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Prevalence of Hepatitis B and Hepatitis C Viruses in Human Urban Population of Bahawalpur District, Pakistan

Rifat-uz-Zaman

The present study examines the prevalence of hepatitis (B and C) infection in population of the urban areas of Bahawalpur district. The study population comprised of 6815 peoples (3924 male, 2891 female) of different age group and professions. The subjects were selected randomly and had blood samples taken. Rapid immunochromatographic tests were conducted to detect hepatitis B and C infections. The prevalence of diseases was: hepatitis B, 9.59% and hepatitis C, 4.41% indicating the spread of infections on rise in Pakistan. The results of the study illustrate the importance of education of the society including health care individuals to observe the protective measures against hepatic viral infections and of maintaining the health care measures.

Key words: Hepatitis B, hepatitis C, blood transfusions, virus, sexual health, population

INTRODUCTION

It has been indicated that hepatitis or inflammation of liver can be caused by different factors e.g., viruses, bacteria, protozoa, drugs/chemicals, surgery, pollution and infected food as well as drinking water etc. The viral hepatitis infections have been found to cause primarily by one of at least five different viruses, each of which has a different epidemiologic pattern of transmission, a different clinical outcomes and a different means of prevention (Takahashi *et al.*, 1993). Of the five known hepatitis viruses, three can cause persistent infection and chronic hepatitis; the Hepatitis B Virus (HBV) (Lau and Wright, 1993), the Hepatitis C Virus (HCV) (Houghton *et al.*, 1994) and the Hepatitis delta (Hepatitis D) virus (HDV) (Hoonagle, 1989). The other two viruses, Hepatitis A and Hepatitis E, can cause acute, self-limited disease only (Siegl, 2004). The finding of an additional form of viral hepatitis, the Hepatitis G Virus (HGV) has also been suggested by Linnen *et al.* (1996). The various forms of chronic viral hepatitis have been found similar in the clinical symptoms, signs, biochemical abnormalities and histological characteristics they produce and in their ability to cause cirrhosis. However the three causative viruses have been shown distinct and the diseases they cause respond differently to the therapy (Hoonagle *et al.*, 1997).

Hepatitis A virus (a picornavirus) and Hepatitis E virus (so far unclassified) have been indicated to be small, non-enveloped and relatively stable RNA viruses with many similar, yet, not identical characteristics. Both viruses transmit preferentially by the fecal-oral route. Consequently, their spread favor by poor personal hygiene and inappropriate sanitary conditions. Infection can pass subclinically, take an acute and self-limiting course and can also manifest as fulminant hepatitis with liver failure. True chronic disease has been reported unknown. Laboratory diagnoses perform preferentially by serology, but can also be complemented by assay for viral RNA in stool or serum. Resolution of infection leads to immunity which, in the case of hepatitis A, known to be fully protective and most likely lifelong (Siegl, 2004).

The invasion of Hepatitis B Virus (HBV) causes Hepatitis-B that has been considered a serious disease of liver. HBV can cause a life-long infection, which can further lead to cirrhosis of the liver, hepatic cancer, hepatic failure and eventually death. HBV infection has been found an important public health problem worldwide with over 350 million carriers of the virus, of this 25-30% will die as a consequence of the infection (CDC, 2005).

An estimated 200,000 new cases of HBV occur annually and 1-1.25 million people being the carriers. The

prevalence of the disease has been reported higher among African Americans and persons of Hispanic or Asian origin. In addition, a higher carrier rate exists among certain sub-populations such as the Alaskan Eskimos, Asian Pacific islanders and Australian aborigines. HBV accounts for 5-10% of cases of chronic end-stage liver disease and 10-15% of cases of hepatocellular carcinoma (CDC, 2005).

HBV has blamed for 5000 deaths annually. Prevalence is low in persons younger than 12 years, but it increases in those older than 12 years. The increased prevalence in persons older than 12 years associates with the initiation of sexual contact (the major mode of transmission), the number of sexual partners and an early age of first intercourse. The cocaine use, high number of sexual partners, divorced or separated marital status, foreign birth and low educational level remain some other means (CDC, 2005).

