

# Frequency of viral hepatitis D among hepatic patients positive for HBV through PCR presenting at Aria Institute of Medical Sciences.

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## ABSTRACT

**Objective:** The purpose of this study is to report frequency of hepatitis D virus among hepatitis B positive patients.

**Methodology:** This observational study was conducted from March 15, 2024 to August 28, 2024; at Aria Institute of Medical Sciences Quetta. Data for the research was collected after approval from IRB approval letter no ARIA-IRB-009-2024. Samples from all patients were collected after written consent permission with reassurance for anonymity of the data and it will be used only for research purpose. Samples were collected from those patients who were not diagnosed previously to hepatitis D.

**Results:** A total 138 positive HBS positive patient's samples were tested for HDV through PCR among total samples 27 were positive and 111 were negative for HDV. Patient were divided into 3 age groups among them HBV positive were most common in 13 - 36 age group and HDV positive were most common in same age group.

**Conclusion:** HDV virus is becoming very common in 13 - 36 age groups which are positive for HBV virus,

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Hepatitis D is the among super severe type of viral hepatitis in humans, HDV is a faulty RNA virus that needs HBsAg to spread; hepatitis D can only occur in people who test positive for the HBV surface antigen (HBsAg). There are eight (1-8) distinct HDV genotypes known to exist, and each has a unique clinical course and distinctive geographic distribution.<sup>1</sup> Coinfection of HBV and HDV can be linked to intricate and shifting patterns of viral dominance. Compared to HBV mono-infection, chronic HDV infection causes more severe liver disease and is linked to early hepatic decompensation, faster progression of fibrosis, and a higher risk of hepatocellular carcinoma.<sup>2</sup> HDV spreads in a similar fashion as HBV, primarily through exposure to infected body fluids. This virus is highly prevalent in Mediterranean countries, the Middle East, Central Africa, and the northern regions of South America.<sup>3,4</sup> In Western countries, there is a high occurrence of HDV infection among individuals with HBV who use intravenous drugs. Globally, over 350 million people are estimated to have chronic HBV infection, with approximately 15-20 million of these individuals believed to

be co-infected or superinfected with HDV. Southern Europe has historically seen a high prevalence of HDV infection.<sup>5</sup> The ratio of hepatitis B is high in the overall population of Pakistan, with an infection rate of 7-9 million people and a prevalence ratio ranging from 3% to 5%. Molecular characteristic shows both viral diseases are genetically diverse. Pakistan is among those developing countries with most burden of viral diseases considering HDV seroprevalence ranging from 16.5% to 58.9%.<sup>6</sup> The worldwide prevalence of HDV poses a significant health risk, affecting approximately 15 to 20 million individuals with chronic HBV infection. The main reason of increasing high ration of HDV among Pakistan population is also Hep B. 58.6% of Pakistanis were found to have anti-HDV antibodies in a 2009 study. Pakistan's high rate of HDV and HBV infection could be brought on by a number of things, including poverty, a high population density, illiteracy, and insufficient HBV vaccination programs.<sup>7</sup> In cases of chronic HDV infection, the complex interaction between the hepatitis B (HBV) and delta (HDV) viruses is not always apparent. Patients who have both HBV and HDV infections in clinical settings usually have a significant decrease in their HBV-DNA viral load.

## Methodology:

This observational study was conducted from March 15, 2024 to August 28, 2024; at Hepatology Clinic of Aria Institute of Medical Sciences Quetta. All patients regardless of age and gender were included in current study. Sampling technique used consecutive convenience sampling. Those who refuse for informed consent were excluded also patients with history of Hepatitis D or B in past were excluded. Data for the research was collected after approval from IRB approval letter no ARIA-IRB-009-2024. Samples from all patients were collected after written consent permission with reassurance for anonymity of the data and it will be used simply for research purpose. Samples were collected from those patients who were not diagnosed previously to hepatitis D.

