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Incidence of HBV and HCV in Young Orphan Students of Swat

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Abstract
Hepatitis remains major public health problem in the country, also one of the leading causes of death. The present work was conducted to define the incidence of HBV and HCV infections and related risk factors among young orphan students from different areas of District Swat. A total of 175 samples within the age group of 5-17 years were studied of which 109 were males and 66 females. Initially, samples were examined by immunochromatographic assay (ICT) and were further analyzed by PCR method. 61(34.85%) subjects were found positive for HCV by ICT strip and 31 of these were confirmed by PCR, having 20 male and 11 female. The prevalence rate was recorded 17.71 % for HCV in selected areas. Similarly, for HBV a total of 7 (4%) patients were recorded positive by ICT and 3 (2.75 %) male subject were confirmed by PCR. No female subject was found HBV positive. Total HBV prevalence rate was 1.71 %. Disease frequency revealed increasing configuration and massive health education campaign is needed in related areas of Swat regarding hepatitis B and C viral infections. Vaccination at all health care channels should be planned to limit its spread.
Keywords: Hepatitis, HCV, HBV, Immunochromatographic assay, vaccination, PCR.

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INTRODUCTION

Globally hepatitis is one of the leading causes of death and spread via contaminated blood and related products. The World Health Organization (WHO) recorded 780,000 deaths with chronic hepatitis B virus infection per year and 240 million infected people worldwide (Harris *et al.,* 2017; Kalim *et al.,* 2017). It was also reported that about 3-4 million people are infected each year via hepatitis C virus among 180 million infected people (Khan and Murad, 2015; Kalim *et al.,* 2017). Theses chronic infections lead to hepatocellular carcinoma (HCC), hematologic diseases, hepatic and renal disorders, and cirrhosis (Ali *et al.,* 2015; Cacoub *et al.,* 2017). Number of studies also reported thyroid dysfunction after interferon treatments (Tomer *et al.,* 2007; Andrade *et al.,* 2011). Lead acetate changes the serum AST and ALT levels by affecting the liver (Khanam *et al.,* 2016). Elevation of liver enzymes is responsible for liver disorders (Toor *et al.,* 2016). It was reported that incidence of HCV and HBV infections in Pakistan is 5% and 4.8% respectively and these are the leading cause of hepatocellular carcinoma (Ali *et al.,* 2011; Imran *et al.,* 2014). Another study reported the prevalence rate of Hepatitis C (HCV) in Pakistan from 4.5% to 8% (about 10 million people) (Irfan *et al.,* 2016). HCC cases (87%) are due to these infections in which HBV contributed 22% and HCV 68% (Parkash and Hamid, 2016).

Transmission route of HBV and HCV is mainly through exposure to contaminated blood and blood products like sharing syringes and needles (93%), sexual contacts with infected victim (82%) and alcohol intake (61%) in different regions of Pakistan (Mansha *et al.,* 2017). Vertical transmission was reported the most prominent one among young individuals for HCV (Benova *et al.,* 2014; Cottrell *et al.,* 2013). The risk of vertical transmission in Pakistan was recorded low at <0.1%. However, the estimated infection of HCV was lesser than past record (Gower *et al.,* 2014). The infection rate of HBV in Pakistan is 2-4% defining Pakistan as an intermediate source of infection (Harris *et al.,* 2017). Overall genotype study of HBV in Pakistan indicated 63.7% predominant exposure for genotype D, followed by B (26.7%), and genotype A (4.9%) (Alam *et al.,* 2007; Ali *et al.,* 2011; Mahmood *et al.,* 2016).

Enormous contribution occurs globally in health extortions due to hepatitis B and C infections, especially in

developing countries where public awareness is limited (Lavanchy, 2009; Rantala and van de Laar, 2008). Several studies were carried out in Pakistan to demonstrate the distribution of HBV and HCV in general population with associated risk factors (Ali *et al.,* 2009; Khan *et al.,* 2003). The aim of this study was to estimate the incidence of HCV and HBV infections in young orphan students of District Swat. The specificity and sensitivity of ICT and PCR techniques were elevated to determine the ratios. This study is important due to targeted urban areas and orphan students considerations.

MATERIALS AND METHODS

Sample screening

Volunteer young orphan students of 5 to 17 years age, were studied from different areas of District Swat. Blood samples were collected by using sterile 5ml syringe under strict sterile conditions. The isolated samples were transferred to laboratory and processed to collect the serum and further to screen out the hepatitis B and C by using screening kits (ICT: ACON, ACON Laboratories Inc., San Diego, CA 92121, USA) (Kalim *et al.,* 2017).

