MEDICAL CHANNEL

Original Article

PREVALENCE OF HEPATITIS B VIRUS (HBV) IN ASSOCIATION OF HEPATITIS DELTA VIRUS (HDV)

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ABSTRACT

Objective: To assess the prevalence of Hepatitis B Virus (HBV) in association of Hepatitis Delta Virus (HDV) as well as to evaluate the viral load and liver function tests of HBV and HDV associated patients.

Materials & Methods: Study Design: Prospective Observational study

Setting: Diagnostic and Research Laboratory, ISRA University Hospital, Hyderabad, Pakistan

Study duration: May 2011 to October 2011.

Sample Size: A total number of 104 HBV DNA positive samples were included in this study. All the patients were of both genders with in the age between 10 to 70 years of age.

Methodology: The blood samples were collected from the different collection points in Hyderabad District and its adjacent areas (Sanghar, Shehdadpur, Kandiyaro, Dadu and Badin) 5.0ml of venous blood samples of patients was collected in serum separation tubes or in sterile tubes using EDTA as the anticoagulant.

Results: From total samples tested 71 patients of HBV positive were male and 33 patients of HBV positive were females. In male population, 68% were HBV positive while in females, 32% were HBV positive. 14 patients were from group I, 38 patients from group II, 22 patients from group III, 18 patients of group IV, 05 patients of group V and only one (01) patient of group VI. As results showed that HBV infection is highly prevalent in group II (36%) followed by 21%, 17%, 13%, 04% and 0.96% in group III, group IV, group I, group V and group VI respectively. The highest viral load 610,000 IU/ml was recorded in a 27 years old female patient. Similarly the lowest viral load (200 IU/ml) was observed in a 15 years old male patient.

Conclusions: The trend of the prevalence of HDV is decreasing globally, and this can not be controlled until the high prevalence of HBV is controlled. There is a dire need to educate public about diseases, their complications, route of transmission and risk factors in this region especially in anterior Sindh.

INTRODUCTION

Hepatitis is inflammation of the liver which can be caused by chemicals, toxins, herbal remedies, alcohol, autoimmune diseases and viruses.[1] Viral hepatitis is usually used for the infection caused by one of following different and distinct agents collectively known as hepatitis viruses and these include hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis C virus (HCV), hepatitis D virus (HDV), hepatitis E virus (HEV) etc. The liver is responsible for remarkable body functions, such as metabolism, exocrine, endocrine, reticuloendothelial and immunologic functions.[1, 2]

Hepatitis B virus (HBV) infection is a major global health problem especially in Asia, Africa, southern Europe and Latin America.[3] About 40% of the world's population has

had contact with or are carriers of the HBV,[2] 400 million among them are suffering from chronic HBV infection, 50 million people pass this disease every year, resulting in 1 to 2 million deaths. Pakistan is highly endemic with HBV with nine million people infected with HBV with 3-5% carrier rate and its infection rate is on a steady rise. The reason may be the lack of proper health facilities, poor economical status and less public awareness about the transmission of major communicable diseases including HBV, HCV and HIV.[3]

Clinical demonstrations of HBV has broad spectrum that variegate among individuals in acute and chronic conditions. Manifestations range from sub clinical hepatitis to anicteric hepatitis, icteric hepatitis and fulminant hepatitis during acute condition while in chronic condition, manifestations range from an asymptomatic carrier condition to chronic hepatitis, leads to cirrhosis and hepatocellular carcinoma.[4] The clinical occurrence of HBV infection mainly depends upon three things i-e the age at infection, the level of HBV replication and the immune status of the host.[5]

Hepatitis D virus, classified as Hepatitis delta virus, is a condition caused by a small, circular RNA virus. Hence it can propagate only in the presence of hepatitis B virus therefore HDV is considered to be a subviral satellite. Transmission of HDV can takes place in two ways i-e coinfection (simultaneous infection with HBV), and superinfection (superimposed on chronic hepatitis B or hepatitis B carrier state). In more severe convolution, both superinfection and coinfection with HDV results is compared to infection with HBV alone. These complications carry a greater chance of, liver failure in acute infection and a rapid evolvement to liver cirrhosis, with an increased contingency of developing liver cancer in chronic infection.[6]

About 20 million people are infected with HDV worldwide and 5% of the HBV carriers have hepatitis D virus (HDV) superinfection.[5,6] "Superinfection of HDV in persons with HBV infection leads to progressive disease and cirrhosis in approximately 80% of cases".[7] Hepatitis D in combination with hepatitis B virus has the highest mortality rate of all the hepatitis infections of 20%. In Pakistan, 16.6% of patients infected with hepatitis B virus also suffered from superimposed delta infection.[8] However, this local data is based on serology of HDV. It is not clear how many of local patients spontaneously recover from the infection and how these patients differ from patients with persistent infection.