## DNA extraction and Amplification

Bosphore® HBV and HDV Kit is composed of R-T PCR reagents and quantitation serum standards which have

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been calibrated against WHO International Standard. One method for amplifying a DNA region is the polymerase chain reaction. Repeated cycles of heating and cooling cause the reaction. The Taq polymerase enzyme, buffer, dNTPs, and primers are the essential ingredients of PCR both a template and a solution. To put it briefly, primers are little synthetic DNA molecules that anneal to the particular sections of the template to initiate the synthesis process. The components that make up the amplified goods. The DNA template is amplified by Taq polymerase. Buffer solution offers the necessary correction. The target region for synthesis is the reaction and template, as mentioned.

#### Statistical analysis

Statistical analysis was done on SPSS V22. Patients were also divided into age groups according to HBV & HDV results.

#### Results:

During study period 323 patient were enrolled. Male out-number (n=201) female (n=122). Majority of the patients were either jobless or house wives (n=234, 72.4%), while 69 (21.4%) were employed and 20 (6.2%) were scholars. Majority of the patients (n=223, 60.37%) were unmarried. The HBV antigen PCR and HDV PCR of patients is shown in table no 1.

**Table No 1. This Table Represents HBV antigen PCR and HDV PCR of Patients.**

Age Group	HBV PCR		HDV PCR	
	+ve	-ve	+ve	-ve
13 to 36	68	93	14	147
37 to 44	15	20	4	31
45 to 70	55	72	9	120
Total	138	185	27	298

#### Discussion:

The frequency of viral infections is increasing. worldwide Recent studies estimate that between 15 million and 20 million chronic HBV positive patients also have HDV. Furthermore, our data indicate that a sizable fraction of patients with HBV also test positive for HDV.<sup>8</sup> 200 serum samples were tested for the presence of the hepatitis B surface antigen in a study carried out in Lahore. 120 (or 60%) of the 200 samples were positive for HBsAg. Seven samples were subsequently verified by sequencing. Of the 100 HBsAg positive samples that were analyzed for HDV RNA using nested PCR, 35 percent were found to be infected with HDV. Three distinct age groups comprised the study sample, of which 138 had positive HBV and 185 had negative HBV. Twenty-seven of the positive samples were positive for HDV, and all positive samples underwent HDV testing.<sup>9</sup> A study with 212 anti-HDV positive patients out of 362 HBSag positive patients was carried out in the Hepatology Clinic.<sup>9</sup> Hep B positive patients were tested for Hep D testing. A HDV PCR test was performed on 65 positive samples, indicating that the individuals had an active virus. In our investigation, out of 138 HBV positive samples, 27 samples were positive for HDV, which is similar to a study done in a liver clinic in Sindh.<sup>10-12</sup> Males and females aged 6-68 were evaluated in a different study carried out at JPMC; all samples Nine of the 129 individuals had acquired HCC, however 58.9% (n=76) of them were not coinfectd. 45 (34.9%) patients tested positive for HBV/HDV and 4 (3.1%) for HBV/HCV, making up the double-active infection group. Our study's sample, which was drawn from three distinct age groups—13-36, 37-44, and 45-70—reveals

that, among those in the 13-36 age group, 68 tested positive for HBV and 14 tested positive for hepatitis HDV. Four people were positive for HDV and fifteen for HBV in the second age group. 55 people in the 45-70 age range tested positive for HBV, while 9 people tested positive for HDV.<sup>13</sup> An epidemiological survey conducted in Pakistan found that the prevalence of HDV was 16.6% among 8721 HBV patients over 14 who were tested for anti-HDV antibodies nationwide. Three distinct age groups comprised the study sample, of which 138 had positive HBV and 185 had negative HBV. Twenty-seven of the positive samples were positive for HDV, and all positive samples underwent HDV testing.<sup>14</sup>

#### Conclusion

The frequency of HDV positive in patient positive for Hepatitis B are very common also every 6<sup>th</sup> person was effected in our study conducted at Aria Institute of Medical Sciences.

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