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Seroprevalence of Hepatitis B infection in Chronic Liver Disease patient in Kota region and comparision between the two different diagnostic techniques current in use

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ABSTRACT

Introduction: Hepatitis B virus (HBV) is one of the most important cause of chronic liver disease (CLD) and hepatocellular carcinoma.

Aims and objectives: To study the seroprevalence of Hepatitis B viral infection in CLD patients in Kota region and detect the infection as early as possible to reduce morbidity, mortality and disease progression. To compare immunochromatographic rapid test and ELISA, for detecting HBsAg.

Materials and method: Serum sample were tested for Hepatitis B virus infection by detecting serological marker HBsAg using immunochromatographic rapid test and sandwich ELISA technique.

Result: Seroprevalence of HBV among CLD patients in present study was 28%. There is a significant difference between the reactivity of rapid card test and ELISA. Maximum numbers of HBV infection were seen in age group of 41-50 years (36.7%), rural, married, illiterate males. The predominant mode of acquiring HBV infection appears to be blood transfusion (36.6%).

Conclusion: All high risk groups & CLD patients should be screened for HBV for early detection of infection, to reduce complications and transmission. Rapid test kits should be used in conjunction with other immunoassay, particularly ELISA technique. There is need to educate the people for proper safety measures.

KEYWORDS: Hepatitis B virus (HBV), Chronic liver disease (CLD), Hepatitis B surface antigen (HBsAg), Enzyme linked immunosorbent assay (ELISA).

INTRODUCTION

Chronic hepatitis represents a series of liver disorders of varying causes and severity in which hepatic inflammation and necrosis continue for at least 6 months.[1] CLD comprises of a spectrum of disease such as chronic hepatitis, liver cirrhosis and HCC.[2] Among the viral causes of hepatitis, hepatitis B virus (HBV) infection accounts for a substantial proportion of liver diseases worldwide.[3] Hepatitis B virus (HBV) and hepatitis C virus (HCV) are endemic in India and have an aetiological role in acute hepatitis, 50-70% of which end up with chronic liver disease.[4]

Hepatitis B Virus is classified in the family Hepadnaviridae and genus orthohepadnavirus. It is a 42 nm spherical DNA virus with an outer envelope and an inner core of 27 nm in diameter enclosing the viral genome and a DNA -polymerase. The genome consist of partially double-stranded circular DNA of approximately 3200 base pairs.[5]

The HBV is highly infectious and is transmitted through contact with the blood or other body fluids of an infected person.[6] The hepatitis B surface antigen (HBsAg) in serum is the first seromarker to indicate active HBV infection, either acute or chronic.[7] Individuals who remain (HBsAg) positive for at least six months are considered to be hepatitis B carriers and have a high probability of developing CLD.[8] In India, HBsAg prevalence in general population ranges from 2% to 8%, placing India in intermediate HBV endemicity zone and the number of HBV carriers is estimated to be 50 million, forming the second largest global pool of chronic HBV infections.[9]

MATERIALS AND METHODS:

The study was carried out at the Department of Microbiology, MBS Hospital, Government Medical College, Kota between July 2015 and July 2016. Patients diagnosed with chronic liver disease & aged 18 years and above were included in the study & failure by patient to give consent to participate in the study or patient other then chronic liver disease were excluded from study.After pretest counseling and informed consent, 5ml of blood is collected in a test tube using all aseptic precautions and is properly labeled. The collected blood is allowed to clot then centrifuged at 3000 rpm for 5 min to separate out serum. The specimen vial is unstoppered and the clear serum is drawn off by sterile pasteur pipette and transferred to a sterile plastic screw capped leak proof tubes and labeled. Serum of 100 CLD cases were tested for Hepatitis B virus infection by detecting serological marker HBsAg, using immunochromatographic rapid test SD Bioline HBsAg immunochromatographic test kit and sandwich ELISA technique using Erba Lisa SEN HBsAg Gen3 by Transasia bio medicals Ltd.

