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Incidence of Anti HbsAg and HCV in the Young Orphans of District Nowshera, Khyber Pakhtunkhwa, Pakistan

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Abstract

This study was conducted to determine the prevalence of HCV, hepatitis B and associated risk factors among the orphans aged 5-17 years of district Nowshera, Khyber Pakhtunkhwa, Pakistan. A total of 93 individuals were screened for HCV and HBV, among them, 73 were males and 20 females. Initially, all the samples were diagnosed for the HBsAg and HCV virus through immune-chromatographic test (ICT) and were further confirmed positive through polymerase chain reaction (PCR). After analysis, 7 individuals were found positive through ICT device, of which 3 were females and 4 were male individuals, but the PCR results were negative for all 7 samples. Similarly, for HBV, a total of 3 (3.22%) patients were recorded positive by ICT and 2 (66.67 %) were male and 1 (33.34%) was female. Among them, a single case was positive on polymerase chain reaction. Disease frequency showed a growing pattern that need awareness of all concerned. A considerable health education campaign and vaccination at health care should be planned to limit its spread.

Keywords: Hepatitis, HCV, HBV, Immuno-chromatographic, PCR, prevalence, virus.



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INTRODUCTION

Hepatitis is one of the main severe diseases worldwide which is usually transmitted through blood and sexual transmission (Walter *et al.*, 2005). Recently, 2 billion peoples were affected by HBS and 350 million people were affected by HCV. Among these cases, 170 million people were chronically infected whereas 3-4 million people are affecting every year (Merican *et al.*, 2000). With the passage of time, a large number of patients goes in HCC (Hepatocellular Carcinoma) and cirrhosis (Furusyo *et al.*, 2002).

The control of HBV and HCV infections is the main challenge and goal to the world health organization (WHO) in the developing countries. The illiteracy rate, lack of awareness and less knowledge about these diseases in the developing countries are the main causes of these diseases (Rantala and Ven De Laar, 2008; Shepard *et al.*, 2005; Lavanchy, 2009). In Pakistan, valuable research work was carried out on Hepatitis B and C in the last 20 years. Unfortunately, these research work were only limited to the clinical data of hospitals and blood banks which could not establish fruitful goals to prevention measures and treatment of the stated diseases in Pakistan (Ali *et al.*, 2009; Khan and Rizvi, 2003).

The present ratio of hepatitis B and C infections in Pakistan is very high and in an alarming situation in Pakistani populations. Different research and surveys indicate that the prevalence %age of HBV and HCV in Pakistan is approximately 3-4% and 5-6% respectively. Nearly 12 million people are affected by HBV and HCV in Pakistan (Raja and Janjua, 2008). Among them, 60-70% of patients were affected by CLD (chronic liver disease). According to province wise data of Pakistan, the HCV, 5% in Sindh and 1% in KP (Ahmad *et al.*, 2009; Khan *et al.*, 2007) whereas the HBV prevalence ratio is 6.7% in Punjab, 4.3% in Baluchistan, 2.4% in Sindh and 1.3% in KP respectively (Ahmad *et al.*, 2009).

The main objectives of our work were to screen out the healthy young orphans of district Nowshera KP for HCV and HBV and further confirmation of these diseases by PCR and ICT and the comparison of them with world reported data.

MATERIALS AND METHODS

Sample Screening

The blood was collected from the young healthy orphans of district Nowshera. The collected blood was further processed to separate the serum and to screen out the anti-HBsAg and anti HCV by using screening kits (ICT: ACON, ACON Laboratories Inc., San Diego, CA 92121, USA).

PCR Amplification of HBV

The positive samples were further processed to isolate DNA through the manufacturer standard protocol of DNAzol® BD. The forward and reverse primers were used in first round amplification: (5'-CATCCTGCTGNNNNCCTCATCT-3' and 5'CGAACCACTNNNNATGGCACT-3') respectively. The 1st round product was used as a template for the nested PCR based amplification by using the following primers: (5'GGTATGTTGNNNNNTGCCTCT-3' and 5'GGCACTAGNNNNNGAGCCA-3') (Kalim *et al.*, 2017). The PCR bands were seen on 1% agarose gel.

