

Original Research Article

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Immunoassay Test for Evaluation of Seroprevalence of HBV and HCV Infection among Patients Attending a Tertiary Care Hospital

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ABSTRACT

Keywords

Hepatitis B Surface Antigen (HBsAg), Anti HCV Ab, Seroprevalence, Immunoassay test

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Viral Hepatitis is a major healthcare problem worldwide and is an important cause of morbidity and mortality. Aim of the study is to assess the seroprevalence of Hepatitis B and Hepatitis C viral infection among patients attending a tertiary care hospital. A prospective study was done from a period of 20 months (May 2016 to December 2017) on patients attending a tertiary care hospital. Serum samples were taken from patients for testing HBV and HCV infection using rapid immunochromatographic test and ELISA. A total number of 39874 patients were screened for HBsAg and anti HCV Ab. Among them; 249 were HBsAg positive (0.62%), 51 were anti HCV Ab positive (0.1%). Majority of the HBsAg positive patients belonged to age group 38-47 years (28.1%) and anti HCV Ab positive patients belonged to age group 58- 67 years (47.0%). Only one patient was found with a co-infection with both HBsAg and HCV positive. The screening of Hepatitis B and Hepatitis C viral infection among the patients attending a tertiary care hospital enlightens the details of seropositive being unnoticed.

Introduction

Viral Hepatitis is a major healthcare problem. Hepatitis B and Hepatitis C virus are the two most common hepatotropic viruses responsible for majority of acute and chronic hepatitis (Wang S *et al.*, 2017). Hepatitis B infection is the 10th leading cause of death and annually one million die from HBV associated liver disease (Teresa L *et al.*, 2006). Worldwide prevalence of HBsAg varies between 0.1 to 11.7% (Koshy J *et al.*, 2014). In India prevalence in general population 2-8% and among the blood donors 1- 2% (Gupta

et al., 2004, Datta *et al.*, 2008, Panda *et al.*, 2008). Every year 100,000 Indians die due to HBV infection related illness (WHO, 2015). Hepatitis C virus affects more than 170 million people around the world (Moosavy *et al.*, 2017). WHO report of Hepatitis C suggests its prevalence 3% in the world which is a significant burden on public health (WHO, 2012). In India, seroprevalence of HCV infection is 1.8% among the general population (Chakravarti *et al.*, 2013).

Hepatitis B and Hepatitis C virus are the main causes of severe liver disease, including

chronic hepatitis, hepatocellular carcinoma and cirrhosis- related end- stage liver disease (Kanodia *et al.*, 2015). They share a common risk factors and mode of transmission includes exposure to infected blood, various body fluids such as saliva, menstrual, vaginal and seminal fluids. Unvaccinated heterosexual and homosexual men are highly prone to hepatitis B infection (WHO, 2017).

Among all body fluids serum sample shows high HBV titre values. The Hepatitis B surface antigen (HBsAg) in serum is the first seromarker to indicate HBV infection, either acute or chronic (Horvat *et al.*, 2011). The presence of anti-hepatitis C virus antibody (anti HCV Ab) indicates exposure to hepatitis C virus. This antibody is found to be association with 95% of chronic infections (Baheti *et al.*, 2000).

The present study was designed to assess the seroprevalence of Hepatitis B and Hepatitis C viral infection among patients attending a tertiary care hospital using rapid immunochromatographic test and ELISA.

Materials and Methods

Hepatitis B and Hepatitis C virus are among the principal causes of severe liver disease. A prospective study was done from a period of 1 year 8 months (May 2016 to December 2017) on patients attending a tertiary care hospital. A total of 39874 patients were included in the study and their samples were taken for testing HBsAg and anti HCV Ab. Samples from the age groups below 18 years and above 67 years were excluded from the study. Samples were collected under aseptic condition and were subjected for anti HCV Ab and HBsAg screening by rapid card method and confirmation was done by ELISA. Using the commercially available rapid test kit (Orchid Biomedical Systems, A Division of Tulip Diagnostics (P) Ltd., Goa, India). Venous