MATERIAL AND METHODS

Objective: To assess the prevalence of Hepatitis B Virus (HBV) in association of Hepatitis Delta Virus (HDV) as well as to evaluate the viral load and liver function tests of HBV and HDV associated patients.

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RESULTS

HBV DNA was detected through polymerase chain reaction using

FIGURE-1:
Distribution of HBV patients according to gender (Males & Females)

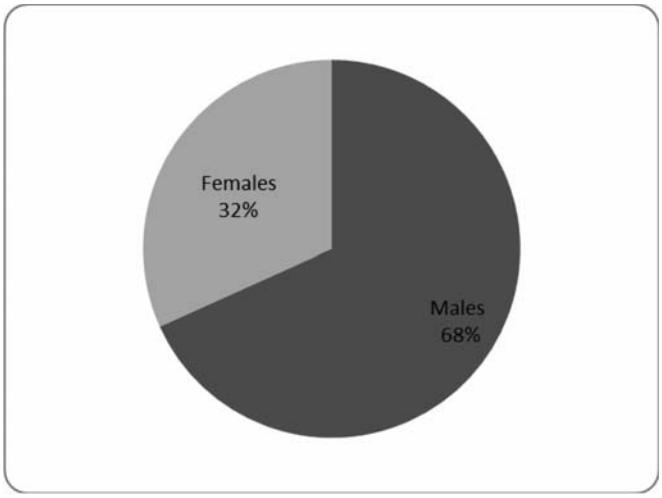


FIGURE-2:
Distribution of HBV patients according to age

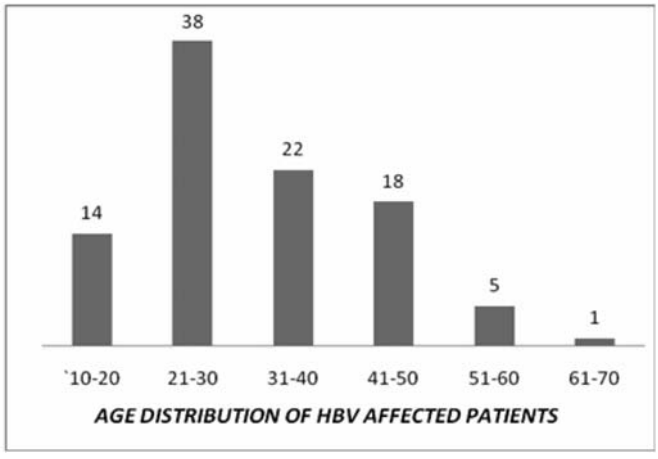


FIGURE-3:
Screening of HBV patients for HDV through ELISA

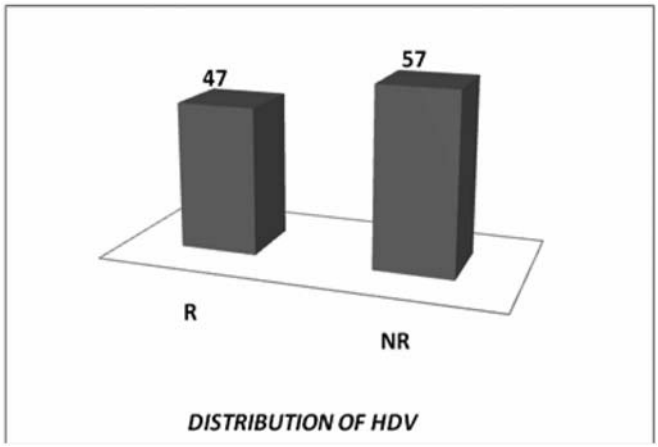


FIGURE-4:
Age Distribution

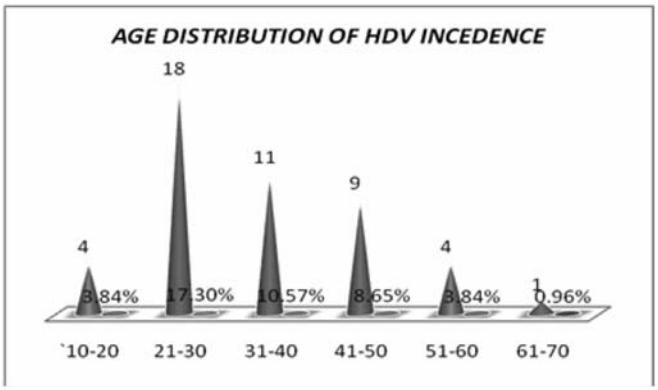


TABLE 1:
Liver Function Tests of HBV positive patients

Age Groups	Liver Function Tests (LFTs)			
	AlbuminMean (U/L)	BilirubinMean (mg/dl)	ALT/SGPTMean (U/L)	AST/SGOTMean (U/L)
Group-I	4.18	0.83	65.33	48.73
Group-II	3.96	0.87	53.69	46.07
Group-III	3.64	0.86	87.14	85.66
Group-IV	3.22	0.997	94.72	69.0
Group-V	3.56	0.742	50.66	44.83
Group-VI	4.2	0.2	11	25
Low & High Limits	3.5 - 4.8 (M & F)	0.1 - 1.0 (M & F)	9 – 40 (M) 6 – 27 (F)	8 – 40 (M) 6 – 34 (F)

instrument COBAS AMPLICOR HBV Monitor test. The PCR positive samples were from both sexes. From total samples tested 71 patients of HBV positive were male and 33 patients of HBV positive were females. In male population, 68% were HBV positive while in females, 32% were HBV positive patients as presented in **Figure-1.**

According to age wise distribution of HBV patients, 14 patients were from group I, 38 patients from group II, 22 patients from group III, 18 patients of group IV, 05 patients of group V and only one (01) patient of group VI. As results showed that HBV infection is highly prevalent in group II (36%) followed by 21%, 17%, 13%, 04% and 0.96% in group III, group IV, group I, group V and group VI respectively as presented in **Figure -2.**

The HBV DNA viral load or quantification of all 104 samples was also done through Abbott m2000rt (RealTime) PCR. According to results, it was observed that viral load was significantly high in individuals below the 30 and almost all these patients with higher viral load showed HDV ELISA non reactive. The highest viral load 410,175,303 IU/ml was recorded in a three years old baby followed by 178,614,658 IU/ml, 98,795,587 IU/ml, 43,132,186 IU/ml in 44 years (male), 27 years (female) and 13 years (male) respectively with non reactive HDV ELISA. Similarly, individuals