JMSCR Vol||04||Issue||07||Page 11429-11434||July 2016

www.jmscr.igmpublication.org
Impact Factor 5.244
Index Copernicus Value: 83.27
ISSN (e)-2347-176x ISSN (p) 2455-0450
crossref DOI: http://dx.doi.org/10.18535/jmscr/v4i7.40

IGM Publication
Journal Of Medical Science And Clinical Research
An Official Publication Of IGM Publication

Sero-Prevalence and Coinfection of Hepatitis C Virus and Hepatitis D Virus in Patients with Hepatitis B Virus Infection in A Tertiary Care Hospital

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Abstract
Background: *Hepatitis virus infection is one of the most prevalent public health problems in developing countries. Hepatitis B (HBV) and Hepatitis C (HCV) viruses are main causative agents of chronic liver disease. This co-infection has been detected among patients with severe chronic hepatitis, cirrhosis, and inactive HBV infection. Hepatitis D virus (HDV) infection occurs either as a co-infection or super-infection in HBV carriers. Early screening of clinically diagnosed cases of viral hepatitis is essential for establishing diagnosis and treatment to prevent long term sequelae. About 380 million persons worldwide carry HBV and among them 5% have delta hepatitis. In association with HBV, HDV produces significantly more severe illness than HBV alone. Hepatic encephalopathy and fulminant hepatic failure were also common in HBV and HDV infected patients compared to those with HBV alone.*
Aims and Objectives: *To find the rate of prevalence of HCV and HDV co-infections in patients with HBV infection in a tertiary care hospital. To do a comparative study of the rate of prevalence of hepatitis viral co-infections among male and female gender groups and also in patients with chronic and acute liver diseases.*
Materials and Methods: *A total of 500 Venous blood samples were collected basing on the inclusion criteria and serum was separated and preserved at -20°C. The samples were tested for detecting HBV, HCV, and HDV markers by ELISA method, for HDV detection, HBV positive samples alone were considered. A total of 40 (8%) samples were HBsAg positive, followed by HCV 12 (2.4%), co-infection with HBV and HCV is 2 (0.4%) and 1(0.2%) with HCV and HIV co-infection.*
Conclusion: *According to our study even though single infections are common, co-infections are seen more in age group of above 50yrs which may increase the risk of developing a chronic infection.*
Key words: *HBV, HCV, HDV, Co-infection.*

Introduction

At present viral hepatitis is a major health problem worldwide, particularly in Asian countries. Hepatitis is caused by different hepatic viruses and it leads to liver related morbidity. Mostly hepatic infection is caused by single hepatic virus but sometimes infection with multiple viruses may occur and it leads to different management problems [1]. HBV and HCV infections account for a substantial proportion of liver diseases worldwide. Majority of those with chronic HBV and HCV infection will develop complications i.e. 15%-40% may develop cirrhosis, liver failure or hepatocellular carcinoma. It has been estimated that over the next 20 years, the proportion of HBV/HCV infected patients with cirrhosis will

increase from 16% to 32% and that other complications will also increase dramatically including hepatic decompensation, hepatocellular carcinoma and liver related deaths [1]. Development of HCC was reportedly found to be associated with anyone of the dominating virus in a triple infection and not because of all three of them [1].

Hepatitis C virus (HCV), hepatitis B virus (HBV) and hepatitis D virus (HDV) are transmitted via similar routes that is through blood or blood products so as a result, dual infection and even triple infection can occur in some cases at same time. Dual and triple infections are commonly referred to as co-infection or super-infection [2].

Hepatitis D virus (HDV) or delta virus is defective virus. It requires the help of another virus that is hepatitis B virus for its multiplication. It occurs with HBV either in the form of co-infection or super-infection. It is very rare, but the most severe form of the viral hepatitis. The virus cannot replicate or multiply in the absence of HBV. Worldwide 15 million people positive for hepatitis B are also infected with hepatitis D. Like HBV and HCV, it also plays a significant role in liver abnormalities [3].

