

Molecular Prevalence of Triple Infection Hepatitis B, C and D Viruses in Sindh Pakistan

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ABSTRACT

INTRODUCTION: Viral hepatitis is one of the major health problems worldwide, particularly in South East Asian countries including Pakistan where hepatitis C virus (HCV) and hepatitis B virus (HBV) infections are highly endemic. Hepatitis delta virus (HDV) is also now emerging world-wide. HCV, HBV, and HDV share parallel routes of transmission due to which dual or triple viral infections can occur in a proportion of patients at the same time. HBV and HCV are important factors in the development of liver cirrhosis and hepatocellular carcinoma, while HDV infection also plays an important role in the liver damage.

PURPOSE/OBJECTIVES: The aim of this study was to find out the molecular prevalence of triple infection HBV, HCV and HDV in Sindh.

STUDY DESIGN: Cross Sectional Descriptive Study.

PLACE AND DURATION OF STUDY: It was carried out at Diagnostic & Research Laboratory LUMHS Hyderabad from June 2017 to September 2018.

MATERIAL AND METHODS/RESULTS: Whole Blood samples were collected in 5 ml vacutainer. Total 21816 samples were received from our laboratories/ collection centers located in 20 different cities of Sindh for detection of different Hepatitis viruses by PCR. Out of that 5335 samples were for HBV, 15366 samples for HCV and 1115 samples for HDV detection. Serum was used to extract DNA & RNA of HBV and HCV respectively by Abbott m2000sp and was amplified on m2000rt automated instrument using commercially available kits of Abbott, while for HDV extraction was done using Roche high pure vial nucleic acid kit & amplification was done using light cycler mix kit (Roche) on instrument z480 (Roche). Out of the total 5335 HBV samples 4275 (80%) were positive and 1060 (20%) were negative, out of the total 15366 HCV samples 8763 (57%) were positive and 6603 (43%) were negative, out of the total 1115 HBD samples 594 (52.3%) were positive and 521 (46.7%) were negative.

CONCLUSION: It is concluded that the only way to prevent these triple infections is by educating the general population by massive awareness programs, extensive vaccination and other preventive measures to stop the spread of these alarming diseases in Sindh, Pakistan.

KEY WORDS: HBV, HCV, HDV, PCR.

INTRODUCTION: Hepatitis is a Latin word which means inflammation of liver. Mostly hepatic infection is caused by single hepatic virus but sometime infection with multiple viruses may occur and it leads to different management problems, which leads to higher incidence of morbidity and mortality¹. Hepatitis B virus (HBV) infection is one of the most common causes of human death worldwide⁷. Approximately 2 billion people are infected with Hepatitis B Virus (HBV) globally, of which 350 million are chronic HBV carrier. Each year approximately 1 to 2 million people die from HBV related complications such as chronic hepatitis, cirrhosis and hepatocellular carcinoma². In Pakistan, it affects 5% or around 12 million people annually, out of which 1 million develop hepatocellular carcinoma while four million, develop chronic hepatitis⁴. The infection rate of HBV has

decreased significantly in developed countries unfortunately, in developing and under-developed countries including Pakistan, the infection rate seems to have not decreased, even to any appreciable level⁶.

Viral hepatitis is one of the major health problems worldwide, particularly in South East Asian countries including Pakistan where hepatitis C virus (HCV) and hepatitis B virus (HBV) infections are highly endemic. Of the six main viruses causing the acute form of the disease, Hepatitis C virus (HCV) causes a prolonged chronic form of the condition in susceptible patients¹¹. Hepatitis delta virus (HDV) is also now emerging world-wide¹.

All Hepatitis viruses (HCV, HBV and HDV) are transmitted via similar routes that are through blood or blood products. Major risk factors include Injection Drug Users (IDUs), blood transfusions, reuse of syringes, facial and armpit shaving by barbers, positive pregnancy, tattooing and unprotected sex, so as a result, dual infection and even triple infection (A condition in which all three viruses occur together in the same patient) can occur in some patients at the same time^{1,5,9}. Co-infection is defined as the simultaneous presence of two or more infections by different pathogens which may increase the severity and duration of one or both⁸. HBV and HCV are important factors in the development of infection which leads to end stage liver diseases like cirrhosis and hepatocellular carcinoma (HCC)³, while HDV infection also plays an important role in the liver damage.