showing HDV ELISA reactive showed low viral load. In case of individual showing HBV with HDV, the highest Hepatitis B viral load 822,509 IU/ml in a male of 52 years old, which also showed hepatitis D viral load 780,00 IU/ml. Out of 104 patients, 33 showed less than 100 IU/ml viral load and 9 individuals of both gender showed the lowest viral load (10 IU/ml). Similarly no relation was observed with minimal viral load with HDV.

To observe the incidence of hepatitis D infection along with HBV infection, HDV ELISA was performed. From all HBV positive patients, 57 were showed non-reactive for HDV infection during HDV ELISA test while 47 patients were found reactive for HDV infection during HDV ELISA. According to results presented in **figure-3**, 45.2% infective is reactive and 54.8% non reactive thus showing 45.2% prevalence of HBV in association with HDV was observed in Hyderabad and adjacent regions.

The ELISA reactive samples were further confirmed through nested PCR. HDV On the basis of HDV ELISA reactive and HDV DNA positive, the incidences of HDV were distributed according to age group. The highest incidences of DHV was observed in age group II in 18 (17.3%) patients followed by group III and group IV in 11 (10.6%) and 9 (8.6%) patients respectively. Similarly, 4 (3.84%) incidences of HDV was observed in both

groups 1 & V while only one (0.96%) patient was from group VI as presented in **figure-4.**

The viral load of HDV ELISA reactive patients was also determined. The highest viral load 610,000 IU/ml was recorded in a 27 years old female patient. Similarly the lowest viral load (200 IU/ml) was observed in a 15 years old male patient. The elevation of LFTs was also observed in HBV and HDV positive patients, e.g. patient showing the highest HDV load also showed elevated values including 160 U/L (SGPT), 107U/L (SGOT) and 3.5 mg/dl (bilirubin).

Liver function tests are imported biochemical assays that indicate the situation of liver. Different LFTs including albumin, bilirubin, ALT/SGPT and AST/SGOT of all 104 HBV positive patients were also performed to observe the condition of liver. The LFT values of patients were varied from patient to patient that indicated the severity of disease. The results were divided in to different groups according to their age groups. According to results presented in **table 1**, mean albumin and bilirubin values were 3.22 – 4.2 U/L and 0.2 – 0.997 mg/dl respectively. Similarly ALT/SGPT and AST/SGOT mean values were 11 – 94.72 U/L and 25 – 85.87 U/L respectively.

