Detection of HBV DNA in ascitic fluid of decompensated cirrhotic patients to assess infectivity: A novel pilot study

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Research Article

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Abstract

Background and aims: Body fluids such as saliva, tears and urine from patients with hepatitis B virus (HBV) infection are well known to be infectious. However, the infectivity of internal body fluids like ascitic fluid from patients with HBV infection has not been established. So, we conducted this study to know the infectivity of ascitic fluid for hepatitis B in decompensated cirrhosis by detecting HBV DNA in it.

Methods: Patients with HBV related cirrhosis with ascites were enrolled. The levels of HBV DNA in the ascitic fluid from these patients were quantified by real-time PCR, and compared with HBV DNA levels in serum. Clinical and laboratory parameters to predict HBV DNA positivity in ascitic fluid were also assessed.

Results : Twenty one patients (mean age 45.43±13 years) with HBV related cirrhosis with ascites were enrolled. HBV DNA in ascitic fluid was detected in 4/21 (19 %) patients. The ascitic fluid HBV DNA levels ranged from 4.8 to 6.4 log copies/mL (mean ± SD = 5.27 ± 0.55). High levels of serum HBV DNA was significantly associated with HBV DNA detectability in ascitic fluid (p=0.001). Patients with HBV DNA detected in ascitic fluid had significantly higher serum protein levels as compared to those having undetectable HBV DNA in ascitic fluid (6.83 ± 0.33 versus 5.70 ± 0.77 g/dl, p=0.011).

Conclusions : HBV DNA is detectable in ascitic fluid in about one fifth of HBV related cirrhosis patients with ascites so it may not be considered an important source for HBV transmission. High serum HBV DNA and high serum protein levels were positively associated with HBV DNA detectability in ascitic fluid.

Introduction

Hepatitis B related cirrhosis is a major public health problem leading to significant morbidity & mortality. About 4% of all cirrhosis is caused by hepatitis B(1). About 20% of patients with cirrhosis have ascites at their first presentation(2). It is estimated that millions of therapeutic and diagnostic ascitic taps are performed each year in hepatitis B related cirrhosis patients worldwide, posing a healthcare hazard. The prevalence HBV in different category of healthcare workers ranges from 6-7% in various studies(3,4). Occupational exposure is a major risk factor. HBV is transmitted efficiently by percutaneous and mucous membrane exposure to infectious body fluids(5).

So, it is important to know the infectivity of ascitic fluid to prevent horizontal transmission to health care workers via this route. As there is no good tissue culture for HBV to determine the infectivity of body fluids, the presence of HBsAg or HBV DNA in them have been extrapolated as surrogate markers for their infectivity. As far as the presence of HBV DNA in ascitic fluid is concerned, the current literature does not provide any useful information. However, presence of HBV DNA has been shown to be present in various other body fluids like serum, saliva, nasopharyngeal fluid, urine, semen, vaginal fluids and tears in the HBV infected patients(6–8). A relationship between HBV DNA levels in different body fluids and blood has also been reported along with a potential of horizontal as well as vertical transmission in different studies(7–9). Despite the availability of the evidence with respect to presence of HBV DNA in different

body fluids and its correlation with serum viral load, possibility of horizontal and vertical transmission and its predictive and prognostic value, there had been no study till date, assessing the HBV DNA in ascitic fluid and its association with clinical profile, predictive value and correlation with serum viral load.

With this background, the present study was carried out with the aim to know the infectivity of ascitic fluid for hepatitis B in patients suffering from decompensated liver cirrhosis and the objectives were to determine the proportion of patients with HBV DNA detectable in ascitic fluid to that with total number of HBV cirrhosis patients with ascites, to study the correlation of HBV DNA level in ascitic fluid with serum viral load and to determine the predictors of HBV DNA detectability in ascitic fluid.

