ORIGINAL ARTICLE

FREQUENCY AND COMPARATIVE ANALYSIS OF HEPATITIS D IN PATIENTS SEEKING TREATMENT FOR HEPATITIS B

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ABSTRACT

Objective: To find the frequency of hepatitis 'D' in patients of hepatitis 'B' seeking treatment and to compare clinical and biochemical features in patients harboring HDV with those who are not.

Design: Cross-sectional study.

Place and Duration of Study: Medical Unit-IV, Civil Hospital Karachi, Medical Unit-VI and Surgical Unit-VII, Lyari General Hospital, Dow University of Health Sciences, Karachi; from July 2003 to June 2005.

Patients and Methods: HBsAg positive patients seeking treatment were enrolled in the study. Anti-HDV was done in all. Patients were split into two groups according to their anti-HDV status into HDV positive and HDV negative groups. Liver biochemistries and viral profile for HCV, anti-HBc IgM and HBeAg were done and compared between the two groups.

Results: A total of 246 patients were selected. HDV was positive in 66 (26.8%) patients. No significant difference was observed in the frequency and stages of cirrhosis between the two groups while significant differences were observed in the mean SGPT (95% CI: -381.09 to -110.74; P = 0.001) and albumin levels (95% CI: 1.87 to 7.73; P = 0.007) and in the frequency of HBeAg (P = 0.001), anti-HBc IgM (P = 0.02) and HBV DNA (P < 0.001).

Conclusion: HDV infection was common in patients with HBV in this cohort of patients. All patients of HBV should be screened for HDV before treatment decision for the former is taken.

KEY WORDS: *Hepatitis. HBV. HDV. Hepatitis delta. Cirrhosis. Lamivudine. Interferone. Nucleoside. Nucleotide.*

INTRODUCTION

Chronic liver disease has become the major burden on health delivery system in our country. The etiological pattern is changing with incorporation of vaccination and better treatment options. Hepatitis D or delta (HDV) is a defective RNA virus which requires hepatitis B (HBV) for its replication and infection.[1] It was first described in Italy in 1977 and is a small 1.7 Kb RNA virus.[2,3] HDV possesses a compact catalytic center; it retains activity at temperatures of about 80º C and in buffer containing either 5 M urea or 18 M formamide.[4,5] The infection could occur as super-infection when it occurs over already infected patients with HBV or can occur simultaneously along with HBV infection when it is termed co-infection.[6] The decreasing trend is being reported about HDV infection from many areas possibly due to HBV vaccination.[7-10] Infection with HDV usually results in severe disease with higher rates of progression to cirrhosis and HCC.[11,12] Varying frequency of HDV seroprevalence (12.6 to 63 %) have been reported in patients with fulminant hepatic failure from India.[13] HDV is associated with higher incidence of hepatocellular carcinoma (HCC).[14] Treatment of HDV is still not satisfactory; the only approved drug interferon-a in higher dose has shown limited response. Although many viruses encode the majority of their replicative and processing enzymes, HDV apparently does not. The sole enzymatic activity that HDV possesses is a ribozyme that autocleaves the circular RNA, producing a linear molecule.[16,17] Other enzyme activities are apparently provided by the host cell. These features make the HDV difficult to eradicate as most of the possible therapeutic targets are normal cellular proteins.

A state of relative ignorance about HDV prevails, owing to lack of exact figures of its epidemiology in Pakistan. Furthermore, HDV status is usually not determined before initiation of therapy for HBV. This study was carried out to document the frequency HDV in patients presenting with HBV at Civil Hospital and Lyari General Hospital associated with Dow University of Health Sciences, Karachi, to compare the clinical and biochemical characteristics of hepatitis B patients also harboring HDV with those who are not.

PATIENTS AND METHODS

All HBsAg positive patients presenting at Civil Hospital and Lyari General Hospital, Karachi, seeking treatment for hepatitis B during the specified period of July 2003 and June 2005 were selected for the study.

Clinical details were recorded in the proforma and patients were segregated according to the HDV status into HDV positive and HDV negative groups. Each patient was subjected to following investigations:

Complete blood counts (CBC); HBsAg; HBeAg; anti-HB core IgM; HBV DNA PCR; anti-HDV; anti-HCV; serum albumin; SGPT; serum bilirubin; ultrasound abdomen. The biochemistries were done by auto analyzer; serological tests were carried out by Elisa.