blood sample (3 ml) was drawn from each patient in a labelled plain vacutainer tube. Blood was allowed to clot and sera was separated and stored at -20°C until tested for HBsAg and anti HCV Ab. The procedures were executed as per the kit as follows: The samples were brought to room temperature before performing the analysis. 50µl of the serum was dispensed in to the sample well of the card. The results were interpreted in 15 minutes. The results are as follows: the presence of two colour bands on "T" test line and "C" control line indicates a positive result. The presence of only one band on "C" (Control) line indicates a negative result. The test should be considered invalid if no coloured band appears on the device. The test should also be considered invalid if a coloured band appears only at the test region 'T' and not at the control region 'C'. In such cases, repeat the test with a new device, ensuring that the test procedure has been followed accurately.

All the samples were tested for HBsAg using the enzyme linked immunosorbent assay (Transasia Bio- Medicals LTD, India). The procedures were executed as per the kit as follows: Bring all reagents and test specimens at room temperature before use. Add 50µl of sample diluent to each well. In each run, there will be one blank (100µl sample diluent plus 50µl conjugate), three negative controls and one positive control. Add 50µl of control and test specimens to the respective wells. Add 50µl of conjugate to each well. Cover the plate with black cover and incubate 60 minutes at 20- 37°C. Wash the plate as per microplate washing procedure. Add 50µl of colour reagent. Cover the plate with black cover and incubate for 15 minutes in dark at 20- 30°C. Add 100µl of stopping buffer to each well. Read absorbancy at 450 nm (using 630nm as reference wavelength). Deduct blank absorbancy from the control and the test wells. The interpretations of the result are as follows: If the absorbancy of the test serum is less than

0.1, then the sample is considered as negative. If the absorbancy of the test serum is equal or greater than 1, then the sample is considered as positive.

All the samples were tested for anti HCV Ab using third generation ELISA method (Qualpro diagnostics. A Division of Tulip Diagnostics (P) Ltd, Goa, India). The procedures were executed as per the kit as follows: Bring all reagents and test specimens at room temperature before use. Add 200µl of sample diluent to separate wells. Add 10µl of control specimen in the wells. Then gently shake the plate to mix thoroughly and apply plate sealer and incubate for 30 minutes at 22-28°C. Wash each well by filling approximately 350µl diluted wash buffer and aspirating off six times. Then blot dry and add 100µl substrate and incubate at 22- 28°C away from light for 30 minutes and stop reaction by adding 100µl stop solution. Then read the absorbance at 450nm with 600- 700nm as reference within 30 minutes of stopping the reaction. Samples with absorbance value less than 0.1 are considered non-reactive. Samples with absorbance value equal to or greater than 1.0 are considered as reactive. The study was approved by Institutional Ethical Committee before commencing the study.

Results and Discussion

A total number of 39874 patients from both inpatient department (IPD) and outpatient department (OPD) departments were enrolled during the study period with fulfilling the inclusion and exclusion criteria. Out of 39874 patients, 30841 (77.3%) were married and 9033 (22.7%) were unmarried. The genderwise distribution of sample source shown in Figure 1. Among them 8,558 (21.5%) were from rural areas, 21,298 (53.4%) from urban areas and 8,376 (21%) from sub urban areas and 1,642 (4.1%) from tribal areas.

Out of these patients, 249 samples were HBsAg positive (0.62%), 191 (76.7%) were males and 58 (23.3%) were females. Majority of the HBsAg positive patients belonged to age group 38- 47 years (28.1%). Age and genderwise distribution of HBsAg positive patients shown in Table 1. The seroprevalence of HBsAg were found to be more or less equal among the OPD (50.2%) and IPD (49.8%).

Among the HBsAg positive, majority were found to be admitted to general medicine (65.5%), followed by ICU (16.9%) and general surgery ward (8.8%).

Wardwise distribution of HBsAg positive patients are shown in Table 2.

Out of 39874 patients, 51 samples were anti HCV Ab positive (0.1%). Among them, 28 (54.9%) were males and 23 (45.1%) were females. Majority of the anti HCV Ab positive patients belonged to age group 58- 67 years (47.0%). Age and gender wise distribution of anti HCV Ab seropositive sample shown in Figure 2.