Methods

Study design, setting, period and duration

This was a pilot observational study in which patients admitted to the indoor facility of the department of Medicine, Medical and Surgical Gastroenterology of our institute from July 2020 to July 2021 were enrolled. Laboratory tests were done in collaboration with the department of Microbiology of our institute.

Patients

Patients more than 18 years with HBV related chronic liver disease with ascites, willing and able to provide consent were included in the study. Those patients who were not giving consent, critically ill, aged less than 18 years, without ascites or any local skin infection like cellulitis, abscess were excluded from the study.

Ascitic fluid and serum collection and transportation

5 ml of ascitic fluid and 5 ml of serum sample were taken into plain vial under aseptic conditions and samples were transferred to the Microbiology lab immediately and stored them at 4°C for 24 hours and then at -20°C for 5 days and processed for detection of HBV DNA viral load.

Detection of HBV DNA in serum and ascitic fluid

Hepatitis B DNA viral load assay in serum and ascitic fluid were performed as per patent molecular protocol as described by Prakash S et al(10). The control were validated before the PCR run. RNAseP was checked for every clinical sample quality. The viral load HBV quantification was done by using standard protocol.

Data collection

Relevant demographic, clinical and laboratory data were collected for all patients on a pre-designed proforma. All patients underwent a detailed history, including presenting complaints, past and personal history, detailed history of recent exposure and risk factors. Findings of complete blood counts, liver and kidney function tests, ultrasound and/or CT abdomen, upper gastrointestinal endoscopy were recorded.

Statistical analysis

Continuous data were summarized as mean ± SE (standard error of the mean) whereas discrete (categorical) in number (n) and percentage (%). Continuous two independent groups were compared by Student's t test. Categorical groups were compared by the chi square ($\chi 2$) test. A two-tailed ($\alpha=2$) $p<0.05$ was considered statistically significant. Fisher's exact test was employed when sample sizes were smaller than 5.

Results

Due to COVID pandemic, only 21 cases could be enrolled. The demographic, clinical & laboratory parameters of patients are shown in Table 1. The majority, 18 (85.7%) were males. Lower class socioeconomic status (SES) was found in maximum (85.7%) cases. The mean age and Body mass index (BMI) were 45.43±13.12 years and 20.20±1.81 kg/m^2 respectively. Out of 21 cases, 6 (28.6%) had a history of alcohol intake. No history of pregnancy, family history and drug history was found in any cases. Hepatic encephalopathy, upper gastrointestinal bleed, hepatomegaly, shrunken liver and splenomegaly were found in 9 (42.9%), 7 (33.3%), 2 (9.5%), 8 (38.1%) and 14 (66.7%) cases respectively. One case expired while the remaining 20 (95.2%) were discharged.

The mean hemoglobin, platelets, total leucocyte count, mean corpuscular volume and mean corpuscular hemoglobin were 9.29±2.65 gm/dl, 7.4±3.7 lac, 65.19±44 cells/mm^3, 88.41±11.16 fL and 29.63±4.58 pg respectively. The mean serum bilirubin, serum protein, serum albumin, ALT, AST, PT and INR, were 5.97±8.91 mg/dl, 5.91±0.84 gm/dl, 2.66±0.52 mg/dl, 93.15±32.11 IU/ml, 84.12±36.11 IU/ml, 23.21±8.64 sec and 1.77±0.66, respectively. The mean urea and creatinine were 45.13±23.81mg/dl and 1.35±0.67 mg/dl respectively. HBsAg was reactive in all 21 cases. Anti-HCV and HIV were negative in all cases.

17 (80%) patients had detectable HBV DNA in serum, 14 (66%) patients had high viral load (>10^5 IU/ml), while 7 (33%) patients had low viral load (<2000IU/ml). The mean CTP and MELD was 9.41±3.4 and 20.32±6.21 respectively. The HBV DNA in ascitic fluid was detected in 4 (19%) cases while in 17 (81%) cases, it was not detected (Table 1). The calculated 95% confidence intervals for the sample size used in the study was in the range 5.45% to 41.91%. In all the cases, serum HBV DNA level was higher than the ascitic fluid level except one case, in which serum HBV DNA was undetectable (Case 2) (Figure 1).