PCR confirmation of HCV

Blood samples were processed for isolation of plasma and further analyzed for total RNA isolation using TRIZOL-REAGENT kit (INVITROGEN) following the manufacture standard protocol. The complementary DNA products were obtained by using RT-PCR analysis tools via reverse polymerases (M-MuLV-Moloney Murine Leukemia Virus) and further analyzed for PCR amplification. The product was amplified in two different rounds. In first round two primers were used as forward and reverse having nucleotide sequence of CCCTGTGNNNNNNCTGTCTTCACGC and ACTCGCAAGCNNNNNAGGCAGTAC. In second round of PCR the following forward and reverse primers were used to polymerize the targeted internal region to confirm the presence of HCV: GAAAGCGTNNNNNTGGCG and CACAAGGNNNNNCGACC-3'. 1% agarose gel was used for loading the sample and bands were observed under UV light.

PCR confirmation of HBV

ICT positive samples were further analyzed to isolate DNA by using *DNAzol*® BD following the procedure described by the manufacturer. These DNA samples were amplified twice in two different rounds. The following forward and reverse primers were used in first round amplification: (5'-CATCCTGCTGNNNNCCTCATCT-3' and 5'-CGAACCACTNNNNATGGCACT-3'). The first round product was used as template for the nested PCR based amplification using the following primers: (5'-GGTATGTTGNNNNTGTCCTCT-3' and 5'-GGCACTAGNNNNNGAGCCA-3'). The second round polymerization targets the inner regions of the DNA fragment amplified in the first PCR reaction. 1% agarose gel was used for analysis.

Statistical analysis

The data obtained was analyzed using computer software SPSS (IBM Inc.) and results were tabulated.

RESULTS

Considering the total population size screened for hepatitis, samples were initially tested by using ICT analysis and 61 (34.85%) were calculated positive for HCV antibodies test. Out of these, 42 (68.85%) male samples were recorded positive and 19 (31.14%) samples of female students were positive (Table 1). To confirm these positive samples were further subjected to PCR analysis (Figure 1) to make sure the positive clones for hepatitis C related RNA and found that 31 individuals (71.71%) were confirmed positive for hepatitis C in the serum. The prevalence ratio was conducted and 20 (18.34%) individuals were males and 11 (16.67%) were recorded females. 38.53% individuals among male students with total percentile incidence of 18.34% as shown in Table 2. By ICT analysis, 28.78% were found positive for hepatitis C viral antibodies detection and later on analyzed by using PCR detection including 16.67% was set positive for HCV. The HCV prevalence was recorded 17.71% in young orphans of District Swat which is alarming for all health professionals.

Table 1. Hepatitis positive patients among total screened samples

Sample	Male	Female	HBV (+)	HCV (+)
175	109	66	7 (4%)	61 (34.8%)

Table 2. Gender wise prevalence of HCV

M/F	Samples	ICT (+)	PCR (+)
Male	109	42 (38.5%)	20 (18.34%)
Female	66	19(28.78%)	11 (16.67%)
Total	175	61 (34.85%)	31 (17.71%)

Incidence of hepatitis B in total population size screened was recorded less than HCV. Total samples were initially screened via ICT test and only 7 (4.0%) individual were found positive for hepatitis B virus. These positive samples were comprised of 6 male students and only one female individual. By confirmation with polymerase chain reaction (Figure 2), we found three male individuals confirmed positive with prevalence ratio of 2.75% and no confirmed results were recorded for female students.

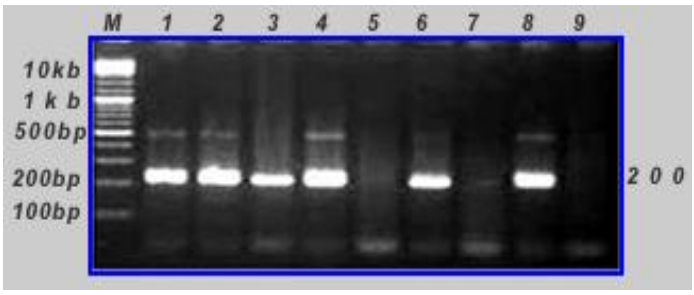

Fig. 1. Detection of HCV through PCR. 2% agarose gel was used for it.
Lane M indicates 1kb plus 100bp DNA ladder marker vol. 250 µl (83 µg) cat# EMB 1003C: Lanes 1-4, 6, 8 show positive results of HCV; Lanes 5, 7, 9 are negative for HCV.