PCR Confirmation of HCV

RNA isolation from ICT positive samples was done with the standard protocol of TRIZOL-REAGENT kit (Invitrogen). Using sequence-specific reverse primer and reverse transcriptase polymerase (M-MuLV-Moloney Murine Leukemia Virus) the cDNA was generated from RNA for further PCR amplification. cDNA was amplified in two round. Two primers forward and reveres having following nucleotide sequence were used in the 1st round of PCR CCCTGTGNNNNNCTGTCTCACGC and ACTCGCAAGCNNNNNAGGCAGTAC. In the 2nd round of PCR, these forward and reverse primer were used to confirm the presence of HCV: GAAAGCGTNNNNNTGGCG and CACAAGGNNNNNCGACC-3'. (Kalim *et al.*, 2017). After PCR 10µl PCR products were loaded on 1% agarose gel and bands were observed.

Statistical analysis

One way analysis of variance by using GraphPad Prism 5 (GraphPad Software; Inc: La Jolla, CA) software and Microsoft Excel was used to evaluate frequencies and incidence of HBV and HCV. The significance level was set at $p \leq 0.05$.

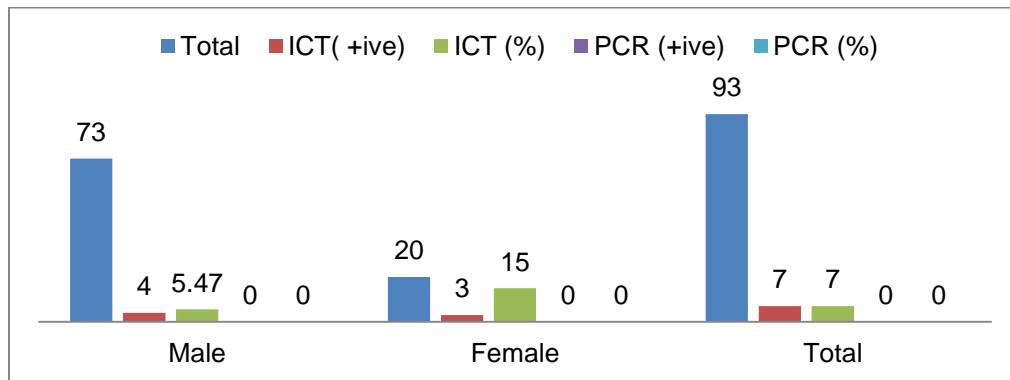
RESULTS AND DISCUSSION

HCV Prevalence

The 93 healthy orphans were checked for HCV viral infection. Among them, 7(7.52%) samples were reported positive for HCV antibodies on ICT test in which 4 (57.14%) were males and 3(42.85%) were females as shown in Table 1. The Samples were further analyzed for confirming the HCV virus on nested PCR method. Among the total 7 ICT positive samples, no viral RNA was found in the serum. The tabular and graphical data of these samples were shown in Table 1 and figure (1).

Table 1. Gender wise Prevalence of HCV

M/F	Total	ICT(+ive)	ICT (%)	PCR (+ive)	PCR (%)
Male	73	4	5.47	0	0
Female	20	3	15	0	0
Total	93	7	7.5	0	0


Fig.1. Graphical presentation of hepatitis C virus prevalence.

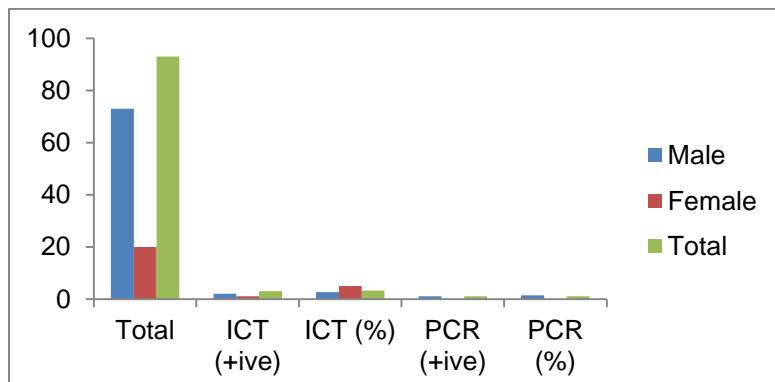
HBV Prevalence

The 93 healthy young orphans aged 5-17 years were checked for HBV viral infection. Among them, the 3 (3.22%) samples were positive for hepatitis B infections. The samples were analyzed through ICT method. In the initial screening of the ICT test, only 1 female sample was found positive and was further confirmed negative when analyzed through PCR. Among the males, 2 (66.67%) were found positive for hepatitis B virus through ICT test

whereas on PCR only 1 person was reported positive for HBV. Among these samples, the 3 samples were reported positive through ICT test were males with prevalence percentage of 66.67% and 33.34% were females. On the other hand, the prevalence %age of HBV viral infection was 1.17% as a whole. After PCR analysis. Table 2, figure 2, figure 3 and figure 4 show the analyzed data of the concerned samples.