Discussion

HBV is one of the most common underlying causes of decompensated liver cirrhosis, especially in tertiary care hospitals where people often report to a healthcare facility only when the disease is in its advanced stage. Ascites is one of the most commonly encountered complications of cirrhosis. It might be proposed that after viral translocation, virus may enter the systemic bloodstream and access ascitic fluid.

In the present study, only a total of 21 patients fulfilling the eligibility criteria could be enrolled owing to COVID-19 pandemic, thus the present study reflects a pilot study to assess the issue in question. We

enrolled patients with decompensated cirrhosis only. The mean age of patients was 45.43 years and mean BMI was 20.2 kg/m^2. The study sample was predominantly male (85.7%) and was dominated by a lower socioeconomic class (85.7%) marked by high prevalence of uneducated patients (61.9%). HBV DNA was found to be positive in 4 (19.0%) ascitic fluid specimens only. Detection of HBV DNA in different body fluids in serum positive patients has been reported to vary substantially(6). Detection rates in different body fluids might be dependent on the interaction of these body fluids with the bloodstream. It would also be pertinent to mention here that in the present study, HBV DNA in serum was found in only 17 (81%) cases. Moreover, there were three cases having low serum HBV DNA expression (<2000 IU/L). It was also observed that one of the patients with undetectable serum HBV DNA had detectable HBV DNA in ascitic fluid. This finding indicates thst it is difficult to state that the detectability of HBV DNA is solely dependent on serum positivity. However further studies are needed to reach any definite conclusion. Although, some case reports like the one by Fagan and Coworkers et. al(11) who detected HBV DNA in the urine, semen and saliva of a HBV patient who failed to have detectable HBV DNA levels in serum. It is important to understand the reason for this discrepancy which might be dependent upon heterogeneity in the assay procedures used for detection of HBV DNA in different body fluids.

As far as replicability is concerned, there seems to be high inconsistencies among different studies(6–8,12–16). In the present study, we could detect HBV DNA in only 19% of ascitic fluid specimens in decompensated cirrhosis patients. The calculated 95% confidence intervals for the sample size used in the present study was in the range 5.45% to 41.91%, thus offering a vast range for replication of results. It must be seen that the previous studies have reported detection rates in different fluids to be highly diversified. Table 2 below shows the HBV DNA detection rates for different body fluids in different studies along with the calculated confidence intervals.

On the other hand van der Ejik *et al*(16) in their series of 27 chronic HBV patients, detected the HBV DNA in 23/27 (85%) of saliva specimens, thus showing a high detectability in saliva. However, there is a high chance of incidental findings as in a subsequent study the same authors failed to get replicable results and reported only 47% of saliva specimens to be positive for HBV DNA. In this study they also assessed HBV DNA in 32% of urine specimens(15).

Compared to the present study, van der Eijk(15,16) carried out their study in chronic hepatitis B patients but did not provide any information regarding the sociodemographic and anthropometric profile except for a male dominance (63%). The study of Heiberg et al.(13) conducted their study among children with chronic hepatitis B. Lin et al.(17) carried out their study in a study population consisting of adult hepatocellular carcinoma, hepatitis and cirrhosis patients. Jain et al.(14) carried out their study on 60 adult patients with a mean age 48.8 years with various chronic hepatitis B conditions including cirrhosis and HCC and a dominance of males (58.3%). The role of demographic profile becomes an important issue as detection of HBV DNA in different body fluids is considered to be transmitted from the bloodstream and thus the patient disease profile, nutritional and hygiene status and other sociodemographic factors are deemed to have a determining role.