Unfortunately the estimated Hepatitis-B carrier rate in Pakistan has also been alarmingly high i.e., approximately 10%. Both the acute and chronic hepatitis B virus infections cause major health problems (CDC, 1990).

The onset and development of infection can be described by dividing in five following stages (WHO, 2000; Lok, 2001; Valla, 2003).

The first stage has been known as immune tolerance. The duration of this stage for healthy adults has been reported approximately 2-4 weeks and represents the incubation period. For newborns, the duration of this period often is decades. Active viral replication has been known to continue despite little or no elevation in the aminotransferase levels and no symptoms of illness.

In the second stage, an inflammatory reaction with a cytopathic effect occurs. HbsAg can be identified in the sera and a decline of the levels of HBV DNA can detect. The duration of this stage for patients with acute infection has been found approximately 3-4 weeks (symptomatic period). For patients with chronic infection, 10 years or more may elapse before cirrhosis develops.

In the third stage, the host can target the infected hepatocytes and the HBV. Viral replication no longer occurs and HbsAg can be detected. The HBV DNA levels remain lower or undetectable and aminotransferase levels within the reference range. In this stage, an integration of the viral genome into the host's hepatocyte genome takes place. HbsAg still is present.

In the fourth stage, the virus cannot be detected and antibodies to various viral antigens have been produced. Different factors have been postulated to influence the evolution of these stages, including age, sex, immunosuppression and co-infection with other viruses.

The final state of the disease is cirrhosis. Patients with cirrhosis and HBV infection have likely been developed hepatocellular carcinoma (Fattovich *et al.*, 1995; Watanabe and Ikeda, 2002).

Infections with HCV have been identified, the most common cause of chronic viral hepatitis world over and ranks only slightly below the chronic alcoholism as a cause of cirrhosis. Hepatitis C affects 100-300 million people worldwide. Chronic hepatitis C has been a leading indication for liver transplantation and a major cause of hepatocellular carcinoma in the USA and Europe (Pianko and McHutchison, 2000). Chronic hepatitis C remains often silent and usually discovers only by routine serological or biochemical testing. Symptoms remain typically absent or minimal unless the severe disease or cirrhosis. The prognosis in chronic HCV infection varies greatly and has been difficult to determine. Probably only 20-30% of patients ultimately have cirrhosis and disability from end-stage liver disease (Hoonagle *et al.*, 1997; Lauer and Walker, 2001).

HCV is a single-strand RNA virus that belongs to the Flaviviridae family. A diagnosis of chronic hepatitis C can be made on the basis of anti-HCV in the serum in a patient with persistently high serum aminotransferase concentration or histological evidence of chronic hepatitis. Most patients have also had detectable HCV RNA in the serum, if assayed by a sensitive and reliable method such as reverse-transcriptase-polymerase chain reaction (Hoonagle *et al.*, 1997). Six major HCV genotypes and numerous subtypes have been identified. Molecular differences between genotypes are relatively large and they have a difference of at least 30% at the nucleotide level. The major HCV genotype worldwide is genotype 1, which accounts for 40-80% of all isolates. Genotypes 1a and 1b are prevalent in the United States, whereas in other countries, genotype 1a is less frequent. Genotypes 2 and 3 are also found globally and account for a significant minority of infections. HCV genotype 1, particularly 1b, does not respond to therapy as well as genotypes 2 and 3. Genotype 1 may also be associated with more severe liver disease and a higher risk of hepatocellular carcinoma (El-Serag *et al.*, 2003).

HCV may be transmitted by means of acupuncture, tattooing and sharing razors. Needle stick injuries in the health care setting result in a 3% risk of HCV transmission, but according to Rischitelli *et al.* (2001), HCV prevalence among health care workers is similar to that of the general population. Nosocomial patient-to-patient transmission may occur by means of a contaminated colonoscope, via dialysis or during surgery, including organ transplantation. Yeung *et al.* (2001) have reported the uncommon routes of transmission of

HCV, which affect less than 5% of the individuals at risk, include high-risk sexual activity and maternal-fetal transmission. Co-infection with HIV type 1 appears to increase the risk of both sexual and maternal-fetal transmission of HCV. Casual household contact and contact with the saliva of those infected are inefficient modes of transmission. No risk factors have identified in approximately 10% of cases.

