# FREQUENCY AND RISK FACTORS OF HEPATITIS B AND HEPATITIS C IN PESHAWAR KHYBER PAKHTUNKHWA

**Muhammad Naoman[1], Muhammad Medrar Hussain[1], Ghadir Ali[1], Muhammad Saqib Ishaq[1], Muhammad Khan[1], Mian M Salman[2], Amir Muhammad[3], Khalid Javed[3], Arshad Parvez[3]**

## ABSTRACT

**Objective:** The Purpose of this research work was to study the Prevalence of Hepatitis B and Hepatitis C and associated risk factors in Peshawar Khyber Pakhtunkhwa.

**Materials and Methods:** A total of 180 individuals were randomly selected and tested for Hepatitis B surface antigen and Hepatitis C antibody on Immunochromatographic technique (ICT) and positive samples were also confirmed on Enzyme Linked Immunosorbent Assay (ELISA).

**Results:** Out of 180 individuals 5 (2.77%) were found positive for Hepatitis B Virus (HBV). These 5 cases had history of surgery (20%), blood transfusion (20%), and unknown reason (60%). The ratio of male to female in the positive cases of HBV was 4:1. While amongst 180 individuals 9 (5%) were positive for Hepatitis C virus (HCV). These 9 cases had different histories of dental treatment (33.3%), traveled abroad (11.1%), surgery (22.2%), blood transfusion (11.1%) and unknown reason (22.2%). The ratio of male to female in the positive cases of HCV was 7.7:2.2.

**Conclusion:** The study concluded that considerable prevalence of Hepatitis B and Hepatitis C was present in Peshawar Khyber Pakhtunkhwa. The ratio of male patients was higher than female in case of both diseases.

**Key Words:** Hepatitis B, Hepatitis C, ELISA, Risk Factors.

## INTRODUCTION

Hepatitis B and hepatitis C has emerged as a major public health issue throughout the world including Pakistan. It has been estimated that there are 350 million people with chronic HBV infection and 170 million people with chronic HCV infection worldwide[1]. Hepatitis B is estimated to result in 563000 deaths and Hepatitis C in 366000 deaths annually[2]. Pakistan is among the worst afflicted nations. Lack of Health Education and information about the safe surgery and dental treatments appear as major risk factors for the transmission of HBV and HCV in our community. Massive health care awareness programs should be arranged for both health care providers and the public to reduce this menace[3].

HBV is a hepadnavirus i.e. attaches to the liver and DNA[4]. It has a circular genome of partially double-stranded DNA. Viruses replicate through RNA intermediate form by reverse transcription[5]. Although replication takes place in the liver, the virus spreads to the blood where viral proteins and antibodies against them are found in infected people. Acute infection with HBV results in symptoms like loss of appetite, nausea, vomiting, body aches, mild fever, and dark urine, and then progresses to development of jaundice. The illness lasts for a few weeks and then gradually improves in most affected people[6]. Chronic infection with hepatitis B virus either may be asymptomatic or may be associated with a chronic inflammation of the liver (chronic hepatitis), leading to cirrhosis over a period of several years[7]. Transmission of hepatitis B virus results from exposure to infectious blood or body fluids containing blood. It includes, blood transfusions, re-use of contaminated needles & syringes, sexual contact and vertical transmission from mother to child during childbirth[8].

HCV is an infectious viral disease that chiefly infects liver. The HCV belongs to Flaviviridae family of viruses. Its genome is single-stranded RNA, enveloped with positive polarity[9]. Genome of HCV was identified in 1989 that was isolated from the plasma of a chimpanzee, experimentally infected with blood borne non-A, non-B Hepatitis virus[10]. Initially the disease is often asymptomatic. However, infection with HCV can lead to chronic liver disease in which scarring of liver (fibrosis) can occur and if it continues to progress then cirrhosis and in some cases hepatocellular carcinoma is the end result that appears after years[11].

The virus spreads through direct contact with an infected person's blood. Previous studies of clinical cases have identified that transfusion of blood products

[1] Department of Microbiology, Faculty of Life Sciences, Abasyn University Peshawar, Pakistan.
[2] Disease Investigation Laboratory Peshawar, Livestock & Dairy Development Department, KPK
[3] Khyber Girls Medical College Peshawar.

