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PREVALENCE OF HEPATITIS D VIRUS AND ITS IMPACT ON CLINICAL PARAMETERS IN QUETTA, PAKISTAN

Akhtar Hamid Tariq[1], Tauseef M Asmat[1*], Mohammad Zahid Mustafa[1] and Shahid Pervaz[2]

[1]Center for Advanced Studies in Vaccinology and Biotechnology (CASVAB), University of Balochistan, Quetta Pakistan
[2]Department of Pathalogy, Agha Khan University Hospital Karachi, Pakistan

[*] **Correspondence Author:** Prof. Dr. Tauseef M Asmat
*Center for Advanced Studies in Vaccinology and Biotechnology (CASVAB), University of Balochistan, Quetta Pakistan. Email: tauseefcasvab@gmail.com

Abstract

Background and Objectives: Hepatitis delta virus (HDV) is a small, 1.7 Kb RNA virus contained in a protein envelope consisting of hepatitis B surface antigen (HbsAg). Hepatitis delta has a worldwide distribution and is often attributed to cause severe and progressive liver disease. HDV infection in Quetta has been reported previously but no comprehensive study is available. The aim of this study was to get first detailed insight of HDV infections and associated risk factors in infected patients.

Methods: It was a cross sectional study. The study was conducted in Balochistan from 2021-22 All the HbsAg positive patients visiting tertiary care hospitals in Quetta were included in the study. The collected blood samples with elevated liver function tests were screened for the detection of HbsAg anti-HDV and anti-HCV. ELISA and qPCR were employed for confirmation and quantification. The collected data was recorded in MS-excel and the standard descriptive analysis was done by using MS-excel and SPSS V20.

Results: The present study showed that 27 participants were tested positive for HDV PCR out of 138 positive HbsAg PCR patients. HbsAg PCR was found to have a significant association with the gender. Similarly, LFT Alkaline Phosphatase, Heamoglobin Levels and WBC Levels were also found to have a significant association with the gender. It was also noted that LFT bilirubin of 133 participants was found increased, LFT SGPT of 284 participants was found increased, 281 participants had increased levels of LFT Alkaline phosphatase, 276 participants had increased LFT y-GT whereas 130 participants were reported with enlarged liver through ultrasound.

Conclusion: This study concludes that every fifth HbsAg positive patients is vulnerable to the development of hepatitis in Quetta, Pakistan.

Introduction

Hepatitis delta virus (HDV) is a small, 1.7 Kb RNA virus contained in a protein envelope consisting of hepatitis B surface antigen (HbsAg) [1]. Hepatitis delta has a worldwide distribution and is often attributed to cause severe and progressive liver disease [2]. The infection was endemic in the 1970s throughout Southern Europe, where it was responsible for a substantial proportion of cases of

HbsAg- positive liver disorders [3, 4]. Although brief reports [5, 6] on the prevalence of HDV infection are available but no latest data has yet been published from Balochistan during past decade.

There has been a dramatic change in the worldwide epidemiology of hepatitis delta, so that the traditionally prevalent areas such as Italy have shown a steady decline in HDV prevalence from 23% in 1987 to 14% in 1992 and 8.3% in 1997 [7-9]. This is contributed by improvement in socioeconomic conditions, increased health awareness fostered by AIDS prevention policy, and vaccination against HBV [10]. Countries such as Pakistan, with a high prevalence of HBV infection and lack of preventative strategies, have become the prevalent areas for HDV infection. The prevalence of HDV infections has been found linked with HBV endemic [11]. Data from Irabu Island, Okinawa, Japan shows high HDV prevalence of 23.6%, a figure that is unusual for the Far East [12]. In Taiwan, there is a relatively high prevalence of hepatitis D infection, particularly in intravenous drug abusers [10]. In a study from Pakistan, younger male subjects (mean age 31 years) showed a higher sero-prevalence of HDV among HbsAg-positive subjects, similar to earlier studies from Italy [8, 10]. One possible explanation could be the higher prevalence of injection drug use in this cohort of patients in the developed countries and that of therapeutic injections with contaminated needles in the developing world [13]. Sexual transmission may be another possible explanation that could be explored in future prospective studies [10].