Cases of triple viral infections are reported from different regions of the world; Mongolia is a country which is highly endemic for triple hepatitis viruses. Taiwan is another country which was previously reported as HBV and HCV endemic area but recent studies showed that now it is also endemic for HDV. It has been reported that triple viral infection does not cause the development of HCC but only one virus that dominates the other two viruses in triple viral infection causes this condition of HCC¹.

OBJECTIVE: The aim of this study was to find out the molecular prevalence of triple infection HBV, HCV and HDV in Sindh province of Pakistan.

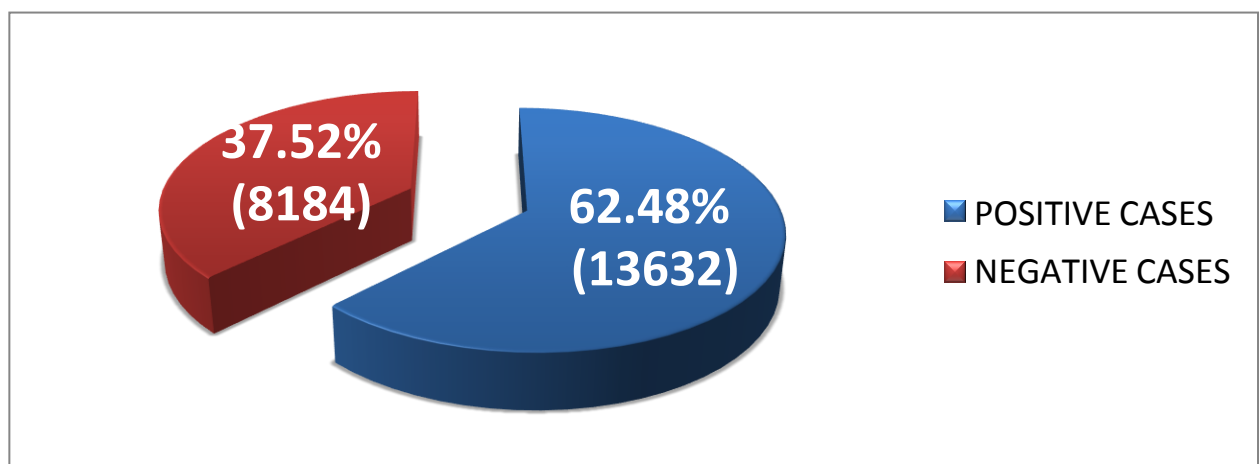
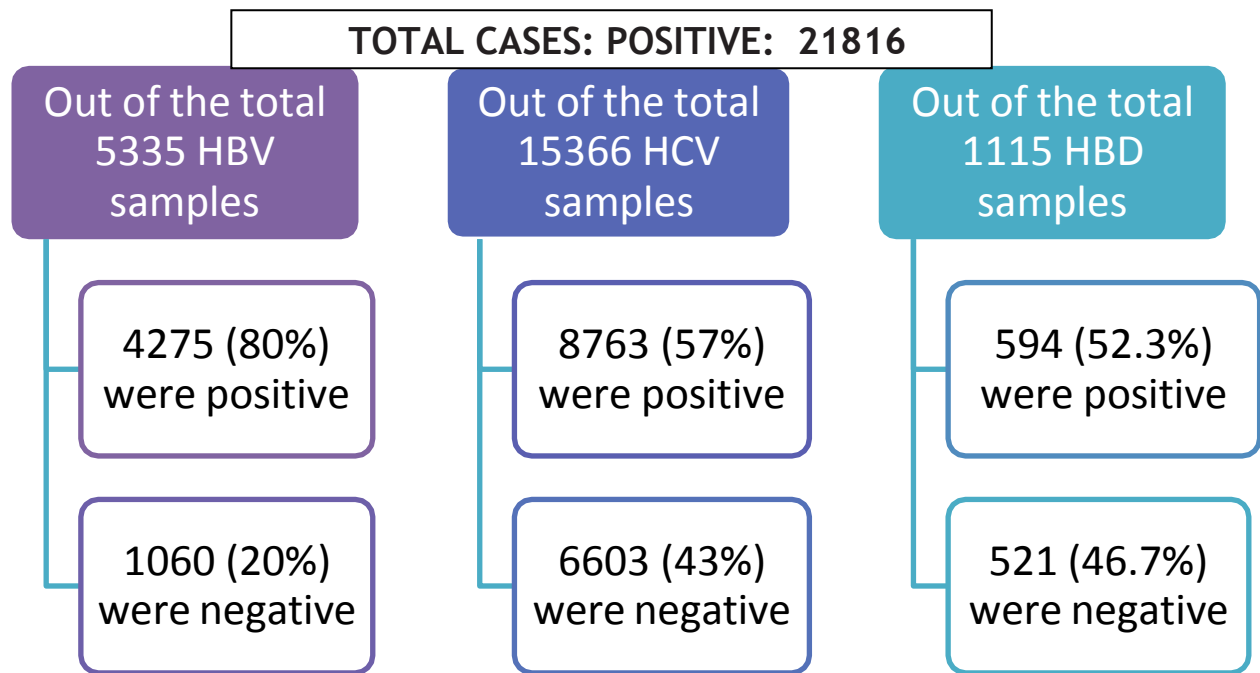
MATERIAL AND METHODS: This Cross Sectional Descriptive Study was conducted at Diagnostic & Research Laboratory LUMHS Hyderabad from June 2017 to Sept 2018.