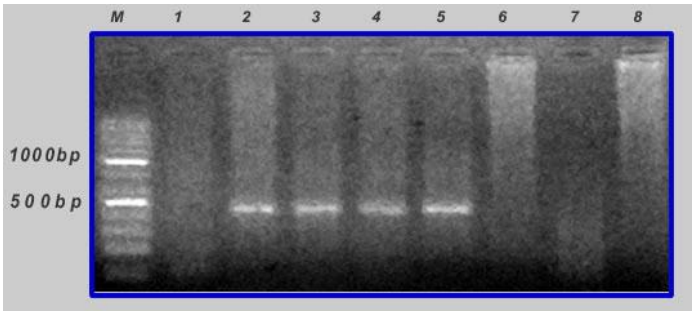

Fig. 2. PCR confirmation of HBV.
Lanes 2-5 show positive results; lane M indicate molecular marker. 1% agarose gel was used for differentiation

The one individual was counted as false positive in initial screening (Table 3). The resulted final prevalence rate for HBV was recoded 1.71% among all tested samples.

Table 3. Gender wise prevalence of HBV

M/F	Total	ICT (+)	PCR (+)
Male	109	6 (5.5%)	3 (2.75%)
Female	66	1 (1.51%)	0 (0%)
Total	175	7 (4%)	3 (1.71%)

DISCUSSION

District Swat of Malakand is located in Khyber Pakhtunkhwa with total population of 1257602 (according to 1998 censuses, Directorate of Information Technology, Government of KP). Our study of prevalence and incidence of HBV and HCV infections was focused on young orphan students of different age groups. All these tested individuals belong to rural areas of Swat where the chances of rapid growth of hepatitis are prominent due to lack of proper treatment and hepatitis awareness and precautions. In studied individuals 4.0% were found to have antibodies in their blood against HBV and 34.85% against HCV related viruses. It was reported that HCV prevalence in Swat is 13.8% with positivity of 4.7% (Ahmad *et al.,* 2009). In another study, 30 (10.71%) patients were positive for Anti HCV, 27 (9.64%) for HBs Ag and no record were found in individuals having both conditions (Kalim *et al.,* 2017). The PCR analysis proved only 1.71% prevalence rate for HBV and 17.71% for HCV from the blood serum.

It was also reported that none of the screened sample hold both hepatitis B and C co-infections. Baig et al. (2009) reported highest frequency of super infection of hepatitis C and D in HBV cirrhosis patients in Pakistan. A previous study reported the prevalence rate of HBV was 0.96% and that of HCV was 7.5% in studied population whose age ranges from 7 years to 70 years (Rauf *et al.,* 2013). Some results of this research do not match with the existing results of different areas of Pakistan. In our study the prevalence of HBV was 1.71% and that of HCV was 17.71%. The results of another study are quite different to current work as reported by Sarwar and co-workers from different area of Abbotabad region (Sarwar *et al.,* 2008). They found co infection of both HBV and HCV which was recorded 40% as in our work there was no co infection recorded. The reported study also coincides with our prevalence analysis that showed higher recorded prevalence rates in male individual as compared to females (Muhammad and Jan, 2005). Similarly the percentage of co-infection HBV with diabetes was reported to be 12.80% (Muhammad *et al.,* 2013). In another study rate of HBV infection was 25% among male malarial patients while in females rate was 23% (Dilshad *et al.,* 2016).

CONCLUSION AND RECOMMENDATIONS

Pakistan is being considered as high prevalence and risk related country of hepatitis infections. The frequency distribution of HBV and HCV showed an alarming pattern of threats that acquires a special attention. Both electronic and printing media related health education campaigns are needed in rural areas of Pakistan with hepatitis and co-infection. Proper arrangement of vaccination must be required and abundance of contaminated needles and blood transfusion ailments need to be eliminated to eradicate hepatitis. The most prominent weapon for hepatitis spread was found the recycling of these sources in different health workplaces. The local medical staffs must be proper trained and well equipped to deliver the proper treatments and also aware the victim against the threats. As in rural areas the surgical tools are not properly sterilized and freely used careless of infection so these must be eliminated. At last but not the least, to avoid use of unsafe blood and blood product transfusion, share blades, and unsterilized instruments in barber shops. It is suggested that all the patients who need surgery or any other treatments from doctors they should be properly screened for HBV and HCV. It is also necessary

that separate operation theaters and instruments should be used for HBV and HCV positive cases. Tremendous efforts are needed in the form of print and electronic media to achieve reduction of these infections especially HCV and HBV.

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CONFLICT OF INTEREST

There is no conflict of interest.

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