Among HbsAg positive samples, screening for co-infection of HDV was one the most important aspect of this current study. Seroprevalence of HDV varies greatly across different countries, for example, in Turkey, Saudi Arabia and Bangladesh prevalence rates were observed 5.2%, 3.3% and 24.4%, respectively [14, 15].

This study comprises a detailed epidemiological survey of HDV infection in patients of Balochistan and shows that the overall prevalence of HDV infection as well as the testing trend.

Methodology and Material

Study Design

It was a cross sectional study.

Study Duration

The study was conducted in Balochistan from 2021-22

Study Population

All the HbsAg positive patients in Balochistan were included in the study.

Inclusion Criteria

Patients having no prior history of viral hepatitis were included in this study. Peripheral blood samples (4.0 mL) were collected by using 5.0 mL sterile BD syringe. A brief centrifugation step was performed for serum separation.

Serological analysis

Patients with elevated liver function tests were included for further evaluation based on relevant serological and molecular parameters. Samples were screened for the detection of HbsAg. Positive samples were evaluated for anti-HBc-IgM, anti-HBc-IgG, anti-HDV and anti-HCV. For this purpose, commercially available ELISA kits (MBS-SRL, Milano, Italy) were used according to the instructions provided by the manufacturer.

2.4. DNA extraction and amplification

Viral DNA was extracted by using commercially available kits (Sacace Biotechnologies S.r.l, Italy). For the amplification of DNA, previously reported sets of primers were used, amplification conditions were essentially the same as previously reported. DNA fragments were resolved on

1% agarose gels and size of each amplified product was estimated by comparing it with 1 kb DNA ladder (Solis BioDyne).

Statistical analysis

The data were recorded in MS-excel and the standard descriptive analysis was done by using MS-excel and SPSS V20. The frequency distributions of various attributes of infections were explored across different demographic variables. Distributions were separately established for the male and female samples along with other demographic characteristics.

Results

The participants were grouped on age, gender, education. Monthly income, occupation, marital status and polygamy as shown in table 1. The results demonstrated no involvement of HDV in patients below age 14. The highest numbers of patients were in age group of 15-24 years of age followed by age group of 25-34 of age. In case of gender distribution male (62.2%) were more affected than female. Surprisingly, literate were more affected when compared to illiterate. Similarly people with above average income were less prone to HDV infection. The detailed numbers and percentage regarding each characteristic is shown in Table 1.

Table 1. Demographic Characteristics and Frequency Distribution

Demographic Characteristic	Frequency (%)
Age (years)	
14 and below	0
15-24	99 (30.7)
25-34	62 (19.2)
35-44	35 (10.8)
45-54	43 (13.3)
55-64	44 (13.6)
65 and above	40 (12.4)
Gender	
Male	201 (62.2)
Female	122 (37.8)
Education Level	
Illiterate	97 (30.0)
Literate	226 (70.0)
Monthly Household Income	
Up to 20000	160 (49.5)
Between 20000 to 30000	121 (37.5)
More than 30000	42 (13.0)
Educational Level	
Illiterate	97 (30.0)
Up to Matric	121 (37.5)
Graduation	69 (21.4)
Post Graduate	36 (11.1)
Occupation	
Student	20 (6.2)
Unemployed	234 (72.4)
Private Job	40 (12.4)

Government Job	29 (9.0)
Marital Status	
Married	100 (30.95)
Unmarried	195 (60.37)
Widow/ Separated	28 (8.66)
Polygamy	
Yes	9 (2.8)
No	314 (97.2)

The patients visiting tertiary hospitals were subjected to HbsAg and HDV PCR, the results are presented in table 2. The results demonstrate that out of 323 patients 138 were positive for HbsAg, however only 27 were found co-morbid with HDV.