METHODOLOGY: Whole Blood samples were collected in 5 ml plain vacutainer. Total 21816 samples were received from our laboratories/ collection centers located in 20 different cities of Sindh for detection of different Hepatitis viruses by PCR. Out of that 5335 samples were for HBV, 15366 samples for HCV and 1115 samples were for HDV detection.

All the samples were centrifuged on 5000 RPM for 10 minutes to get the serum; serum was used to extract DNA & RNA of HBV and HCV respectively by Abbott m2000sp and was amplified on m2000rt automated instrument using commercially available kits of Abbott. While HDV extraction was done using Roche high pure vial nucleic acid kit & amplification was done using light cycler mix kit (Roche) according to the manufacturer's protocol on instrument z480 (Roche)²³.

Statistics: Basic statistical tools were used for the analysis of data

RESULTS: Out of the total 5335 HBV samples: 4275 (80%) were positive and 1060 (20%) were negative, from 15366 HCV samples: 8763 (57%) were positive and 6603 (43%) were negative and from 1115 HBD samples: 594 (52.3%) were positive and 521 (46.7%) were negative.



TOTAL360 cases were requested for dual hepatitis i-e HBV and HDV from them HBV+VE were 228 (63.5%) and HBV-VE= 132 (36.5%), HDV+VE=203 (56.3%) and HDV-VE=157 (43.7%).

TOTAL390 cases were requested for dual infection of HCV and HBV from them HCV+VE were 219 (56.2%) and HCV-VE were 171 (43.8%), HBV+VE were 241 (61.8%) and HBV-VE were149 (38.2%)

While 12 cases already positive as HBV were requested for HCV and HBD from that HCV and HDV +VE were 4 (33.33%) respectively and HCV-VE were 8 (66.66%) respectively

While only 10 samples were send from different centers for detection of all three types of infections at one time from which 2 cases one each from Sukkur and Dadu were reported triple infection 4 cases 2 from Dadu and one each from Jamshoro and Hyderabad Saddar branch were reported having dual infection i-e HBV & HDV, 3 cases were reported single hepatitis HBV- 2 at our main branch in Hyderabad and one from Ghotki while a single sample were not detected any virus.