The individual patients showed significant differences with elevated values of LFTs. The highest albumin value (5.5 U/L) was observed in a patient of 40 years HBV positive and HDV negative patient. Overall 12 HBV positive patients showed elevated values above 4.8 U/L. while 32 patients showed lower values below 3.5 U/L. In patients showing elevated values, 4 were HDV positive while 8 were HDV negative. In case of bilirubin, 15 patients showed elevated values i.e. above 1.0mg/dl, in which 7 were HDV positive and 8 were HDV negative.

In case of SGPT test, the highest elevated value was 730 U/L in 40 years of age male. Among all patients 62 showed elevated SGPT value, in which 40 patients were males with 18 HDV positive and 22 were HDV negative patients. Similarly 22 were females showed elevated values with 14 HDV positive and 8 HDV negative patients. From all 62 patients with elevated values, 13 patients showed the elevated values above 100 U/L in which 8 from males and 5 females. Among males, 5 were above 40 years of age and 3 below 40, while in case of females, 4 females were below 40 and only one was above 40 ages that showed above 100 U/L elevated values.

The AST/ SGOT test was also performed. In AST, the highest elevated value (923 U/L) was recorded in the same 40 years of male that showed the highest elevated value (730 U/L) of ALT/SGPT. From all patients, 56 individuals showed elevated values of ALT. Among these, 39 were males with 24 HDV positive and 15 HDV negative while 17 females showed elevated values, with 7 HDV positive and 10 HDV negative. Similarly 7 individuals showed above 100 U/L elevated values with 4 males, all 40 – 45 years of age and 3 females, all 26 – 30 years of age.

DISCUSSION

This is the first detail report about HBV/HDV con-infection in Hyderabad and adjacent regions of Sindh. HBV patients can be simultaneously infected by HDV resulting in a severe form of liver disease. There are variable reports, as to which virus is actively responsible for liver disease in patients with chronic HDV infection – HBV/ HDV.[9] According to results from 104 patients HBV, 68% were males and 32% were females, 45% patients showed HDV co-infection. The highest infection rate was observed in individuals between 20 – 40 years of age. This was first detailed study at Hyderabad and adjacent regions showing the association of HBV with HDV. This kind of research has already been done by Mumtaz et al,[10] but the samples were collected from all over the Pakistan. Mumtaz et al reported 35% HBV/HDV coinfection, which is lower than reported in our results (45%). Similarly Zaidi et al[11] also reported the co-infection of HBV and HDV in the largest province of Pakistan but this is the first detailed work in Hyderabad region. The percentage of co-infection in reported region is higher in males (64%) as compared to female patients (36%). The viral load of HBV single infection was the highest as compared to the viral load in patients of HBV/HDV co-infection. The results showed that HDV infection may reduced the viral load of HBV significantly. Our results may also be confirmed from previous report. Mumtaz et al., [10] also reported the suppression of HBV DNA due to HBV/HDV co-infection. The average of HBV DNA viral load for HDV reactive patients was 19393.541667 U/L while average viral load of HBV DNA for HDV non-reactive patients was 15520811.316 U/L. A fair proportion of HBV/HDV coinfected patients with HBeAg negative have active hepatitis B infection and cirrhosis as compared to those with mono-infection. HBV/HDV co-infection produces a more aggressive disease as compared to mono-infection with hepatitis B.

Our studies also include liver function test (LFT) of patients which is important biochemical marker, used to detect the liver diseases. The liver function test includes serum analysis of SGPT, SGOT, Albumin and Billirubin. The normal ranges of LFT vary from laboratory to laboratory, and the standard values of ISRA University Hospital are SGPT 3-40U/L, SGOT upto 37U/L, Albumin 3.5-5.5g/dL and bilirubin 0.3-1.2 mg/dl, and elevations in these values show disruption of normal functioning of liver cells. The highest elevated values of SGPT 94.72 U/L was observed in age group of 41-50 years, followed by 87.14 and 53.59 U/L in age groups between 31 – 40 and 21 – 30 years. The highest elevated SGOT value 85.66 U/L was observed in age group between 31 – 40 years followed by 69.0 U/L in age group IV (41 – 50 years). Mumtaz et al[10] also reported the elevation of ALT and co-infection as compared to mono-infection. Our results are agreed with this report. The average values of LFT of our samples were found to be not much elevated, which is due to the reason that our patients were those who were under treatment.[8,9] The HDV infection occurs in two states along with hepatitis B infection; HBV-HDV co-infection that occurs simultaneously while the other is HDV super infection that occurs in those patients who already having hepatitis B infection.[12,13,14] The HDV infection reduces the HBV DNA viral load as it requires HBV surface protein for encapsidation of its genome. [15,16,17]

CONCLUSION

The co-infection of HBV/HDV (45%) was found higher with 64% males and 36% females. The co-infection as well as mono-infection was also higher in age groups between 21 – 40 years. The co-infection of HBV/HDV also suppressed the HBV DNA in the patients as well as elevated the values of SGOP, SGPT and bilirubin. Due to limited and ineffective treatment options for the treatment of HBV/HDV infection our main focus must be on the prevention of the disease.