RESULTS

Age Most of the CLD patients were in the age group of 41-50 years of age (30%) followed by 23% in 31-40 years, 19% in 51-60 years, 8% in 71-80 years, 4% in 20-30years, & 2% in >80 years. Seroprevalence of HBV among 100 CLD patients in present study was 28%.(Graph No.1) HBV infection was seen mostly in age group 41-50 yrs (36.7%). (Graph No.2)

Gender Among the 100 analyzed CLD patients, 86% were male while 14% were female and male to female ratio was 6:1. The prevalence of HBV infection was higher in male (29%) then female (21.4%).

Education level 24% of the study participants were illiterate, 35% had primary education, 20% had upto secondary education & 17% were up to high school. Only 4% was graduate.HBV infection was maximum in illiterate 9/24 (37.5%) & patients

having education upto primary level, 12/35 (34.2%). (Table 1)

Residence In relation to living area, 42% of the CLD patients were urban dwellers and the remaining 58% were rural dwellers. HBV positivity was more in rural area(31%) then in urban area(26.1%)

Marital Status Most of the clients were found to be married. Among the 100 analyzed CLD Patients, 91% were married, 9% were single. Among CLD patients, HBV infection was maximum in married 28.5% as compared to single 22.2%.

Alcohol intake 62% gave the history of harmful alcohol intake. Among them, 30% were HBsAg positive as compared to 18.7% of non alcoholics. (Table 2)

Possible risk factors In 12 % CLD cases, no possible route of transmission of HBV could be elucidated as they could not adequately recall any significant past history. Among the known risk factors, blood transfusion 36.6% and previous surgical intervention 33.3% were most common for HBsAg positive patients. (Table 3)

Comparision of results between rapid card test & ELISA- There is a significant difference between the reactivity of HBV rapid card test & ELISA. Among 100 study subjects, 24 were reactive for HBV infection by rapid card test while 28 were positive by ELISA. (Table 4)

Table 1- ***Education level of Chronic Liver Disease, Positive HBV Patients***

Educational status	CLD		HBV +ve	
	No	%	No	%
Illiterate	24	24	9	37.5
Primary school	35	35	12	34.2
Secondary school	20	20	3	15
High school	17	17	3	17.6
Graduate	4	4	1	25
Total	100		28	

Table 2- Alcohol abuse among CLD & HBV positive CLD patients

History of Alcohol abuse	CLD		HBV +ve	
	No	%	No	%
Alcoholic	62	62	19	30
Non alcoholic	38	38	9	23.6
Total	100		28	

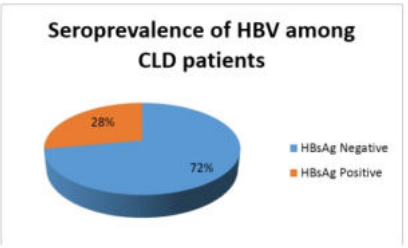
Table 3- Possible route of transmission of HBV among CLD patients

Risk factors	CLD	HBV		
	No	%	No	%
History of Blood Transfusion	30	30	11	36.6
Multiple Sexual partner	10	10	3	30
Major/Minor surgery	15	15	5	33.3
Tattooing	17	17	4	23.5
History Quack treatment	10	10	2	20
Frequent visit to barber shop (shaving)	6	6	0	0
No risk identified	12	12	3	25
IV drug abuse	0	0	0	0
Total	100		28	

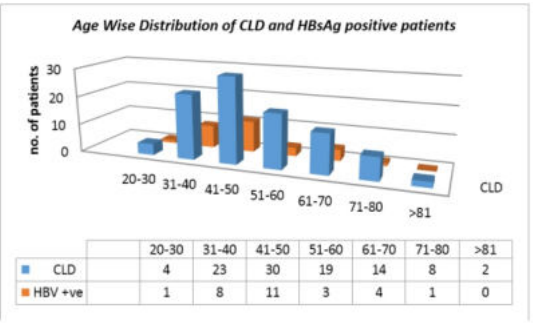
Table 4- Comparitive evaluation of HBV rapid card & ELISA test

Total	HBV positive by card	HBV positive by ELISA
100	24	28

Graph No.1



Graph No.2



DISCUSSION:

Among 100 CLD patient 28 patients shown HBsAg positivity. Similar results were found in other studies by Sanjay S *et al*[12] (HBV-36.3%), S. Saravanan *et al*[13] (HBV-9%), but variable results were observed by Singh V et al14 (30% HBV), and Chakravarti A *et al*[15] (60% HBV). Variable results may be due to the fact that in these studies various viral markers like HBsAg, HBV DNA, were detected. The variations are also due to geo-cultural, epidemiological, education level & population related variations in prevalence of hepatitis B

A high prevalence of HBV was recorded in the age group 41-50 with a prevalence of 36.7% which correlates with other study by Anirban K et al10 (41-50 years) but contradicts from Shanmugam S et al16 (55 years). The cause of high prevalence of HBV in younger age may be early childhood infection with the hepatitis viruses. HBV vaccine was not a part of universal vaccination program till 2009 so most of the patients reported, were from the pre-vaccination era. Also, there is no HBV vaccination schedule for adults in the current ongoing national vaccination program to prevent it.

The Gender wise distribution showed higher prevelence of HBsAg in male patients 29% as compared to female patients 21%, which correlates with studies done by Tessemma B et al17 (4.9% male, 3.3% female), but differs from the study done by Sanjay S et al12 (HBV-70.6% male, 29.4% female). High prevalence of Hepatitis in males might be due to fact that Males more frequently visits to barber and thus have more chances to get cuts from infected/contaminated & shared equipment. Moreover, men also are more likely to have multiple sex partners and follow unprotected sex. Most of the patients are from rural area, where due to social cultural environment, male are more concerned about their health & female health is not prioritised in the family. That can be probable causes of more males are coming to health facility for treatment.

In relation to residence area, 31% of HBV positive CLD patients were from rural area & 26.1% patients were from urban area. Comparable study from Anirban K et al10 Haryana also reported higher prevalence in rural area than urban area.Rural poor population are still dependent upon the untrained paramedics for their treatment needs which follow unsafe injection practice. Unfortunately, the sterilization of minor surgical instruments is often improperly done in rural areas. Also, there may be probable reuse of the disposable syringes by the quacks.

A significant proportion (62%, all male) of the participants had

history of excessive alcohol use. Out of them, 30% were HBsAg positive. Similar results are seen in other studies like by Saigal S et al18 (100% alcoholic, 23% HBV). Alcohol abuse and concurrent HBV infection are synergistic in causing CLD, causing hepatocellular damage and accelerating disease progression.

As seen with the marital status, the present study concludes that the prevalence of HBsAg is more in married CLD patients. These result are in agreement with study conducted in Anirban K et al123 2015, Haryana. It might be due to lack of awareness or education in people that had exposure prior to marriage which is then transmitted to her or his spouse

While comparing rapid card test & ELISA, in the present study, ELISA was inferred as much better method then the Immunochromatographic test strip as it shows 28% HBV positivity as compared to 24% by card. Similar results were observed in other studies by Mohammad K et al19 (card positivity of HBV-22 cases and ELISA positivity of HBV 30 cases).

CONCLUSION

We conclude that, HBV infection is high among CLD patients (28%). The risk is more in patients with 41-50yrs age group, married, male, poorly educated or illiterate, from rural area & alcoholic individuals . Blood transfusion appears to be as one of the major means of transmission of HBV infection, as most of the patients were giving the history of blood transfusion. All high risk groups and CLD patients should be screened for HBV infection so as for early detection of infection, thus to reduce the complications and to reduce the transmission. Rapid test kits are not very sensitive enough to confirm the hepatitis status of a CLD patient. Therefore, it is recommended that, rapid test kit should be used in conjunction with other immunoassay particularly ELISA technique. It is required to educate the people for proper safety measures such that- to avoid common use of syringes, avoid intravenous drug abuse, proper disposable of infected biohazards, wearing gloves on handling the infected samples, so that they aware about these infectious disease to reduce its transmission. There is need to add vaccination schedule for HBV in immunization schedule for adults also, so as to reduce its prevalence in community. Other modes of transmission like tattooing, major/minor surgery are also important and should be given attention.

So, all CLD patients should be screened for HBV infection for early detection of infection, thus to reduce the complications and transmission.

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