HCV	HDV	HBV
17080101367 Not Detected	17080101367 Not Detected	17080101367 Detected 39
		MAIN
18010101876 Not Detected	18010101876 Not Detected	18010101876 Detected 146
		MAIN
18020601605 Not Detected	18020601605 Detected 1850000	18020601605 Detected 445
	DADU	
18021400088 Not Detected	18021400088 Not Detected	18021400088 Detected 13
		GHOTKI
17110902432 Detected 400	17110902432 Detected	17110902432 Detected 54,892
SUKKUR		
17090303455 Not Detected	17090303455 Detected 2,163,384	17090303455 Detected
	JAMSHORO	
18040601643 Not Detected	18040601643 Not Detected	18040601643 Detected 184
	DADU	
18040602154 Detected 4,300	18040602154 Detected < 12	18040602154 Detected < 10
DADU		
18080202096 Not Detected	18080202096 Detected 396232	18080202096 Detected
	SADDAR	
18100901434 Not Detected	18100901434 Not Detected	18100901434 Not Detected

DISCUSSION / COMPARATIVE STUDIES/DISCUSSION: Traditionally, viruses have been classified according to antigenic characteristics²⁰. There is a wide variation in the prevalence of Hepatitis worldwide¹⁰. Drug resistant viral strains are evolved due to increased use of anti-viral drugs to treat chronic hepatitis¹⁷. There is a geographical difference in patients infected with Hepatitis¹⁵. Pakistan is highly endemic with to all types of hepatitis. Studies are too limited to give a clear picture of the prevalence at the national level, especially among otherwise healthy individuals. Most previous studies targeted different small groups of individuals with some clinical indications, so they do not accurately reflect the overall prevalence in Pakistan.

The overall prevalence of HBV and HCV (Dual infection) in this study was 26.15%. A review study conducted by Ziauddin et al⁸ at Peshawar showed an overall prevalence of 13.5% in KPK. Another study conducted by Syed Saad Naeem et al¹⁴ reported an overall prevalence of 12.9% in general population of Karachi, while a study by Rubina Ghani et al²⁰ shows prevalence in largest province of Pakistan i-e Punjab as 54.93%.

The prevalence of only HBV in this study is 80% while a study conducted by Zia Ur Rahman Awan et al² in KPK showed 50%.

The prevalence of only HCV in this study is 57% while a study conducted by Hakim St et al¹⁸ in Karachi showed 5.2% which is very low as compared to our study.

The prevalence of only HDV in our study is 52.3% which is less as compared to a study in Punjab by Zaidiet al²² that stat 88.81%.

While dual infection of HBV and HDV comes in our study as 56.3% in contrast to 70.5% stated by Crispim et al²¹ in Brazilian population.

CONCLUSION: It is concluded that the only way to prevent these triple infections is by educating the general population by massive awareness programs and by extensive routine vaccination / immunization for infants and high-risk individuals to stop the spread of these alarming diseases in Sindh, Pakistan. The other preventive measures which should be carried out in order to avoid development of triple infection can be: Screening of donated blood, Practicing safe sex, Avoiding sharing razors, syringes, tooth brushes, nail clippers, or needles, when getting a manicure, a tattoo, or having any body part pierced

LIMITATIONS: The inclusion of only those patients who came to our centers prescribed by the physician's, who presented with symptoms. Non-availability of any medical history regarding already having any hepatitis virus and came for other types. The findings may, therefore, not be generalizable to other patient populations, the study findings should be useful to health professionals and planners underscoring the importance of HBV/HCV co-infection during screening, monitoring, planning treatment and prognosis

FUTURE DIRECTIONS: Further research is needed to better understand the pathophysiology, clinical presentations, developing proper screening tools, best possible treatment regimens for patients with HBV/HCV co-infection in order to reduce morbidity and mortality associated with condition. Where the HCV genotypes determination facilities are available, the particular genotypes involved in causing infection must be determined before starting treatment. We also need to have similar studies at a national level to determine the overall prevalence and incidence of hepatitis infections in Pakistan.

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