Majority of the anti HCV Ab seropositive patients were from general surgery ((39.2%), followed by ICU (21.6%) and general medicine (19.6%). Samples from ENT and Ophthalmology department were seronegative for anti HCV Ab. Wardwise distribution of anti HCV Ab seropositive sample given in Table 3. Among 300 seropositive samples, only one sample (0.3%) was positive for both HBsAg and anti HCV Ab.

Hepatitis B virus and Hepatitis C virus infections are the most common viral infections known to humanity worldwide. An estimated 2 billion and 180 million people globally have been infected with HBV and HCV are at risk of developing liver cirrhosis or liver cancer (Franco *et al.*, 2012 and Averhoff *et al.*, 2012).

Table.1 Age and genderwise distribution of HBsAg positive sample source

<i>Characteristics</i>	<i>HBsAg Positive</i>		
	<i>Total n=249 (%)</i>	<i>Male n=191</i>	<i>Female n=58</i>
Age group in years 18- 27	48(19.3)	37	11
28- 37	46(18.5)	33	13
38- 47	70(28.1)	64	6
48- 57	41(16.5)	27	14
58- 67	44(17.6)	30	14

Table.2 Wardwise distribution of HBsAg positive patients

<i>Name of the ward</i>	<i>HBsAg Positive, n=249 (%)</i>	<i>HBsAg negative, n=39625 (%)</i>
General Surgery	22(8.8)	8717(22)
General Medicine	163(65.5)	21387(54)
ENT	1(0.4)	467(1.2)
Ophthalmology	2(0.8)	861(2.1)
Obstetrics and Gynaecology	4(1.6)	4359(11.0)
ICU	42(16.9)	993(2.5)
Orthopaedic Surgery	15(6.0)	2841(7.2)

Table.3 Wardwise distribution of anti HCV Ab seropositive sample

<i>Name of the ward</i>	<i>HCV Positive, n= 51 (%)</i>
General Surgery	20 (39.2)
General Medicine	10 (19.6)
Obstetrics and Gynaecology	6 (11.8)
ICU	11 (21.6)
Orthopaedic Surgery	4 (7.8)

Fig.1 Genderwise distribution of sample source

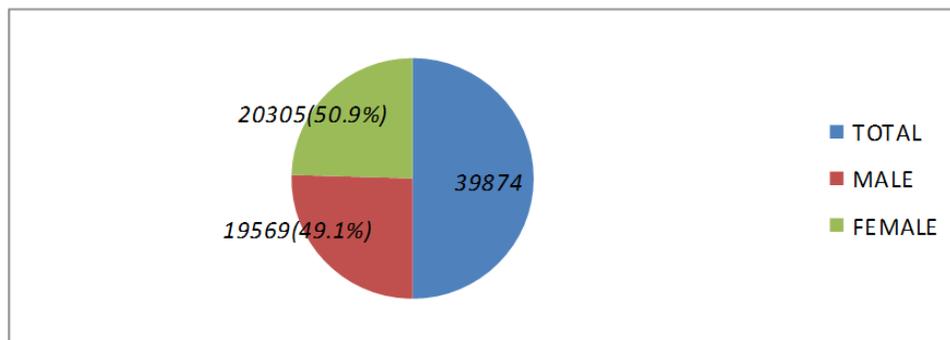
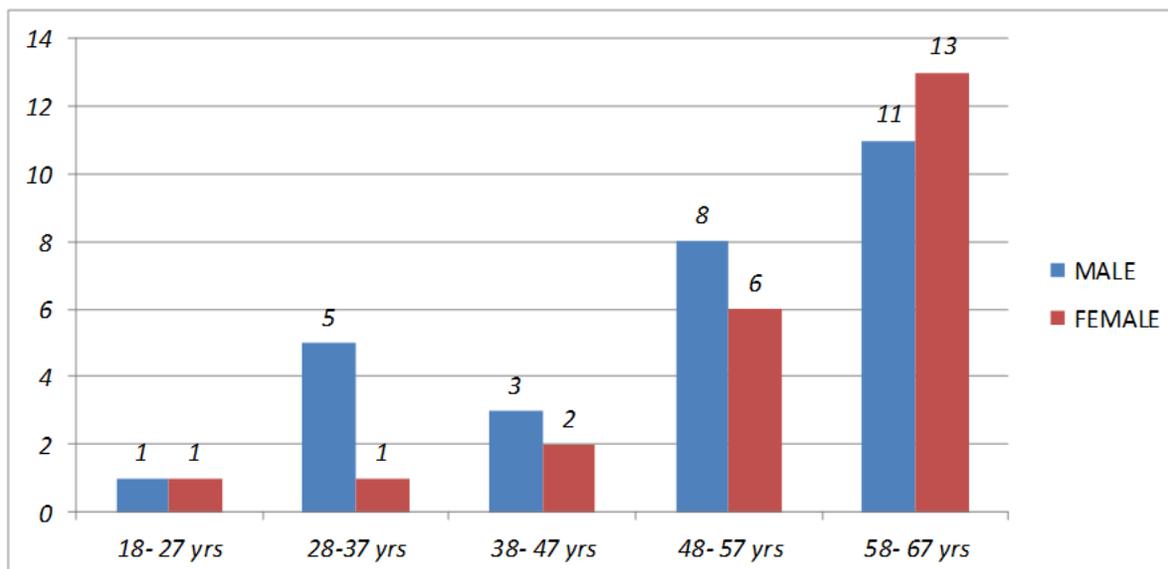