Table 2. Age wise Frequency Distribution of HbsAg PCR and HDV PCR Participants

Age (years)	HbsAg PCR		HDV PCR	
	Positive	Negative	Positive	Negative
14 and below	0	0	0	0
15 to 24	42	57	7	92
25 to 34	26	36	7	55
35 to 44	15	20	4	31
45 to 54	25	18	6	37
55 to 64	15	29	3	41
65 and above	15	25	0	40
Total	138	185	27	296

Next we analyzed age wise frequency distribution of HbsAg and HDV by PCR analysis, the results are shown in figure 1. The highest comorbidity of both antigens was found in age group of 15-24 years of age. The lowest frequency of comorbidity was found in elderly people (above 65 years of age).

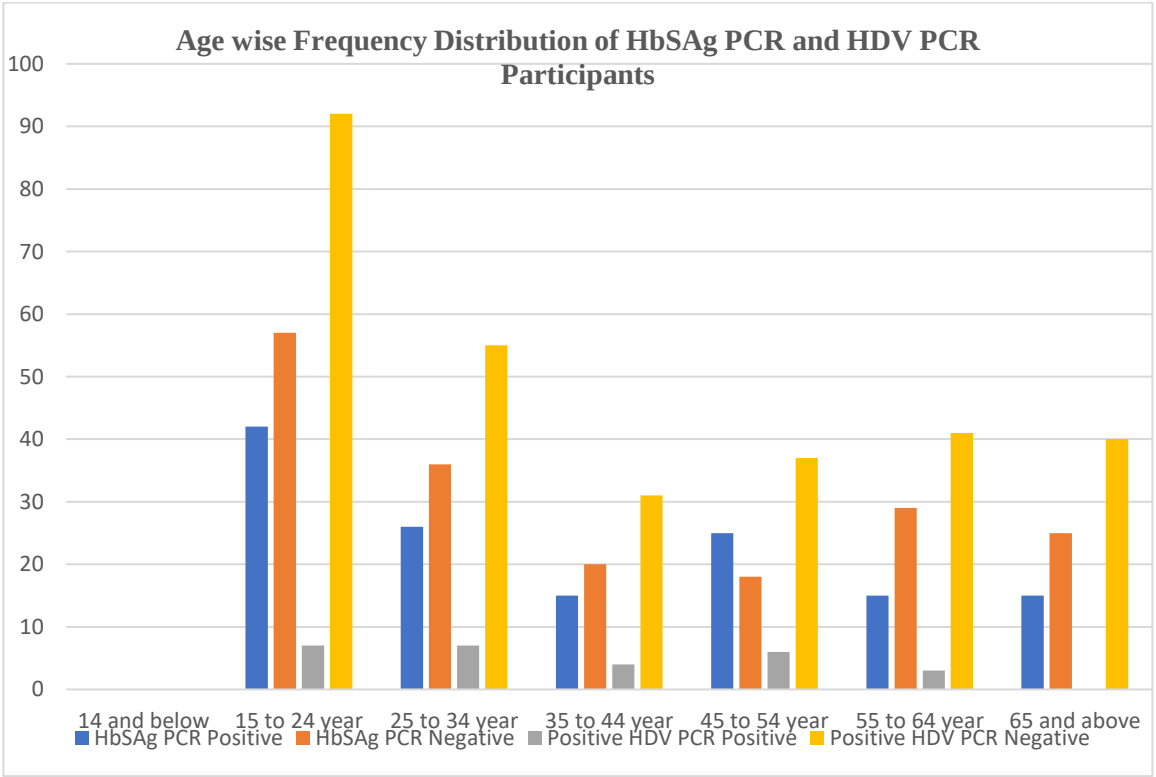

Figure 1. Age wise Frequency Distribution of HbsAg PCR and HDV PCR Participants

Further we were interested to evaluate the clinical data and its difference in male and female as shown in table 3. The results demonstrate that all the clinical parameters mentioned in table 3 were significantly affected in male when compared to female patients. For example the hemoglobin levels were too high (87gm/dL) when compared to female patients (34gm/dL).