A number of previous studies have tried to explore the predictive or prognostic role of detection of HBV DNA in body fluids(6). In the present study, we also assessed the relationship of HBV DNA detection in ascitic fluid with various clinico-demographic and outcome factors but failed to find a significant association except a significant difference in serum protein and HBV DNA levels. Serum protein levels of those with HBV DNA positivity in ascitic fluid were significantly higher as compared to those having undetectable HBV DNA in ascitic fluid.

Given the fewer number of cases with detectable HBV DNA in ascitic fluid (n=4/21), the statistical significance of any association needs to be viewed with caution. Most of the previous studies did not report an association of HBV DNA detection in different body fluids with clinico demographic profile or outcome.

Other internal body fluids like pleural fluid, crebrospinal fluid and pericardial fluids may be considered equivalent to ascitic fluid. One case report described presence of HBsAg in pleural fluid(18). However, no attempts have been made to look for presence of HBV DNA in them and their potential for infectivity may be explored in further studies.

Only raised serum protein and high serum HBV DNA level were positively associated with detection of HBV DNA in ascitic fluid. All other clinical and laboratory parameters were found not significantly associated. Further studies are needed to find the pathophysiological significance of this finding.

Detection of HBV DNA in ascitic fluid is comparable to detection of complete virion in it, as no good virus culuture is available. However, studies aimed to detect complete virion in body fluids including ascitic fluid may be planned to further substantiate the concept of body fluids as potential sources of horizontal transmission.

There were several limitations of the study. Smaller sample size, mostly descriptive data, absence of HBeAg and ascitic fluid parameters were few of them. However, the findings of the present study, within limitations, provide the first evidence of detectability of HBV DNA in ascitic fluid in patients with decompensated cirrhosis. Further studies with larger sample size are required to explore the replicability of these results and their clinical implications to establish the usefulness of such investigations in clinical or epidemiological context.

Conclusions

Our study concluded that, HBV DNA is detectable in ascitic fluid in about one fifth of patients with HBV related decompensated cirrhosis with ascites. So ascitic fluid may not be considered an important source for HBV transmission for epidemiological purposes. However, healthcare workers and laboratory staff may be warned while handling ascitic fluid of HBV infected persons. Serum HBV DNA and protein levels are the determining factors for HBV DNA positivity in the ascitic fluid.

Abbreviations

ALT
Alanine aminotransferase
AST
Aspartate aminotransferase
BMI
Body mass index
CDC
Centers for Disease Control and Prevention
CLD
Chronic liver disease
COVID
Coronavirus disease
DNA
Deoxyribonucleic acid
Hb
Hemoglobin
HBeAg
Hepatitis B envelope antigen
HBeAg
Hepatitis B envelope antigen
HBsAg
Hepatitis B surface antigen
HBV
Hepatitis B virus
INR
International normalized ratio
PT
Prothrombin time
RT-PCR
Real time polymerase chain reaction
RNAseP
Ribonuclease phosphodiesterase
TLC
Total leucocyte count
WHO
World Health Organization

Declarations

Ethics declaration

The project was approved by the ethical committee of King George Medical University, Chowk, Lucknow, India (No.148/Ethics/2021, dated 02-02-2021). Written informed consent for collection of ascitic fluid and blood was taken from each patient. The study was performed as per guidelines of Helsinki declaration and the Guideline for Good Clinical Practice. This article does not contain any studies with animals performed by any of the authors. Any issue related to plant reproducibility was not involved with the article.

Guaranter of the article

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Data Availability

Data will be available from corresponding author upon reasonable request.

Role of medical writer

None

Conflict of interests

The authors declare no conflict of interests.