**Address for Correspondence:**
**Dr. Muhammad Saqib Ishaq**
Lecturer
Department of Microbiology,
Faculty of Life Sciences
Abasyn University, Peshawar
Mobile: 0332-9116867
E-Mail: saqibishaq87@yahoo.com
saqib.ishaq@abasyn.edu.pk

is the major factors in the transmission of hepatitis C infection. Similarly, homosexuality and intravenous drug abuse are the other sources of HCV infection which are on the rise in Pakistan although recently they contribute minimally to the huge burden of the disease[12]. Other routs of transmission of HCV as evident from a number of epidemiological studies include unsafe injections (reuse of glass syringes or needles), vertical transmission, non-sexual contact in households, face or armpit shaving at community barber shops, ear piercing, tattooing and inadequately sterilized surgical or dental instruments[13,14].

## MATERIALS AND METHODS

This study was conducted in Abaseen Laboratory at Abaseen hospital Dabgari Peshawar from 5th March to 20th April 2012. A total of 180 blood samples were collected from the population of different areas of Peshawar and tested for HBS antigen and HCV antibodies.

The data was collected on a (prescribed) structured Performa. Individuals were evaluated carefully and detailed medical history was taken. 5 ml of blood sample was collected from each patient with a disposable syringe under aseptic conditions and was allowed to clot. Blood was centrifuged at 5000 rpm for 5 minutes and serum was transferred to separate test tubes for further testing. The initial screening was carried out by chromatographic immunoassay for the qualitative detection of Hepatitis B surface antigen (HBS-Ag) and HCV antibodies in serum pr plasma. All the positive samples on ICT were also tested on ELISA for confirmation.

**Hepatitis B surface antigen detection on ICT:**

For Detection of HBS-Ag we used ICT strips. Before proceeding with the assay, all reagents and specimens were brought to room temperature. The test strip was removed from the foil pouch and placed on a clean dry surface, than 100$\mu$l serum sample was dispensed in the strip. The results were interpreted after 15 minutes according to appearance of color bands. Control was also run to check the validity of the strip. Both purplish red test bands and purplish red control band appeared on the membrane of the strip which indicates positive result. One red line appears on the membrane of the strip in the control region (C). Appearance of no red line in the test region indicates negative result[15].

**Hepatitis C antibodies detection on ICT:**

The test strip was removed from the foil pouch and placed on a clean dry surface. 2.5$\mu$l serum sample was dispensed on the sample pad and two drops of buffer were added to it. The results were interpreted after 15 minutes according to the presence of color bands. Control was also run to check the validity of the kit. Both purplish red test band and purplish red control band appeared on the membrane of the strip which indicates positive result. One purplish line appears on the membrane of the strip in the control region (C). Appearance of no red line test region indicates negative result[16].

**HBS Ag detection on ELISA:**

The HBsAg ELISA is a direct immunoenzymatic method in which rarefied anti-HBS monoclonal antibody coated on microplate wells act as the capture antibody and anti- HBS antibodies marked with peroxidase serve as conjugate antibody.

All reagents and specimens were brought to room temperature (18-25°C) before beginning the test assay. Three coated wells of HBs Ag were taken and place the wells in a holder. Reserve one well for negative control, one well for positive control and one well for sample to, then add 50$\mu$l positive control to positive control well add 50$\mu$l negative control to negative control well and add 50$\mu$l of serum to sample well. Then add 50$\mu$l of enzyme conjugate to each of the three wells. The adhesive slip was applied to each well. The wells were incubated at 37°C in incubator for 30 minutes. After incubation, the adhesive slip was removed from wells and washed 5 times. Dispense 50$\mu$l of chromogen solution into each well. Dispense 50$\mu$l of substrate solution into each well. Gently mix for 15 seconds. Incubate the plates at 37°C in dark for 10 minute without shaking. Stop solution of 50 $\mu$l was added to stop the reaction. The absorbance of controls and test specimens was determined within 15 minutes with a spectrophotometer. Positive sample showed blue color before the addition of stop solution. After addition of stop solution it showed yellow color, Negative samples have clear water like appearance before and after addition of stop solution. Specimen with absorbance values equal to or greater than the cut-off value (2.00) were considered HBs Ag positive (reactive) and Specimen with absorbance values less than the cut-off value were considered HBs Ag negative (non-reactive)[17].

**HCV Ab detection on ELISA:**

Multiple epitopes of HCV proteins are bounded to the microtiter plate. When antibodies of HCV are Present in test sample, they react with recombinant proteins and attach to solid phase.

