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Detection of HBV and HCV by ICT and ELISA Method in Different Areas of District Malakand

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Abstract
Hepatitis B and hepatitis C virus are two major public health problems in the country. The purpose of this study was to compare the prevalence of hepatitis B and hepatitis C in different areas of District Malakand. A total of 280 individuals were bled. Primary screenings were performed by using ICT SD Strip for anti HCV and anti-HBsAg. The final diagnosis of hepatitis was done using Enzyme linked immunosorbent assay (ELISA) kit. Anti HCV was found positive in 30 (10.71%), HBs Ag was positive in 27 (9.64%) and no record were found in individuals having both conditions. This study showed a similar prevalence of HCV and HBV infection in blood donors. The prevalence of hepatitis B and C virus can be minimized by the screening of all donors for anti-HCV and HBs Ag and discouraging the use of unsterilized syringes.
Keywords: Hepatitis, HBV, HCV, Anti-HCV and HBsAg, Enzyme linked immunosorbent assay.

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INTRODUCTION

Hepatitis B virus (HBC) and Hepatitis C virus (HCV) are the dynamic and projecting source of morbidity and mortality worldwide especially in developing countries like Pakistan (Shah and Shabbir, 2002). Hepatitis B virus was first isolated and confirmed in 1963 and infected over 2 billion people around the globe (Cusheri, 2002). This virus is vital source of cirrhosis, HCC, and hepatitis (Abdolsamadi, 2009), and was reported by World Health Organization (WHO) that almost 3% of all world population was infected by this minute agent (Fisman *et al.,* 2002). Approximately 780,000 deaths were recorded by WHO among 240 million chronic HBV individuals (WHO, 2015). According to WHO reports, about 180 million people are infected by hepatitis C virus in world and 3-4 million people are infected every year. Prevalence rate of Hepatitis C (HCV) in Pakistan is from 4.5% to 8 % (about 10 million people), that is second highest in the world (Irfan *et al.,* 2016). First serological marker for hepatitis B virus was first revealed by Blumberg and colleagues in 1963 (Blumberg, 1977) and that of hepatitis C virus were cloned in 1989 (Choo *et al.,* 1989; Kuo *et al.,* 1989). About 6-8% patients of chronic condition of HCV develop HCC. Hepatitis B virus (HBV) and hepatitis C virus (HCV) are risk factor for Hepatocellular Carcinoma (HCC) and cirrhosis. Chronic infection of HCV leads to extra-hepatic disorders, hepatocellular carcinoma, renal disorders, hematologic diseases and cirrhosis (Ali *et al.,* 2015). It is reported that elevation of liver enzymes responsible for liver disorders (Toor *et al.,* 2016). These complications also develop in IFN-α cured patient. Number of studies also reported thyroid dysfunction after interferon treatments (Tomer *et al.,* 2007; Andrade *et al.,* 2011). Lead acetate alters the serum AST and ALT levels by affecting the liver (Khanam *et al.,* 2016). Blood transfusions, unsterilized surgical instruments, outdoor barber shops, untrained medical instructors, unsafe sexual contacts and vertical transmission are regarded as the chief routs of transmission. Vertical transmission was reported the most prominent route of

transmission of hepatitis C viral infection Worldwide (Cottrell *et al.,* 2013; Benova *et al.,* 2014). The risk of vertical transmission was recorded at <0.1% in Pakistan (UNAIDS, 2016).

Occurrence of hepatitis C virus infection has been increased in Pakistan showing 60 to 70 percent HCV positive cases (Jafri *et al.,* 2006), and was recorded second after Egypt in HCV with total infection of 10 million people (Waheed, 2015). Genotype 3a and 2 are mostly reported in Pakistan (Umer and Iqbal, 2016; Khan *et al.,* 2014).

This study was directed to evaluate the prevalence of hepatitis B and C virus in general people of District Malakand, Khyber Pakhtunkhwa, and to investigate the common risk factors.

MATERIALS AND METHODS

Collection of blood samples

Blood samples were collected from all the tested individuals by authorized technician, kept in heparanized tubes and transported to laboratory in the insulated ice boxes. Samples were centrifuged at 4000 rpm for 10 min to isolate the plasma and stored at -20°C.

ICT for HBV and HCV

The samples were screened primarily by using ICT devices to separate out the positive samples of hepatitis B and C virus. Three full drops were loaded under strict sterile condition in to screening device wells and reaction color was produced due to coated antigens. Two lines indicated positive results as shown in figure 1. Single line showed negative results.

Fig. 1. ICT SD Strip Method

Enzyme linked immunosorbent assay (ELISA) for hepatitis B

ICT positive samples of HBV were further analyzed and confirmed by using HBsAg kit (Equipar, Italy) based on one-step “Sandwich” ELISA. This device uses two separate antibodies directed to two different epitopes. One absorbed onto walls of plate while another one labeled to horseradish peroxidase (HRP) enzymes. Both samples and conjugate were loaded at the same time to micro-plate and incubated at 37°C. Second incubation was done with chromogen which detects the specific immune-complex on the surface of solid phase. Enzyme generated color intensity was proportional to its antigen in isolated blood samples which was further analyzed by ELISA reader.