Financial disclosure

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Specific author contribution

Concept design, data collection, analysis, manuscript writing: Ajay Kumar Patwa; Data collection, analysis, manuscript writing: Shiv Pratap Singh; Manuscript writing, critical analysis of data: Virendra Atam; Manuscript writing, critical analysis of data: M L Patel; Manuscript writing, critical analysis of data: Harish Gupta; Manuscript writing, critical analysis of data: Sudhir Kumar Verma; Microbiological analysis: Amita Jain; Microbiological analysis: Suruchi Shukla; Data collection, critical analysis: Sumit Rungta; Data collection, critical analysis: Abhijit Chandra

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Tables

Table 1 : Demographic, clinical and laboratory parameters in cases with HBV DNA detected and not detected in ascitic fluid

Variable	Overall (N=21)	HBV DNA in ascitic fluid not detected (N=17)	HBV DNA in ascitic fluid detected (N=04)	p-value
Age (years)	45.4 ± 13.1	44.47 ± 13.34	49.50 ± 13.08	0.504
Gender (M/F)	18/03	15/02	3/1	0.489
Alcohol (Yes/No)	15/06	14/3	1/3	0.053
Splenomegaly (Yes/No)	7/14	7/10	0/4	0.255
Esophageal varices (Yes/No))	8/13	8/9	0/4	0.131
Hepatic encephalopathy (Yes/no)	09/12	6/11	3/1	0.468
Jaundice (Yes/no)	11/10	9/8	2/2	0.391
UGI bleed (Yes/no)	7/14	7/10	0/4	0.862
Shrunken liver (Yes/no)	13/08	12/5	1/3	0.642
Outcome (Discharge/Expiry)	20/01	16/1	4/0	0.510
HB (gm %)	9.29 ± 2.65	9.04 ±2.59	10.38 ± 3.06	0.377
TLC (10^2/mm^3)	65 ± 44	6915.47 ± 4797.25	4625.00 ± 1241.97	0.364
Platelets (10^3/mm^3)	74 ± 37	0.74 ± 0.41	0.76 ± 0.06	0.937
S.Bil (mg/dl)	5.97 ± 8.91	6.30 ± 9.62	4.57 ± 5.68	0.736
S. Protein (gm/dl)	5.91 ± 0.84	5.70 ± 0.77	6.83 ± 0.33	0.011
S. Albumin (gm/dl)	2.66 ± 0.52	2.63 ± 0.54	2.78 ± 0.52	0.636
PT/INR (sec)	23.21±8.64/1.77 ± 0.66	24.01 ± 9.12/1.82 ±0.70	19.83 ± 5.92 /1.53 ± 0.41	0.397/0.438
Creatinine (mg/dl)	1.35 ± 0.67	1.38 ± 0.68	1.26 ± 0.72	0.767
S. HBV DNA (IU/ml)	2.4×10^8 ± 1.06×10^9	8.7×10^6 ±2.8×10^7	1.2×10^9±2.4×10^9	0.001
CTP	9.41 ± 3.42	9.23 ± 3.21	10.20 ± 2.20	0.618
MELD	20.32 ± 6.21	21.32 ± 6.21	19.23 ± 3.21	0.496

Note: Continuous data were presented as mean±SD and discrete data as absolute numbers.

Table 2: HBV DNA detection rates for different body fluids in different studies

SN	Body fluid	Author (Year)	HBV marker studied	Sample size	Detection rate	95% Confidence intervals
1.	Saliva	van der Ejik *et al.* (2004)(13)	HBV DNA	27	85%	66.3%-95.8%
		van der Ejik *et al.* (2005)(12)	HBV DNA	147	47%	38.7%-55.3%
		Kidd *et al.* (2006) (18)	HBV DNA	80	10%	4.4%-18.8%
		Helberg *et al.*(2010) (7)	HBV DNA	43	0%	-
		Komatsu *et al.*(2012) (6)	HBV DNA	38	87%	71.9%-95.6%
		Kamimura *et al.*(2021) (8)	HBV DNA	48	40%	25.8%-54.7%
2.	Urine	van der Ejik *et al.* (2005) (12)	HBV DNA	147	32%	24.5%-40.2%
		Komatsu *et al.*(2012) (6)	HBV DNA	19	74%	48.8%-90.9%
		Jain *et al.* (2018)(11)	HBV DNA	59	45.8%	32.7%-59.3%
		Lin *et al.* (2021)(9)	HBV DNA	32	100%	-
3.	Tears	Kidd *et al.*(2006) (18)	HBV DNA	7	57%	18.4%-90.1%
		Komatsu *et al.*(2012) (6)	HBV DNA	11	100%	-
4.	Vaginal Fluid	Shu-Lin Zhang *et.al* (2004) (10)	HBV DNA	59	52.5%	-
5.	Semen	Xuefeng Huang,*et.al* (2015) (14)	HBV DNA	151	43%	-
6.	Ascitic fluid	Present study (2021)	HBV DNA	21	19.0%	5.45%-41.91%,