Table 2. Prevalence of HBV

M/F	Total	ICT (+ive)	ICT (%)	PCR (+ive)	PCR (%)
Male	73	2	2.7	1	1.36
Female	20	1	5	0	0.0
Total	93	3	3.22	1	1.07


Fig. 2. Graphical presentation of hepatitis B virus prevalence.

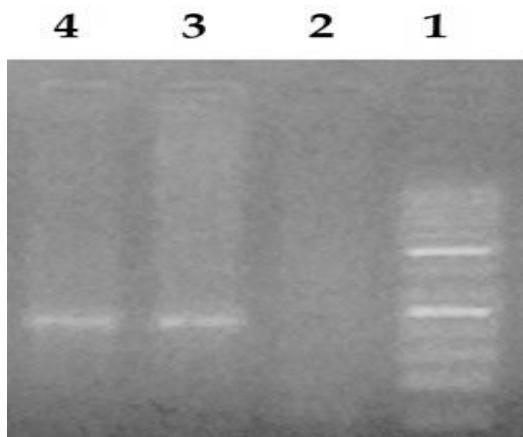


Fig. 3. 1st Round PCR gel Picture lane No indicate 100bp marker, lane 2: negative sample, lane 3: positive sample, lane 4: positive Control.

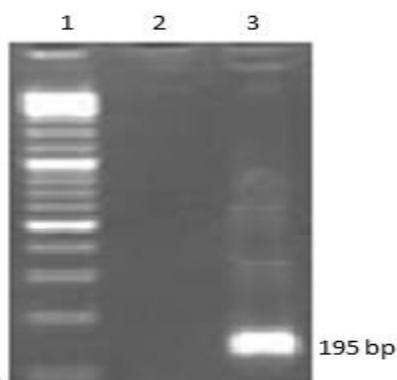


Fig.4. 2nd Round PCR gel Picture lane No, 1 indicate DNA marker, lane 2: negative sample, lane 3: positive sample.

DISCUSSION

The study showed that both spinosad and flubex have high efficacy against the larval stage and adult emergence of *Ae.aegypti* mosquitoes.

HCV and HBV viruses are the most causative agents of viral infections worldwide including Pakistan. Hepatitis virus directly attacks the human liver and cause infections. A lot of research studies were carried out on the HBV and HCV in Pakistan. Present research study and was carried out to calculate the prevalence of HBV and HCV in the young orphans of district Nowshera KP.

This study shows the prevalence ratio of active viral infections in the young healthy orphan population of District Nowshera. The total 93 healthy orphans aged 5-17 years were diagnosed for active viral infections of HBV virus and

HCV virus. Among the samples 73 were males and 20 were females. Our study shows that (22%) individuals were positive for HBV and 7.52% were reported positive for HCV infection. A similar finding was also reported by (Meri *et al.*, 2001), (Costa *et al.*, 2009), (Alam & Ahmed, 2009).

Similarly, a single hepatitis B positive case was detected through PCR whereas, for HCV infection, the PCR result was negative for all of them. Our research study was to diagnose the HBV and HCV infections in the young orphans of district Nowshera which is new research for the first time in Nowshera orphan population. Different studies were reported different result carried (Ali *et al.*, 2009) Raja & Janjua, 2008), (Khan & Rizvi, 2003), (Khan *et al.*, 2007) and (Farooqi, 2000) show that during last several years different epidemiological, diverse methodologies, different time frames, mostly work on high affected and hospitalized patient shows the high prevalence rate in Pakistan ranked it in the list of HBV and HCV infections endemic countries. Lack of awareness, low literacy rate and lack of sufficient information about these diseases increases the spread of viral infections in Pakistan. (Khokhar *et al.*, 2004) (Ali *et al.*, 2002) (Kakepoto *et al.*, 1996).

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CONCLUSION

The high incidence and prevalence of viral infections especially HBC and HCV needed the attention of all concerned. The health education promotions throughout district Nowshera about viral infection of hepatitis B and Hepatitis C should be practiced, as well as vaccination at all health department should be organized to limit its spread.

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CONFLICT OF INTEREST

The authors declare that no there is no conflict of interest for this study.

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