



---

## Identification of Viral Hepatitis by Real Time PCR and Its Gender Association in Punjab, Pakistan

Nadia Jamil<sup>a\*</sup>, Irfan Ali<sup>b</sup>, Rakhshanda Bilal<sup>c</sup>

<sup>a,b,c</sup>Centres of Excellence in Science and Applied Technology, Islamabad, Pakistan

<sup>a,b,c</sup>[diaraj@hotmail.com](mailto:diaraj@hotmail.com)

### Abstract

In the developing countries including Pakistan, hepatitis B virus (HBV) and hepatitis C virus (HCV) cause serious health problems. A range of risk factors are responsible for this infectious disease's spread. This study was carried out to detect virus type in suspected patients from Punjab and evaluate the viral association with gender. Identification of viral hepatitis type was done by real time PCR (Cepheid, USA). Association of Hepatitis viruses (Hepatitis B & C) infection with gender was analyzed, among suspected patients reported in a Local Hospital at Islamabad. A total of 827 suspected patients were screened for Hepatitis B (n=180) and Hepatitis C (n=647). Total pervasiveness of Hepatitis B and Hepatitis C was found to be more common in male by percentage analysis but statistical analysis results differ from these. Further studies are needed to characterize HBV and HCV viruses, causes of their spread and transmission with reference to Pakistan.

**Key Words:** Hepatitis B; Hepatitis C; Real Time PCR; pervasiveness; Punjab.

### 1. Introduction

Viral hepatitis is a major health problem found in almost every country. Due to its asymptomatic nature mainly, it is a silent epidemic where most people are oblivious of their infection. It is a group of infectious diseases that affects hundreds of millions of people globally.

---

\*Corresponding author

To date six distinctive viruses like A, B, C, D, E and G are identified which are capable of causing human liver infections [1,5,15]. Hepatitis A and E (HAV and HEV) viruses are transmitted orally. Hepatitis B, C, D and G (HBV, HCV and HGV) viruses are transmitted, mostly as a result of blood to blood contact as well as injury with contaminated sharp instruments and sharing of needle or by sexual contact and also through prenatal transmission [12]. Hepatitis A, B, C, E and G cause severe illness and death. In particular, due to genotype B and C 350-400 million people are infected and suffering from chronic disease respectively [2,7,13]. Together these two are the most common cause of damage to the liver causing cirrhosis and liver cancer [4]. According to the Global Burden of Disease estimates, HBV and HCV together have caused 1.4 million deaths in 2010, which also include deaths from acute infection, liver cancer and cirrhosis [11]. If we compare these figures in the context of other major infectious diseases, we will come to know that malaria caused 660,000 deaths in 2010 [18], and tuberculosis and HIV caused 1.4 and 1.7 million deaths, respectively, in 2011 [14,18], these estimations put HBV and HCV in group where every country should take a step forward for its eradication. In Pakistan HBV and HCV pervasiveness is proven in different studies and Pakistan is the second country having high rates of chronic infections. Out of the 10 reportable diseases in Pakistan Hepatitis is one. According to estimates the pervasiveness of hepatitis C is 1%–4.6%, with levels as high as 20% or may be more in some parts of Pakistan [15]. The annual liver damage reports and mortality rate in reputable hospitals ranges from 25-35% in different parts of the country, where most of the patients with chronic infections are asymptomatic but appear to be infectious in spite of normal liver enzymes levels in some of them [12]. According to a survey of Health Department of Punjab, Pakistan (2014) overall prevalence of Hepatitis B and C in Pakistan is ~6 million infections and ~7 million infections respectively. Pakistan is currently facing an epidemic of viral hepatitis [2, 13]. Most of the literature shows that HBV and HCV are common in male. Present study was planned to know the pervasiveness of Hepatitis C and Hepatitis B in suspected male and female patients from Punjab, and also the viral association with gender.