The feelings of tiredness and sick to stomach, body temperature elevation, loss of appetite, pain in stomach, indigestion and alternate diarrhea have been reported the main symptoms of hepatitis virus's infection. Some time dark yellow urine, light-colored stools and yellowish eyes and skin can be accompanied. But some people remain free of any symptom (Lauer and Walker, 2001). The final state of the disease is cirrhosis. Patients with cirrhosis and HBV infection are likely to develop HCC. Four different stages have been identified in the viral life cycle. The first stage is immune tolerance. The duration of this stage for healthy adults is approximately 2-4 weeks and represents the incubation period. For newborns, the duration of this period often is decades. Active viral replication is known to continue despite little or no elevation in the aminotransferase levels and no symptoms of illness. In the second stage, an inflammatory reaction with a cytopathic effect occurs. HBeAg can be identified in the sera and a decline of the levels of HBV DNA is seen. The duration of this stage for patients with acute infection is approximately 3-4 weeks (symptomatic period). For patients with chronic infection, 10 years or more may elapse before cirrhosis develops. In the third stage, the host can target the infected hepatocytes and the HBV. Viral replication no longer occurs and HBeAb can be detected. The HBV DNA levels are lower or undetectable and aminotransferase levels are within the reference range. In this stage, an integration of the viral genome into the host's hepatocyte genome takes place. HbsAg still is present. In the fourth stage, the virus cannot be detected and antibodies to various viral antigens have been produced. Different factors have been postulated to influence the evolution of these stages, including age, sex, immunosuppression and co-infection with other viruses (Lauer and Walker, 2001).

Major routes of transmission for hepatitis B are more often associated with vertical transmission, sexual contact and both household and occupational contacts (Lemon *et al.*, 1999). Hepatitis C is primarily transmitted through percutaneous exposures (particularly in the context of injection drug use) though it appears that it can be transmitted sexually as well as vertically (Lemon *et al.*, 1999).

MATERIALS AND METHODS

The present study was carried out from Feb. 01, 2004 to Nov. 30, 2004. A total of 5813 peoples (3924 male, 1889 female) of different age groups of urban areas of Bahawalpur, Pakistan were screened for hepatitis B and hepatitis C.

Selection and division of volunteers: Healthy individuals of either sex, age (13-60 years) without any previous diagnosis were included in this study. The population was divided into three age groups i.e. old male/female (age above 50 years), mature male/female (age 17-50 years) and young male/female (age below 17 years). The male and female of same ages were grouped separately. A willingness certificate for cooperation in carrying out the purpose of present study was obtained, signed by each individual before his/her inclusion in the study (Tassaduqe *et al.*, 2004).

Specimen collection and evaluation: 3.0 mL of fresh blood sample was withdrawn from each volunteer by venepuncture arm vein. Specimen was allowed to clot without hemolysis and was centrifuged for 20 min at 3500 rpm (Labofuge, Heraeus, Germany). Serum was tested for HBsAg and anti-HCV evaluation. The samples were processed immediately following their collections by using rapid chromatographic immunoassay from Acon Laboratories USA (Tassaduqe *et al.*, 2004).

Rapid chromatographic immunoassay for hepatitis B and hepatitis C: The qualitative, solid phase, single test devices (Acon) were used to detect the infections with HBV and HCV. The Acon devices are two-site sandwich immunoassay for the detection of hepatitis B surface antigen (HBsAg) and HCV antibodies in serum/plasma.