The results of hepatitis serology were interpreted as follows: HBsAg positive was taken as exposure to HBV, presence of anti-HB core IgM was taken as recent infection of HBV.

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Presence of HBV DNA was taken as patient harboring live HBV virus, along with HBeAg, the interpretations were:

HBV DNA positive and HBeAg positive: Active replicates.
HBV DNA positive and HBeAg negative: Pre-core mutants.

Absence of HBV DNA with HBsAg positive was taken as inactive HBV infection/carrier state not requiring treatment. Presence of anti-HDV depicted hepatitis D infection whereas presence of anti-HCV was taken as hepatitis C infection.

Continuous variables were analysed by comparison of means using Student's 't'-test. And their 95% confidence intervals (CI) were calculated. Non-continuous variables were analyzed using Chi-square test with application of Fisher's test where applicable. Software used was SPSS ver.13.0.

RESULTS

Two hundred forty-six HBsAg positive patients fulfilling the selection criteria were selected in the study. Mean age ± SD was 26.7 ±11.9 years. Study included 138 (56.1%) males (mean age 24.9 ±11.7 years) and 108 (43.9%) females (mean age 29.1 ±12.1 years). Ascites was present in 48 (19.5%) while edema was present in 18 (7.3%). Mean ±SD of SGPT values were 207.5 ±402.6 IU/dl and that of albumin were 3.7 ±0.5 mg/dl. Recent hepatitis B infection (anti-HBc IgM positive) was found in 18 (7.3%), HBeAg was positive in 72 (29.3%), HBV DNA was positive in 94 (38.2%). Anti-HCV was present in 42 (17.1%) patients with HBV. The frequency of hepatitis D in patients of HBV was 66 (26.8%). Serological markers for all three viruses (HBV, HCV and HDV) were present in 15 (6.1%) patients (Table I).

TableI: Characteristics of studied population.

Variable	Count	%
Males	138	56.1
Females	108	43.9
Ascites	48	19.5
Edema	18	7.3
HBV DNA	94	38.2
HBeAg	72	29.3
Anti-HBc IgM	18	7.3
Anti-HCV	42	17.1
Anti-HDV	66	26.8
Non-cirrhotic	177	72
Cirrhosis Class-A	21	8.5
Cirrhosis Class-B	33	13.4
Cirrhosis Class-C	15	6.1

Two groups were formed on the basis of HDV status; 66 patients who tested positive to anti-HDV were allocated to HDV positive group and 180 patients who tested negative to anti-HDV were allocated to HDV negative group. Comparative analysis of HDV positive group and HDV negative groups were done. Analysis of continuous variables age, SGPT and albumin was done by comparison of means using Student's 't' test. Compared to HDV negative group, the Male: Female ratio was significantly more in HDV positive group 15:7 vs. 31:29. Statistical significance was detected in SGPT values which were higher in HDV positive group reflecting significantly higher inflammation (95% CI: -381.09 to -110.74; P = 0.001) while serum albumin levels were lower in HDV positive group (95% CI: 1.87 to 7.73; P = 0.007) (Table II).

No statistical difference was found in the frequency of ascites and edema between HDV positive and HDV negative groups. HBV DNA was detected in 94 (38.2%) patients analyzing for HDV status it was present in 48 (72.7%) of HDV positive group while its frequency in HDV negative group was 46 (25.6%). Frequency of HBV DNA was statistically significantly higher in HDV positive group (Pearson Chi-square test: df = 1, P < 0.001). HBeAg was detected in 72 (29.3%) patients. Among the patients who were HDV positive, the presence of HBeAg was detected in 30 (45.5%) patients as compared to the HDV negative patients in whom it was detected in 42(23.3%). The difference in the frequency was statistically significant (Pearson Chi-square test: df = 1, P = 0.001) (Table III).

Table II: Comparison of SGPT, age and albumin on HDV status.

		Mean	SD*	*P* value †	95% CI ‡
SGPT	HDV absent	141.5	327.3		-381.09 to -110.74
	HDV present	387.4	515.5	0.001	
Age	HDV absent	28.0	12.5		1.87 to 7.73
	HDV present	23.2	9.3	0.001	
Albumin	HDV absent	3.7	0.5		0.44 to 0.26
	HDV present	3.6	0.3	0.007	

*SD= Standard Deviation † Significant Level ≤ 0.05 ‡ CI = Confidence Interval.