Fig.2 Age and genderwise distribution of anti HCV Ab seropositive sample



The relative importance of HBV and HCV infections varies greatly from one part of the world to another and changes over time (Te H.S *et al.*, 2010). Prevalence of Hepatitis B varies from country to country and depends upon a complex mixture of behavioural, environmental and host factors. Our study showed 0.62% of HBsAg prevalence. Another study conducted in Karnataka is similar to our prevalence rate (Singh *et al.*, 2009). According to Khatoon *et al.*, 2016, the HBsAg prevalence was 3.9% and their study was higher than the prevalence rate found in our study. In our study male population showed higher prevalence (76.7%) than females (23.3%) (Table 1). Similar observation was reported by other studies Khatoon *et al.*, 2016, Sood *et al.*, 2010, Quadri S *et al.*, 2013. The reason for the higher prevalence in males in general population is not yet clearly explained (Qamer *et al.*, 2004).

In this study, the seroprevalence of HBsAg was highest among age groups 38- 47 years (28.1%), followed by 18- 27 years (19.3%), 18.5% in 28- 37 years (18.5%) and the

prevalence decreased among age 58- 67 years (17.6%), followed by 48- 57 years (16.5%) (Table 1). Study conducted by Khatoon *et al.*, in North India showed number of positive cases was highest among age groups 28- 37 years (37.7%), followed by 38- 47 years (29.5%) and the prevalence decreased being 13.1% among 48- 57 years and 1.6% among 58-67 years age group. In our study married population showed higher seropositive (76.7%) when compared with unmarried population and our study in concurrence with Khatoon *et al.*, (2016).

Our study showed that the seropositive of anti-HCV Ab was found to be 0.1%. This seroprevalence is lower than the seroprevalence (4.8%) reported by Bhattacharya *et al.*, 2003. In our study, the seroprevalence of anti HCV Ab was highest among the age groups 58- 67 years shown in (Figure 2) was in concordance with study of Ahmad *et al.*, reporting the Hepatitis C to be highly prevalent in the age group between 55-64 years (Ahmad *et al.*, 2004). Study of Sood *et al.*, 2010 and Abdel-Aziz *et al.*, 2000 is in concurrence with our study for the higher

prevalence of anti HCV Ab among the male gender (Figure 2). In our study, only one sample (0.3%) was positive for both HBsAg and anti HCV Ab. This similar seropositive for both HBsAg and anti HCV Ab was documented by study of Naeem *et al.*, (2012).

The screening of Hepatitis B and Hepatitis C viral infection among the patients attending a tertiary care hospital enlightens the details of seropositive being unnoticed. People must be educated about HBV and HCV infections and their mode of transmission to prevent the spreading of disease. This study provided much important information concerning the prevalence of Hepatitis B and C and these data will serve as an important source of data for the evaluation as well as for future preventive measures.

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