All reagents and specimens were brought to room temperature (18-25°C) before beginning the test assay. Three coated wells of HCV Ab were taken and place the wells in a holder. Reserve one well for negative control, one well for positive control and one well for sample, then add 10$\mu$l positive control to positive control well add 10$\mu$l negative control to negative control well and add 10$\mu$l of serum to sample well then add 100 $\mu$l of sample diluents to each three wells. The adhesive slip was applied to each well. The wells were incubated at 37°C in incubator for 30 minutes. After incubation, the adhesive slip was removed from wells and washed 5 times. Then 100 $\mu$l of Enzyme conjugate was added into each reaction well. The adhesive slip was applied

again to each well. The wells were incubated at 37°C in incubator for 30 minutes. Dispense 50$\mu$l of chromogen solution into each well. Dispense 50$\mu$l of substrate solution into each well. Gently mix for 15 seconds. Incubate the plates at 37°C in dark for 10 minute without shaking. Stop solution of 50 $\mu$l was added to stop the reaction. The absorbance of controls and test specimens was determined within 15 minutes with a spectrophotometer. The blue color turns yellow after reactio n is stopped with sulfuric acid. The amount of color intensity can be measured and it is proportional to the amount of antigen in the samples. Wells containing samples negative Anti-HCV remains colorless. The result was read on spectrophotometer (Strip Reader, America) Specimen with absorbance values equal to or greater than the cut-off value (1.00) was considered Anti-HCV positive (reactive) and specimen with absorbance values less than the cut-off value was considered Anti-HCV negative (non-reactive)[18].

## RESULTS

A total of 180 individuals randomly selected were tested for Hepatitis B and C Viruses, out of 180 individuals 5 (2.77%) were found positive for HBV (Table 1 and Fig 1). These 5 cases had history of surgery (20%), blood transfusion (20%), and unknown reason (60%), (Table 4.3). The ratio of male to female in the positive cases was 4:1 (Table 1).

In 180 individuals 9 (5%) were positive for Hepatitis C (Table 2 and Fig 2). These 9 cases had different histories of dental treatment (33.3%), traveled abroad (11.1%), surgery (22.2%), blood transfusion (11.1%) and unknown reason (22.2%); (Table 4). The ratio of male to female in the positive cases was 7.7:2.2 (Table 2).

**Table 1: Total Number of Hepatitis B Positive Cases and its percentage**

| Parameter | Total Samples | Total Number of Negative Cases | Total Number of Positive Cases | | Positive Percentage |
|---|---|---|---|---|---|
| Hbs-Ag | 180 | 175 | 5 | | 2.77% |
| | | | Male | Female | |
| | | | 4 | 1 | |

**Table 2: Total Number of Hepatitis C Positive Cases and its percentage**

| Parameter | Total Samples | Total Number of Negative Cases | Total Number of Positive Cases | | Positive Percentage |
|---|---|---|---|---|---|
| Anti-HCV | 180 | 171 | 9 | | 5% |
| | | | Male | Female | |
| | | | 7 | 2 | |

**Table 3: Percentage of Different Risks Factors of HBV**

| S. No. | Risk Factors | Number of Positive Cases | Percentage (%) |
|---|---|---|---|
| 01 | Unknown Reason | 3 | 60% |
| 02 | Surgery | 1 | 20% |
| 03 | Blood Transfusion | 1 | 20% |
| 04 | Travel Abroad | 0 | 0% |
| 05 | Dental Treatment | 0 | 0% |

**Table 4: Percentage of Different Risks Factors of HCV**

| S. No. | Risk Factors | Number of Positive Cases | Percentage (%) |
|---|---|---|---|
| 01 | Dental Treatment | 3 | 33.33 |
| 02 | Surgery | 2 | 22.22 |
| 03 | Unknown Reason | 2 | 22.22 |
| 04 | Travel Abroad | 1 | 11.11 |
| 05 | Blood Transfusion | 1 | 11.11 |