Acute infection with hepatitis B virus is associated with acute viral hepatitis, an illness that begins with ill-health, loss of appetite, nausea, vomiting, body aches, mild fever, and dark urine, and then progresses to development of jaundice. It has been noted that itchy skin has been an indication as a possible symptom of all types of Hepatitis virus. The illness lasts for a few weeks and then gradually improves in most affected people. A few people may have more liver disease (fulminant hepatic failure), and may die as a result. The infection may be entirely asymptomatic and may go unrecognised [4].

Chronic infection with hepatitis B virus either may be asymptomatic or may be associated with a chronic inflammation of the liver, leading to cirrhosis over a period of several years. This type of infection dramatically increases the incidence of hepatocellular carcinoma (liver cancer). Chronic carriers are encouraged to avoid consuming alcohol as it increases the risk for cirrhosis and liver cancer. Hepatitis B virus has been linked to the development of Membranous glomerulonephritis (MGN) [3].

Non-specific symptoms are present in 1-10% of HBV-infected people and include serum-sickness-like syndrome, acute necrotising vasculitis, membranous glomerulonephritis, and papular acrodermatitis of childhood. The serum-sickness-like syndrome occurs, often preceding the onset of jaundice. Other clinical manifestations like fever, skin rash, and polyarthritis can also occur[4].

The present study includes the prevalence of blood-borne Hepatitis viral infection and co-infection among clinically diagnosed cases among patients with liver disease in a tertiary care hospital.

Materials and Methods

In our study, sample collection criteria for acute liver disease includes the presence of acute jaundice, anorexia, malaise, extreme fatigue, right upper quadrant tenderness and dark urine, increased urobilinogen and impaired LFT such as raised serum alanine amine transferase. Inclusion criteria for chronic liver disease includes ascites, hepatomegaly, splenomegaly, Jaundice, palmar erythema, clubbing, oedema, axillary and pubic hair loss, spider nevi, flapping of tremors, drowsiness, confusion and coma along with impaired LFT such as raised ALT.

The study was performed by screening 500 clinically diagnosed cases of liver disease patients from June 2012 to August 2014. The blood samples were screened for Hepatitis B virus (HBsAg, anti-HBsAg), Hepatitis C virus (anti-HCV, HCV RNA), HIV virus (anti-HIV1 and anti-HIV2) using commercially available kits. All the liver diseases were clinically diagnosed by the Gastroenterologist based on the presenting signs and symptoms and on clinical examination of the patients. Blood samples were collected aseptically by vein-puncture technique using evacuated tubes as per the guidelines of the BD Vacutainer Blood

Collection System with the help of a phlebotomist, the serum was separated and stored at -20°c until use.

The blood samples were screened for various markers of hepatitis viruses by using third generation ELISA kits and other rapid immunochromatographic tests. Hepatitis B surface antigen (HBsAg) was first screened by an in-vitro immunochrmatographic assay called the SD BIOLINE HBsAg test as a presumptive diagnosis. Then for confirmatory diagnosis a 3rd generation ELISA ErbaLisa, supplied by Transasia Bio-Medicals Ltd, India. Antibodies to HCV (anti-HCV) was screened by the 4th generation HCV TRI_DOT test which is a rapid visual test for the qualitative detection of antibodies to HCV in human serum/plasma. Antibodies to HIV 1 and HIV2 were screened using RETROQUIC immunoconcentration test and then by HIV ELISA.