The membrane HBsAg one-step test device is pre-coated with anti-HBsAg antibodies on the test line region of the device and anti-mouse antibodies on the control line region. During testing, the serum/plasma samples are allowed to react with the particle coated with anti-HBsAg antibody. The mixture moves upward on the membrane chromatographically by capillary action to react with anti HBsAg antibodies on the membrane and generate a red-color line. Presence of this red-color line in the test region indicates a positive result, while its absence indicates a negative result. Regardless of the presence of HBsAg, as the mixture continues to migrate across the membrane to the immobilized control region, a red-color line always appears. The presence of this red line serves as verification for sufficient sample volume and proper flow and as control for the reagents (Blumberg, 1971).

The membrane of HCV one-step test device is pre-coated with anti-HBsAg antigen on the test line region of the device and antibodies HCV on the control line region. During testing, the serum/plasma samples react with the protein coated particles. The mixture migrates upward on the membrane chromatographically by capillary action to react with recombinant HCV antigen on the membrane and generate a red-color line. Presence of this red-color line in the test region indicates a positive result, while its absence indicates a negative result. Regardless of the presence of HCV antibodies, as the mixture continues to migrate across the membrane to the immobilized control region, a red-color line always appears. The presence of this red-color line serves as verification for sufficient sample volume and proper flow and as control for the reagents (Wilber, 1993).

RESULTS

The results of present study indicated that among the observed urban population of Bahawalpur, District (n = 6815), prevalence of HBV in male population was 9.63% (Table 1) and in female population 9.54% (Table 2). The prevalence of HBV when studied in different age groups of both sexes, it was found that prevalence of HBV was higher (11.47%) in old males as compared to mature males (8.86%) and young males (8.58%) (Table 1). The results suggested that among the observed female population, prevalence of HBV was maximum (10.39%) in old females as compared to mature females (9.65%) and young females (8.59%) (Table 2).

The prevalence of HCV in male population was 4.87% (Table 3) and in female population 4.45% (Table 4). Maximum prevalence (7.36%) was observed in the old male in comparison to mature (4.17%) and young (3.92%) male populations (Table 3). Similarly higher prevalence of HCV 6.52% was found in old female in comparison to the mature (4.70%) and young (3.45%) female population (Table 4).

Table 1: Prevalence of HBsAg in different age groups of male population

Age group	Age (years)	Total No.	HbsAg negative (normal)	HBsAg positive (infected)	% age
Old	>50	924	818	106	11.47
Mature	17-50	2133	1944	189	8.86
Young	<17	867	784	83	8.58
Total		3924	3546	378	9.63

Table 2: Prevalence of HBsAg in different age groups of female population

Age group	Age (years)	Total No.	HbsAg negative (normal)	HBsAg positive (infected)	% age
Old	>50	491	440	51	10.39
Mature	17-50	1213	1096	117	9.65
Young	<17	1187	1085	102	8.59
Total		2891	2621	270	9.54

Table 3: Prevalence of anti-HCV in different age groups of male population

Age group	Age (years)	Total No.	Anti-HCV negative (normal)	Anti-HCV positive (infected)	% age
Old	>50	924	856	68	7.36
Mature	17-50	2133	2044	89	4.17
Young	<17	867	883	34	3.92
Total		3924	3733	191	4.87

Table 4: Prevalence of anti-HCV in different age groups of female population

Age group	Age (years)	Total No.	Anti-HCV negative (normal)	Anti-HCV positive (infected)	% age
Old	>50	491	450	32	6.52
Mature	17-50	1213	1156	57	4.70
Young	<17	1187	1155	41	3.45
Total		2891	2761	130	4.50