## **2. Materials and Methods**

**Serum Sample Collection:** A total of 827 hepatitis B and C suspected serum samples were received from a local hospital at Islamabad between March 2013 –September 2014 for the detection of HBV and HCV by PCR. Out of 827, 180 were suspected for HBV and 647 were suspected for HCV. Standard laboratory protocols for reducing contamination were followed strictly. **DNA/RNA Extraction:** HBV DNA and HCV RNA were extracted according to the instructions given by manufacturers of Sacace Biotechnologies, Italy, kits. **PCR Amplification:** HBV and HCV PCRs were carried out via Real-time PCR (Cepheid, USA) by using kits (Sacace Biotechnologies, Italy). Highly pure RNA/DNA was mixed with the master mix provided in the kit and its amplification was analyzed by real time PCR (Cepheid, USA). **PCR Conditions for HBV:** Preheating was done for 95° C for 15 minutes followed by 42 cycles consisting of two steps one at 95° C for 20 sec and second at 60° C for 40 sec. **PCR Conditions for HCV:** PCR was initially run for 50° C for 30 minutes followed by preheating at 95° C for 15 minutes followed by 42 cycles consisting of two steps one at 95° C for 20 sec and second at 60° C for 40 sec. Positive, negative and internal controls for both HBV and HCV were provided with the kit and were used in comparison with the samples to rule out any false positive/negative result.

**Statistical Analysis:** Statistical analysis was carried out by data analyzing via a statistical package, SPSS version

17.0 (Windows). Chi-square test of association was for both HBV and HCV. Two hypothesis were formulated, a null hypothesis (Ho) and an alternate hypothesis (Ha).

Ho states: There is no association between gender and HBV/HCV pervasiveness OR There is no difference in the pervasiveness of HBV/HCV in males and females

Ha states: There is a significant association between gender and HBV/HCV pervasiveness OR There is significant difference in the pervasiveness of HBV/HCV in males and females. The computed value of the chi-square statistic was compared with the critical value to determine the likelihood that the observed deviations are due to random chance alone.

### 3. Results

Total 827 suspected patients were screened for HCV (647 Patients) and HBV (180 Patients) (Table 1 & 2). For HCV 85 out of 647 suspected patients, 325 (50.23 %) were females and 322 (49.77 %) were male. While for HBV out of 180 86 suspected patients (31.1 %) were females and 124 (68.9 %) were male. Statistical studies showed the chi-square value for HBV was 2.495 and p value was 0.114 while chi-square value for HCV is 1.997 and p value is 0.158.

**Table 1:** PCR Results of Patients Suspected for HBV Gender HBV

Gender	Total Samples	Pos	Neg
Male	124	72	52
Female	56	23	33
Total	180	95	85

**Table 2:** PCR Results of Patients Suspected for HCV Gender HCV

Gender	Total Samples	Pos	Neg
Male	322	157	165
Female	325	140	185
Total	647	297	350

### 4. Discussion

Hepatitis infections are a major public health problem affecting around 400 million people in the world [9, 17] with its persistently growing burden on the developing countries like Pakistan [8]. 647 suspected patients were screened for HCV and 180 for HBV. Out of these samples 72 (40 %) males were positive for HBV and 157 (24.26 %) males were positive for HCV, while 23 (12.8 %) females were positive for HBV and 140 (21.64 %) for HCV. On the basis of percentage, the prevalence of hepatitis B and C was found more common in male

patients. It was also observed that in comparison to HBV, HCV was more common. These results are in concordance with other researchers' work [4,6,7,8,11,12]. For further confirmation of results Statistical studies via SPSS version 22 showed the chi-square value for HBV was 4.470 and p value was 0.034 while chi-square value for HCV is 2.102 and p value is 0.147. In case of HBV the p value is less than 0.05 therefore we reject null hypothesis which suggests that there is significant difference in prevalence of HBV in gender same results were found by [10] and [3]. Though the percentage values for the suspected patients show more percentage of male patient positive for HBV and HCV but p values for HCV are more than 0.05. Therefore we failed to reject null hypothesis for HCV which means there is no association between gender and prevalence of HCV, which is contrary to the results found by [4,6,7,8,11,12]. This could be due to the reason that number of suspected male patients was more than number of suspected female patients (Table 1& 2), also males are more likely to get exposed because of shaving at community barbers, increasing trend of tattooing so the percentage ratio was showing more male positive while Chi square differentiated on the basis of probabilities which allow us to determine the likelihood that the observed deviations are due to random chance alone. In conclusion by real time PCR the virus type was detected and gender association was also analyzed. There are different factors like lack of appropriate blood screening facilities and lack of knowledge about the probable spread routes of HBV and HCV that are contributing a great deal towards the spread of the infection among the population.

## **5. Conclusion**

On the basis of these results it is concluded that Hepatitis C is more common in Punjab population as compared to hepatitis B, furthermore study also suggests that pervasiveness of HCV is not gender associated, high number of male patients can be due to the exposure to the virus and not due to the affinity of virus for male gender, but HBV seems to have some association or impact on gender. In this regard further studies are needed to characterize HBV and HCV, causes, transmission, and association with gender especially in Pakistan.