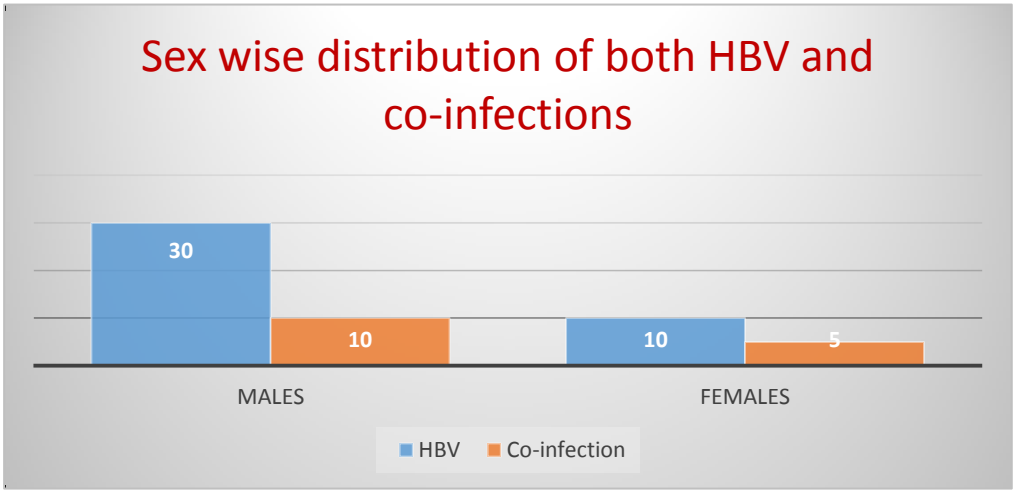
Results

Among the 500 samples screened, 40 (8%) were positive for HBsAg, 12 (2.4%) were positive for HCV, co-infection was seen in 2 (0.4%) for HBsAg and anti-HCV antibodies,1 (0.2%) was positive for both anti-HCV and anti-HIV antibodies. In our study none of the samples were positive for both HBV and HIV.

Table 1

Type of infection	Total no of positive samples	Percentage
HBsAg	40	8%
Anti-HCV	12	2.40%
Co-infection of HBV-HIV	0	0%
Co-infection of HBV-HCV	2	0.40%
Co-infection of HCV-HIV	1	0.20%
Total no samples	500	100%

Graph 1



According to sex wise distribution out of 40 (8%) samples positive for HBsAg, 30 (75%) were from males and 10 (25%) were from females. Out of 12 (2.4%) samples positive for anti HCV antibodies, 7(58%) in males and 5(42%) in females were positive. Among 2 samples which were positive for both HBsAg and anti HCV antibodies all the samples were from males. In case of HCV and HIV co-infection, only one sample collected from male was positive, hence overall Hepatitis viral infection in males was 73% and in females is 27%.

Table 2 Association of clinical conditions with HBV infection and co-infection.

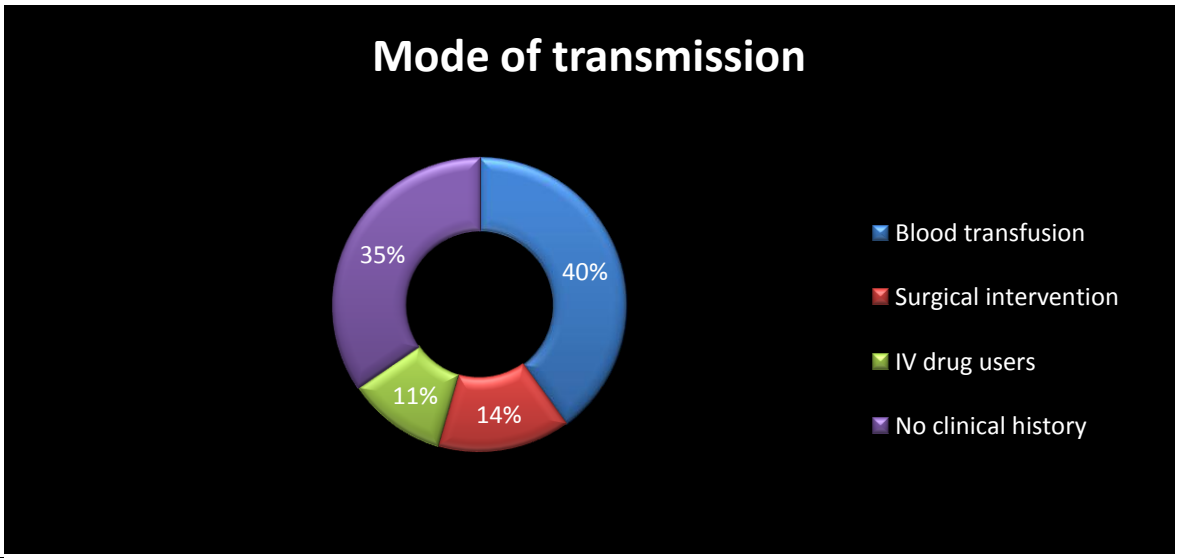
Clinical conditions	Viral infections				Total
	HBV	HCV	HBV-HCV	HCV-HIV	
Acute liver disease	25	3	0	0	28
Chronic liver disease	13	6	2	1	22

In our study single infections with HBsAg alone were 25 and HCV alone were 3 and none of the samples showed co-infection in acute liver disease. On the other hand, in chronic liver disease 13 samples alone were positive for HBV infection and 6 were positive for HCV alone, HBV and HCV co-infections were 2 and HCV –HIV was only 1.

