)
56–60	50	0 (0%)	0 (0%)

Percentages are calculated on the basis of 50 employees in each age group.

DISCUSSION

The immune-chromatographic test (ICT) is quicker, easy to perform and is less expensive.⁹ This test is for determination of the anti HBsAg positive samples. It is not necessary that all anti HBsAg positive employees will have active infection of HBV.

PCR method is expensive, but more accurate and confirmatory method for active infection of HBV.⁹ That is why when these 200 employees were tested with PCR, only 4 (2%) were actively infected with HBV instead of 6 (3%) by ICT. Our findings, based on both ICT and PCR methods are in fair agreement with the published prevalence data in Pakistan and Khyber Pakhtunkhwa.

Though the prevalence of HBV in the employees of the University of Agriculture, Peshawar is in the reported range of prevalence of HBV in Pakistan and KPK, but we feel that this prevalence is high as the

university employees are more educated people, alert to health care and have all the facilities for health care. We recommend that the administration of the University may distribute a poster containing the risk factors for HBV among the employees. Also yearly check up for hepatitis should be compulsory for the university employees.

There are no reports on the prevalence of hepatitis B infection in the different genders in the university employees in KPK. However, studies performed in some selected populations may still be suitable for comparative purposes¹⁰ reported a positivity ratio of 2.73:1 for male to female ratio which is quite matching with our value of 3:1 male to female ratio. It was reported that male were more frequently infected as compared to the female with a positivity ratio of 2.14:1.¹¹ Higher HBV infection in males as compared to female may be due their being employed outside their homes, visiting barber shops and also their involvement in blood transfusion practices, while women are mostly involved in household activities or confined to their duty place.

Our data shows an age effect on the prevalence of HBV and this is supported by the published work of others. A study indicated significantly higher infection in age group between 21–40 years followed by 41–60 years. Very young and old individuals were less frequently infected by HBV.¹² Another study also reported that prevalence of HBV infection was higher in patients up to the age of 40 years.¹³ The higher HBV infection in the age group 25–35 years in our study may be due to their greater exposures to risk factors and interaction in society as compared to children and aged persons.

CONCLUSION

The infection with HBV in the employees of the University of Agriculture, Peshawar was 3% as determined by ICT method and 2%. It was more in male than female and was more in younger age than

older age. Screening and vaccination of the employees is recommended.

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