Table 3. Frequency Distribution of Clinical Values and its Correlation/Gender wise

Tests		**Male**	**Female**
HbsAg PCR	Positive	95	43
	Negative	106	79
Positive HDV PCR	Positive	17	10
	Negative	184	112
LFT Bilirubin	Positive	122	68
	Negative	79	54
LFT SGPT	Normal	17	22
	Increased	184	100
LFT Alkaline Phosphatase	Increased	173	108
	Normal	28	14
LFT y-GT	Increased	25	22
	Normal	176	100
Liver Ultrasound	Enlarged	76	54
	Normal	125	68
Heamoglobin Levels	>10gm/dL	87	34
	<10gm/dL	114	88
WBC Levels	>4-11 cu	190	93
	<400	11	29
Platelets Count	150000-450000	137	89
	<150000	64	33

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Discussion

HDV infected patients showed less severe liver disease than HDV negative/HbsAg positive patients in this study, there are two possible explanations for this. First, HBV replication has been shown to be suppressed to very low levels by the actively replicating HDV [16]. Liver damage in such patients is essentially due to HDV replication only, which is of less intensity as compared to HBV. Occasionally however, HBV and HDV replicate simultaneously, each virus contributing to liver damage, thereby resulting in more severe liver disease [16-18]. Second, the pathogenesis of hepatitis D related liver disease appears to depend on the HDV genotype and the host immune response [19]. Whether super-imposed HDV infection impacts upon the development of hepatocellular cancer in patients with HbsAg-positive cirrhosis remains a controversial question. A study suggests that there was a trend toward lesser development of HCC in HDV infected patients compared to HDV-negative/HbsAgpositive patients [20]. The sero-prevalence of hepatitis delta virus infection in Pakistan is 16.6% [10]. The worldwide epidemiology of HDV is changing, consistent with successful efforts to control hepatitis B virus infection in the developed countries, whereas the infection remains endemic in south Asian countries such as Pakistan. The present study also found that the HDV positive prevalence was around 8.35%.

Clinical studies of HDV disease worldwide indicate that there is a wide variation in the clinical presentation [21]. The manifestations of HDV infection vary from benign acute hepatitis to

fulminant hepatitis and from rapidly progressive chronic liver disease to an asymptomatic carrier state [21]. The clinical course is often influenced by several factors, including the HDV genotype [22]. A previous study indicates, that in Pakistan, there is a predominant genotype of Hepatitis B is genotype D [23] . The association of genotype I of HDV with genotype D of HBV has been reported from other countries where HBV genotype D is predominant as is in Italy, Turkey and Egypt [21]. This genotype may cause a wide spectrum of pathogenicity [21]. In the South East Asia, the predominant HDV genotype is II and HBV genotype B and C. Genotype II is associated with a less rapidly progressive course of chronic liver disease [22].

In the present study the demographic characteristics of the participants showed that the most of the participants belonged to the age group 55-64 years of age. It was also noted that majority of the participants were male which corresponds to a previous study which showed that similar demographic characteristics. In this study, clinically enlarged liver was present in almost all of the patients who were HbsAg positive. This showed the patients the severity of liver disease associated with HDV.

The present study showed that 27 participants were tested positive for HDV PCR out of 138 positive HbsAg PCR patients. HbsAg PCR was found to have a significant association with the gender. Similarly, LFT Alkaline Phosphatase, Heamoglobin Levels and WBC Levels were also found to have a significant association with the gender. It was also noted that LFT bilirubin of 133 participants was found increased, LFT SGPT of 284 participants was found increased, 281 participants had increased levels of LFT Alkaline phosphatase, 276 participants had increased LFT y-GT whereas 130 participants were reported with enlarged liver through ultrasound.

Conclusion

This study concludes that the seroprevalence of HDV in HbsAg positive patients was 19% which means that every fifth HbsAg positive patients is vulnerable to the development of hepatitis in Quetta, Pakistan. This shows that there is an urgent need of precautionary measures for alleviation of the hepatitis through hepatitis control programs at provincial level.

Author Contributions: The authors' contributions and responsibilities are summarized as follows: **AHT, TMA** were involved in conceptualization; **AHT, TMA, MZM, SP** contributed to the study design; **AHT, TMA** were responsible for results analysis investigation was carried out by **AHT. AHT, TMA, MZM, SP** participated in writing, reviewing, and editing of Manuscript. All authors approved the final manuscript.

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Conflicts of Interest: The authors declare no conflict of interest

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