Eighteen patients tested positive for anti-HBc IgM reflecting acute/recent hepatitis B infection. Analyzing its relation to HDV status, revealed 9 (13.6%) out of the HDV positive group to be positive for anti-HBc IgM as compared to HDV negative group in which 9 (5%) were anti-HBc IgM positive. This shows that co-infection of HBV and HDV is significantly more common in new and recent HBV infection. (Fisher's exact test: df = 1, P = 0.02), (Table III). Anti-HCV was present in 42 (17.1%) patients. Out of these 15 (22.7%) also tested positive to HDV while the remaining 27 (15%) tested negative for HDV. The difference in the frequency was not statistically significant (df = 1, P = 0.15), (Table III).

Table III: Comparison between HDV positive and HDV negative groups).

	Anti-HDV				Total n=246	
	Negative n=180		Positive n=66			
	Count	%	Count	%	Count	%
Female *	87	48.3%	21	31.8%	108	43.9%
Male*	93	51.7%	45	68.2%	138	56.1%
Ascites	36	20.0%	12	18.2%	48	19.5%
Edema	15	8.3%	3	4.5%	18	7.3%
HBV DNA*	46	25.6%	48	72.7%	94	38.2%
HBeAg*	42	23.3%	30	45.5%	72	29.3%
anti-HBc IgM*	9	5.0%	9	13.6%	18	7.3%
anti-HCV	27	15.0%	15	22.7%	42	17.1%
Cirrhosis absent	129	71.7%	48	72.7%	177	72.0%
Class-A cirrhosis	15	8.3%	6	9.1%	21	8.5%
Class-B cirrhosis	21	11.7%	12	18.2%	33	13.4%
Class-C cirrhosis	15	8.3%	0	0%	15	6.1%

* Statistically significant with $p < 0.05$

In our studied population, 177 (72%) did not have evidence of cirrhosis while cirrhosis according to child's class A, B and C was present in 21 (8.5%), 33 (13.4%) and 15 (6.1%) patients respectively. Comparing the results according to HDV status it was observed that the percentage of non-cirrhotic patients in HDV positive vs. HDV negative groups was 72.7% vs. 71.7%, child class A cirrhosis 9.1% vs. 8.3%, child class B cirrhosis 18.2% vs. 11.7% and child class C cirrhosis 0% vs. 8.3%. No significant difference was detected between the frequency and stage of cirrhosis in HDV positive and HDV negative groups

(Pearson Chi-square test: df = 3, P = 0.07), (Table III).

DISCUSSION

Hepatitis B is one of the commonest infections worldwide with prevalence of about 2 billion out of which over 350 million are chronically infected.[18] Annual mortality rate of HBV related chronic liver disease is more than a million.[19] We are witnessing the decrease in prevalence of HBV after introduction of vaccination against HBV.[20] The incidence of acute hepatitis B decreased by 67% across all age groups, while in population under 20 years, incidence decreased by 89%.[20] The current report documenting the high frequency of HDV at 26.8% is alarming. This is much higher than the study of Mumtaz et al. where the seroprevalence in Pakistan is reported as 16.6.%[21] Currently in our population majority of the patients of HBV who seek treatment for this infection do not fulfill the criteria for treatment, as they do not have active virus detected in their serum. In this study, we found only 94 (38.2%) of the total patients seeking treatment eligible to receive HBV treatment as they were HBV DNA positive whereas the rest were having non-active infection/carriers. Among these 94 patients, 48 were HDV positive, thus in majority of the patients the treatment options were limited due to the presence of HDV.

The treatment options for patients who are both HDV and HBeAg negative are oral nucleosides or oral nucleotides for at least one year and for those who are HDV negative but HBeAg positive are Peg interferon alfa-2a 180 MIU weekly for 48 weeks.[22-23] In patients with HDV oral nucleosides and nucleotides are not effective. When treated with interferon; the response rate of HDV is slightly better with co-infection (anti-HBc IgM positive) 15% compared to super infection with a response rate of less than 5%.[24,25] These results are often short-lived with reappearance of virus after stopping of therapy.[26] In our study co-infection was present in only 13.6% of cases while in the rest of 86.4% of cases it was super infection. At present we do not have the facility of PCR for HDV RNA so we used only anti-HDV assay for diagnostic purpose.

CONCLUSION

HDV infection was common in this group of patients with HBV. All patients of HBV should be screened for HDV before treatment decision for the former is taken.

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