REFERENCES

1. "Hepatitis B – the facts: IDEAS – Victorian Government Health Information, Australia". State of Victoria. 2009-07-28. Retrieved 2010-09-19.
2. "Hepatitis B". World Health Organization. Retrieved 2009-09-19.
3. Abbott Molecular, Available from: http://www.abbottmolecular.com/products/infectious-diseases/realtime-pcr/hepatitis-hbv-assay.html 2010. Retrieved 2010-09-19.
4. Abe A, Kazuaki I, Take AT, Junko K, Nooki K, Satoshi T, Mkoto Y,Michinori K: Quantification of Hepatitis B virus genomic DNA by Real-Time detection. Journal of Clinical Microbiology 1999, 37(9):2899-2903.
5. Aguesse-Germon S, Liu H, Chevallier M et al. Inhibititory effect of 2´fl uoro-5-methyl-beta-L-arabinofuranosyl uracil on duck hepatitis B virus replication. Antimicrob Agents Chemother 1998;42:369–76.
6. Alam MM, Zaidi SZ, Malik SA, Naeem A, Shaukat S, Sharif S, Angez M, Khan A, Butt JA: Serology based disease status of Pakistani population infected with Hepatitis B virus. BMC Infect Dis 2007, 7:64.
7. Ali M, Idrees M, Ali L, Hussain A, Rehman I, Saleem S, Afzal S, Butt S. Hepatitis B virus in Pakistan: A systematic review of prevalence, risk factors, awareness status and genotypes. Virology Journal, 2011; 8:102
8. Alter MJ, Hadler SC, Margolis HS, et al,1990. The changing epidemiology of hepatitis B in the United States. Need for alternative vaccination strategies. JAMA 1990; 263:1218.
9. Sum SS, Wong DK, Yuen JC, et al. Comparison of the COBAS Taq Man HBV test with the COBAS Amplicor monitor test for measurement of hepatitis B virus DNA in serum. JMed Virol 2005;77:486–90.
10. Mumtaz K, Ahmed US, Memon S, Khawaja A, Usmani MT, Moatter T, Hamid S, Jafri W. Virological and clinical characteristics of hepatitis delta virus in South Asia. Virology Journal 2011, 8:312
11. Zaidi G, Idrees M, Malik FA, Amin I, Shahid M, Younas S, et al. Prevalence of hepatitis delta virus infection among hepatitis b virus surface antigen positive patients circulating in the largest province of Pakistan. Virology Journal, 2010: 7:283.
12. Wu JC, Choo KB, Chen CM, Chen TZ, Huo TI, Lee SD. Genotyping of hepatitis D virus by restriction-fragment length polymorphism and relation to outcome of hepatitis D. Lancet, 1995; 346: 939-941.
13. Xia Y-P, Chang M-F, Wei D, Govindarajan S, Lai MMC. Heterogeneity of hepatitis delta antigen. Virology 1990;178:331–6.
14. Yamashiro T, Nagayama K, Enomoto N, et al. Quantification of the level of hepatitis delta virus RNA in serum, by real-time polymerase chain reaction and its possible correlation with the clinical stage of liver disease. J Infect Dis., 2004; 189:1151-1157.
15. Zhou XJ, Lim SG, Lai CL, Murphy R, Pow DM, Myers MW. Phase 1 dose-escalation pharmacokinetics of L-deoxithymidine in patients with chronic hepatitis B virus infection. Hepatology. 2001;34:629A.
16. Zhu R, Zhang H, Yu H, Li H, Ling YQ, Hu XQ, Zhu HG: Hepatitis B virus mutations associated with in situ expression of hepatitis B core antigen, viral load and prognosis in chronic hepatitis B patients. Pathol Res Pract 2008, 204:731-742.
17. Zuckerman JN, Cockcroft A.,Zuckerman AJ. Site of infection for vaccination. BMJ, 1992; 305: 1158-9.