Table 3 Age wise distribution of patients with Hepatitis B viral infections and co-infections.

Age group (in years)	HBV	HCV	HBV-HCV	HCV-HIV	Total no. of positive samples	%
1-10	1	0	0	0	1	1.8%
11-20	3	0	0	0	3	5.4%
21-30	3	0	0	0	3	5.4%
31-40	10	2	0	0	12	21.8%
41-50	19	7	0	0	26	47.3%
51-60	1	2	1	1	5	9.1%
61 and above	0	1	1	0	2	3.6%
Total	40	12	2	1	55	100%

According to the age-wise distribution, 29 samples alone were HBsAg positive, 9 samples alone were HCV positive between the age group of 30 to 50 years, and co-infections with HBV - HCV the positive samples were 2 and only 1 sample was positive for HCV-HIV above the age group of 50 years.



Graph 2

Retrospective analysis of the Hepatitis B infected and co-infected patients were done by studying their clinical case history from Medical Records Department. The study reveals 22 patients (40%) of HBV positive (alone and with co-infection) patients had history of blood transfusion, 8 patients (14.5%/) had surgical intervention, 6 patients (11%) were IV drug users, while 19 (34.5%) had no evidence of historical record. Blood transfusion was the predominant risk factor in all the patients positive for co-infection.

Discussion

Among the 500 samples collected, 40 (8%) were HBsAg positive, followed by HCV 12 (2.4%), co-infections with HBV and HCV is 2 (0.4%), and only 1(0.2%) with HCV and HIV co-infection. The present study shows that single infections are much common when compared to co-infections. In a study by A Ganesh kumar, out of 175 samples tested 21% were HBV positive, 1.7% showed co-infection with HBV and HCV, which is similar to our study [5].

In our study out of 40 samples positive for HBsAg, 30 (75%) were from males, 10 (25%) samples were from females. Out of 12 cases which were HCV positive, 7 (58%) were from males and 5 (42%) were from females. The two cases which showed co-infection of HBV and HCV were from males. In a study by Suresh B Sonth et al, out of 100 HIV infected individuals, 13(23.63%) samples from males were positive for HBsAg, and 8 (17.7%) were positive in samples from female patients [6].

In our study co-infections are seen more in chronic liver disease when compared to acute liver disease similar to a study by A Ganesh. According to his study most of HBV positive cases had acute liver disease and co-infection of HBV and HCV is found to be among drug users, haemodialysis patients, patients undergoing organ transplantations etc. In our study single infections of HBV and HCV are common between the age group of 30-50 whereas co-infections are common above 50 years. According to A. E. O. Otedo, the average range of patients with only HBsAg positive was 34 $\pm$ 14 years, while those with co-infection was 38$\pm$ 12 years [7].

Conclusion

The present study included 500 liver disease patients who were screened for HBV and its coinfections with other Hepatitis viruses such as HCV and HIV. A total of 40 HBV positive patients and 12 HCV positive patients were reported. The aim of the study was to find the prevalence of HBV infection alone and its co-infections and also to clinically compare the severity of the two in terms of acute or chronic. We found that the patients with co-infections have more risk of getting chronic infections than those with single infections. Improved surveillance and periodical epidemiological studies will have to be undertaken to monitor and prevent these blood borne viruses. It is highly important that we test patients coming with one hepatic infection with other infections as they share common mode of spread. Hence it is strongly recommending that routine tests of the blood for HCV in addition to HBV and HIV made mandatory in routine serological investigations when suspecting liver disease of viral origin.

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