DISCUSSION

Hepatitis is a common public health problem and viral hepatitis has been found major cause of chronic liver disease world over (Gow and Mutimer, 2001). Both male and female in childhood and adolescence have been found at equal risk of hepatic viral infections (Beasley and Hwang, 1983). A variety of means including perinatal HBV and HCV infections and person-to-person transmission have been identified for the transmission of infection in children. The risk of perinatal hepatic viral infections among infants born to infected mothers ranges from 10-85% (Stevens *et al.*, 1985). The children of non-infected mothers also remain at high risk of acquiring chronic HBV and HCV infections by person-to-person transmission during the first five years of life (Beasley and Hwang, 1984). The most adults acquire the infection by several specific modes of transmission including sexual contacts, parenteral drug use, occupational exposure, household contact with carrier or infected persons, administration of blood products and hemodialysis etc. However, over 1/3rd of patients with acute hepatitis B and hepatitis C do not have readily identifiable risk factors (Alter *et al.*, 1990). Moreover, in the presence of active HBV infection, other infection especially Hepatitis Delta Virus can invade which has been identified as either co-infection with HBV infection or super-infection of an HBV carrier (Rizzetto, 1983; US Department of Labor, US Department of Health and Human Services, 1987).

According to Shah *et al.* (2002) the prevalence of hepatitis is generally found highest during young and middle adulthood years. In the present study the prevalence of both hepatitis B and hepatitis C was also observed in the different age groups of both male and females. The data indicated the presence of lesser rate of infection in the different females in comparison to the different male age groups. The hepatitis B cases were found with highest prevalence among old males and

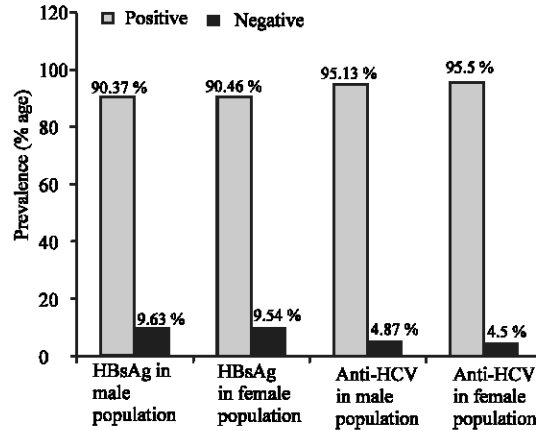


Fig. 1: Comparison of % age of HBsAg, Anti-HCV positive and negative populations

females of age group >50 years (11.47 and 10.39%, respectively) while intermediate prevalence (8.86 and 8.65%) and lowest (8.58 and 8.59%) in the mature males and females of age group 17-50 years and young male and female age group <17 years, respectively. The results further indicated that maximum rate of HCV was found in the old males and females of age group >50 years (7.36 and 6.52%, respectively). The rate of this infection was detected intermediate (4.17 and 4.70%) and minimum (3.92, 3.45%) in the mature and young males and females of age group 17-50 years and age group <17, respectively. The prevalence of both HBV and HCV was higher in old age groups of male than female. The infection with HBV was observed higher in the female than that of male mature groups. This infection was also slightly higher in the female than the male young groups. The presence of HCV in female found greater in comparison to the male mature groups while slightly lesser in respective young age groups. It is a possibility therefore, that these age groups may be exposed highly to risk factors like surgery, blood transfusion, multiple injection and razor trauma of shaving. Moreover, the ratios of infected peoples either with HBV or HCV among male population detected greater in comparison to the female population. The findings remained in-agreement of the study of Remis (1998), Shah *et al.* (2002) who found that prevalence of hepatitis higher in males than females. The higher rate of infections with either HBV or HCV indicated further, the possibility of the development of weaker immunities against the infections. The presence of lesser rate of infections with HBV or HCV in the mature and young age groups in comparison to the old age groups pointed out the possibility of less frequent and lesser-prolonged exposures of population to causative factors as well as

presence of slightly stronger immunities against the diseases. The results are in accord with the findings of Tassaduqe *et al.* (2004).

Ahmad *et al.* (2004) have been pointed out the prevalence rate of HBV (2.21%) and HCV (4.1%) in blood donors in Punjab, Pakistan. The prevalence of HBV reported by Tassaduqe *et al.*, (2004) was 7.94%. The prevalence of HBV and HCV reported by the present study remained much higher (Fig. 1). Therefore, it can be assumed that the spread of infection in Pakistan is on rise, suggesting the education of the society including health care individuals to observe the protective measures against hepatic viral infections.

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