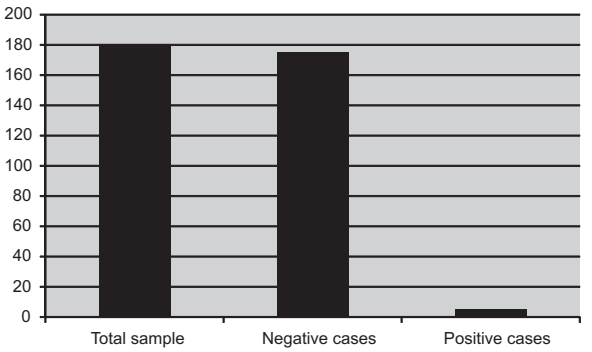

Figure 1: Prevalence of Hepatitis B

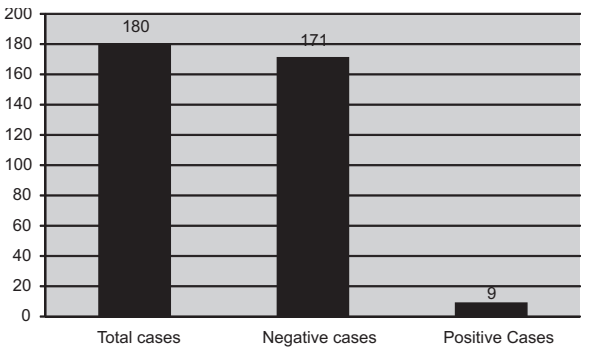

Figure 2: Prevalence of Hepatitis C

## DISCUSSION

In Pakistan the literacy rate is very low due to which the people are unaware of most of the health related problems including HBV and HCV. HBV and HCV in most of the patients is diagnosed when they came to doctors for other problems like surgery, dental treatment etc. so in our study most of the diagnosed positive cases of HBV and HCV were those who were unaware of the disease and came to doctors with other health problems.

The finding of this study was that 2.77% individuals were positive for hepatitis B and 5% for Hepatitis C which was quite similar to the finding of Khokar et al[19] who studied a total of 47,538 individuals. Out of these, 2528 (5.31%) were positive for anti-HCV and 1221 (2.56%) individuals were positive for HBs Ag. 94% individuals were males and 6% were females. The male to female ratio was different from that of my study in which it was 80% in male and 20% in female. The rate of positive cases of HBV was lower and HCV was higher as compared to finding of Khattak et al[17] who studied 103858 blood donors from civilian and armed forces and observed that 3.3% were HBs Ag, 4.0% were anti HCV positive. The reason may be the due to the living standards of peoples of different areas. Finding of this studies was similar with those of Aziz et al[20] conducted a cross sectional survey in which 250 samples of health workers in civil hospital Karachi were studied and observed that prevalence rate was 5-6% for antibodies to HCV and 2.4% for HBs Ag. Ali et al[21] reported incidence of HCV (1.87%) in healthy blood donors from Quetta. The decline in HCV incidence rates might be due the accumulative effect of increasing public awareness of the infection with virus, leading to a decrease in new cases, implementation of stringent donor selection and self deferral by high risk individuals. In contrast to our study, Ali et al[21] observed the high seroprevalence of 6.8% from Karachi in blood donors.

Our study identified the common risk factor associated with HBV in normal population which include history of blood transfusion (20%) surgery (20%) and unknown reasons (60%). Ali et al[22] studied the risk factors associated with HBV and observed that percentage of hepatitis B virus infection in general population was 4.3318% ± 1.644%, in healthy blood donors was 3.93% ± 1.58%, in military recruits was 4.276% ± 1.646%, in healthcare persons was 3.25% ± 1.202%, in pregnant women was 5.872% ± 4.984, in prisoners was 5.75% ± 0.212%, in surgical patients was 7.397% ± 2.012%, in patients with cirrhosis was 28.87% ± 11.90%, in patients with HCV was 22% ± 2.645%, in patients with hepatitis was 15.896% ± 14.824%, in patients with liver diseases was 27.54% ± 6.385%, in multiple transfused patients was 6.223% ± 2.121%, in ophthalmic patients was 3.89% ± 1.004% and users of injectable drugs was 14.95% ± 10.536%.The finding of this study was different in sense that other factors were also identified by Ali et al[22]. The reason was that the most of patient were reluctant in giving the complete history.

Our study indicated the most common identified potential risk exposures that might be the cause of HCV. These included the history of dental treatment (33.33%), traveling abroad (11.11%), surgery in the past (22.22%), blood transfusion (11.11%) and unknown reason (22.22%). There is one donor (11.11%) who had history of blood transfusion. Chowdary et al[23] studied 103 patients of HBV and HCV in which 12 (11.65%) were suffering from HBV and 91 (88.35%) were suffering from HCV, with significantly higher male prevalence. The history of injection was very high in both groups of patients i.e. in HBV (83.3%) and in HCV (86.8%). History of extra marital sex was higher in HBV patients (25%) as compared to HCV (17.6%). The important contributors for different types of hepatitis were blood transfusion (HBV = 16.7%, HCV = 48.4%), surgical procedure (HBV=8.3%, HCV=38.5%) and history of piercing in last six months (HBV=41.5%, HCV=70.3%). History of dental procedure in last 6 month was higher in HCV patients than HBV patients (HCV=45.1% vs. HBV=16.7%).

## CONCLUSION

From the observed findings, it can be concluded that considerable prevalence of Hepatitis B and Hepatitis C was present in Peshawar Khyber Pakhtunkhwa in higher ratio in males than in females population.

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