## **Acknowledgements**

The authors thankfully acknowledge Mr. Ali Dino Sial and Mr. M. Zohaib Khan for providing assistance in sample receiving and preparation.

## **References**

- [1] Aires R.S., Matos M.A.D., Lopes C.L.R., Teles S.A., Kozłowski A.G., Silvaa A.M.C., Filhoa J.A.A., Lago B.V., Mello F.C.A., Martins R.M.B. "Prevalence of hepatitis B virus infection among tuberculosis patients with or without HIV in Goiânia City, Brazil." *Journal of Clinical Virology*, 54, pp 327–331, 2012.
- [2] Badar N., Farooq U., Ali S., Nisar N., Abubakar M. and Qureshi J.A. "A molecular approach for genotyping of hepatitis B virus using restriction pattern analysis of samplicon in Pakistan." *Open Journal of Medical Microbiology*, 2, pp 16-23, 2012.
- [3] Baig S. "Gender disparity in Infections of hepatitis B virus" *Journal of the College of Physicians and Surgeons Pakistan*, 19, pp 598-600, 2009.

- [4] Balasubramanian J. "Current status of the hepatitis around the world: An overview on World hepatitis day, July 28," *Disease*, 4, pp 3, 2013.
- [5] Balew M., Moges F., Yismaw G., Unakal C. "Assessment of hepatitis B virus and hepatitis C virus infections and associated risk factors in HIV infected patients at Debretabor hospital, South Gondar, Northwest Ethiopia." *Asian Pac J Trop Dis*. 4, pp 1–7, 2014.
- [6] Hanafiah K.M., Groeger J., Flaxman A.D., Wiersma S.T. "Global epidemiology of hepatitis C virus infection: new estimates of age-specific antibody to HCV sero-pervasiveness." *Hepatology*, 57, pp 1333–1342, 2013.
- [7] Irshad M., Raza M.N., Anwar Z., Ikram N., Afroz A., Iqbal H.M.N. "Pervasiveness of Viral HBV and HCV among different group patients in Gujrat Pakistan." *Advances in Bioscience and Biotechnology*, 3, pp 423-436, 2012.
- [8] Khan F., Shams S., Qureshi I.D., Israr M., Khan H., Sarwar M.T. and Ilyas M. "Hepatitis B virus infection among different sex and age groups in Pakistani Punjab." *Virology Journal*, 8, pp 225-230, 2011.
- [9] Kurdistan I., A.A. Velayati, M.A. Merza, P. Farnia. "Association of P2X7 Gene Polymorphisms with Chronic Hepatitis B Virus Infection in Duhok." *International Journal of Sciences: Basic and Applied Research*, 16 pp 131-137, 2014.
- [10] Li W.C., Lee Y.Y., Chen I.C., Wang S.H., Hsiao C.T., Loke S.S. "Age and gender differences in the relationship between hepatitis C infection and all stages of Chronic kidney disease." *Journal of Viral Hepatitis*, 21, pp 706–715, 2014.
- [11] Lozano R., Naghavi M., Foreman K., Lim S., Shibuya K., Aboyans V. et al. "Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: A systematic analysis for the Global Burden of Disease Study 2010." *Lancet*, 380 pp 2095–2128, 2012.
- [12] Malik N., Butt T., Mansoor M.N., Khan T.G., Akbar M.S., Asl M. "Percentage of Hepatitis B And C Among Young Adult Males From Interior Sindh." *Pakistan Armed Forces Medical Journal*, 3, 2008.
- [13] Noah D.N., Njouom R., Bonny A., Pirsou, Meli J. and Sida M.B. "HBs antigen pervasiveness in blood donors and the risk of transfusion of hepatitis B at the central hospital of yaounde, Cameroon." *Open Journal of Gastroenterology*, 1, pp 23-27, 2011.
- [14] UNAIDS Report on the global AIDS epidemic 2012. Geneva, 2012.
- [15] WHO<sup>a</sup>, "Global Tuberculosis Report 2012". Geneva, 2013.
- [16] WHO<sup>b</sup>. "Prevention and control of viral hepatitis infection: framework for global action." Geneva, 2012.

[17] WHO<sup>c</sup>. “World Hepatitis Day.” 28<sup>th</sup> July 2014.

[18] WHO<sup>d</sup>. “World Malaria Report 2012.” Geneva, 2012.