Figures

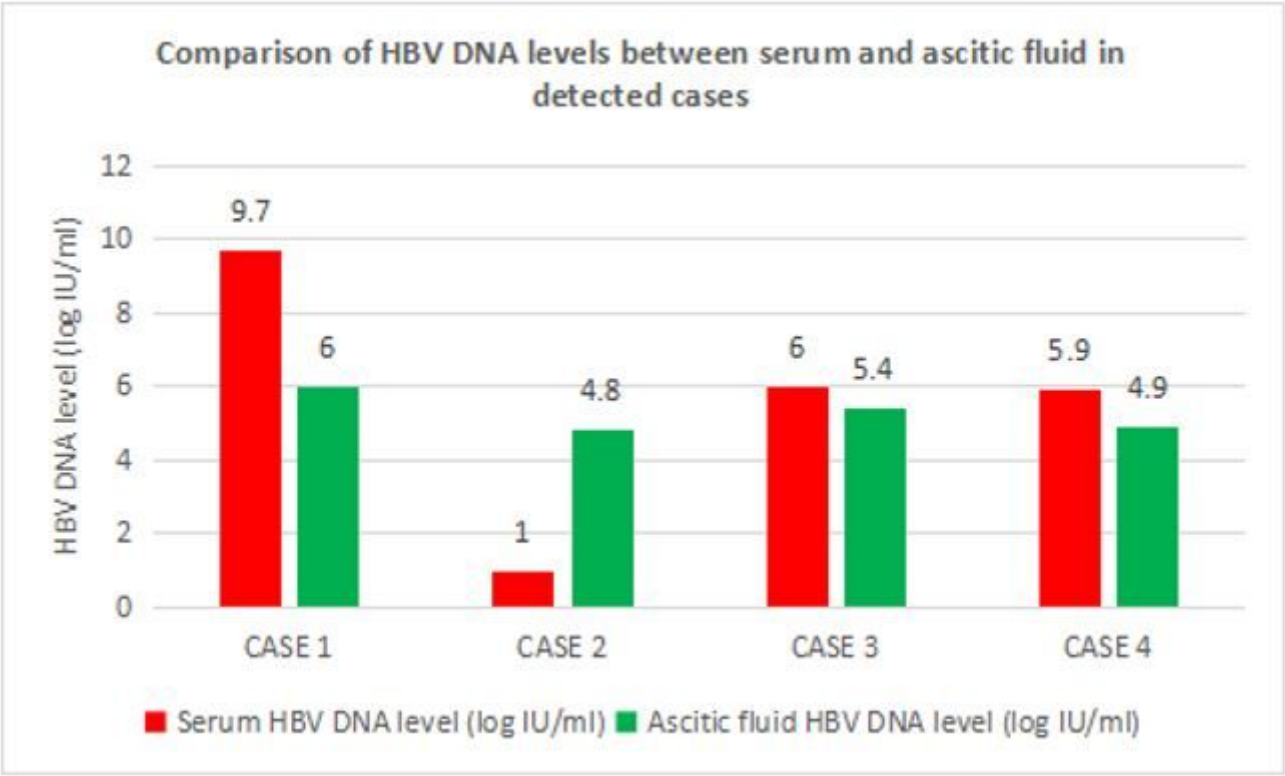

Figure 1

Comparison of HBV DNA levels between serum and ascitic fluid in detected cases