Enzyme linked immunosorbent assay (ELISA) for hepatitis C

The hepatitis C kit (Equipar, Italy) contained the micro plate that was coated with specific recombinant protein analogues of antigens of hepatitis C virus. The plate was first treated with liquid sample then with horseradish labeled monoclonal antibody anti-H-IgG, and further incubated to generate color. Enzymatic activity that creates color indication was proportional to hepatitis C antibodies present in tested samples. The result was evaluated by ELISA reader.

RESULTS AND DISCUSSION

In this study, a total of 280 patients were screened for hepatitis B and C virus. Total of 144 (51.42%) samples were recorded as male patient and 136 (48.57%) were females of different age groups ranging from 13 to 85 years as shown in figure 2. All these patients belonged to rural areas of District Malakand. Total of 57 patients were found positive for both hepatitis B and C viruses. Out of these positive, 27 (9.64%) were recorded hepatitis B positive and 30 (10.71%) for hepatitis C. Total occurrence of male HBV positive were recorded 14 (5.0%) and 13 (4.64%) were female patients. 17 (6.07%) were HCV positive male while 13 (4.6%) were female patients. Both hepatitis B and C were found in 57 (20.35%) patients. Amongst them 31 (11.07%) were male and 26 (9.28%) were females (Figure 3).

HBV and HCV achieved an endemic situation in many countries of the world, especially in underdeveloped countries. In Pakistan, it has been recorded an alarming threat especially in rural areas of country, as can be perceived from our results too. A large proportion of population is already affected with HBV and HCV with prevalence rate of 10% for HBV and 4-7% for HCV. While in rural areas the percentage has been recorded significantly higher, than quoted one (Malik *et al.,* 1999).

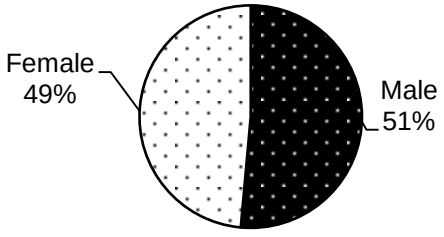

Fig. 2. Sex wise distribution among 280 patients screened for hepatitis.

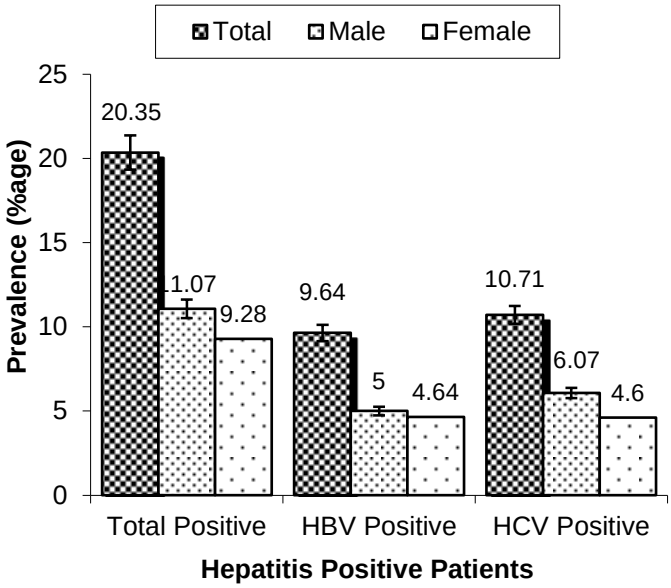

Fig. 3. Prevalence of Hepatitis in different areas of Malakand

The major route of this transmission was found due to blood transfusion and the use of unsterilized syringes or instruments especially dental instruments, surgical and un-screened blood transfusion (Todorova *et al.,* 2015). Other factors involved in the spread of infection are persons who have their armpits or face shaved by street barber or those involved in sexual abuse (Luby, 1997; Khwaja *et al.,* 2002; Thornburn *et al.,* 2003). HCV percentage prevalence in the adult population was 11.55%. HCV genotype 3a prevalence was found to be 63.45%, the highest of all genotypes. The percentage prevalence of HCV found for all of the provinces was Punjab: 5.46%, Sindh: 2.55%, Khyber Pakhtoonkhaw: 6.07%, Balochistan: 25.77%, and federally administrated tribal areas: 3.37% (Arshad and Ashfaq, 2017). Similarly the percentage of co-infection HBV with diabetes was reported to be 12.80% (Muhammad *et al.,* 2013). In another study the prevalence of HBV was found in the 46-60 years age group for malarial patients. Among male patients, rate of infection was 25% while in females rate was 23% (Dilshad *et al.,* 2016).

CONCLUSION

The study concluded that Hepatitis B virus (HBC) and Hepatitis C virus (HCV) are major cause of hepatitis and liver cirrhosis and achieved an alarming situation in Pakistan especially in those areas which lack education and awareness. The use of unsterilized surgical tools, unscreened blood transfusion procedure, unqualified medical trainees medication, unsafe barber shops and reuse of razors and shaving tools shows full efficiency in the transmission of these viruses. Our study contributes to identify and explore these sources and point the occurrence and spread of the disease in less educated areas of Pakistan. This study also provides a clue to high authorities to control the spread of these threats.

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CONFLICT OF INTEREST

The authors verify having no interest in competition and have no conflicts of interest.

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