



RESEARCH PAPER

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HCV prevalence in the volunteer blood donors in Peshawar, Khyber Pakhtunkhwa

Sajid Ali*¹, Nourin Mahmood¹, Jehan Zeb Afridi², Fazal Jalil¹, Ihtisham Ul Haq¹, Izaz Ali¹, Fazal Jalil¹, Hamza Hameed¹, Tanveer Ahmad¹, Najeeb Ur Rehman³, Bashir Ahmad⁴

¹Department of Biotechnology, Abdul Wali University, Mardan, Mardan, Khyber-Pakhtunkhwa, Pakistan

²Department of Medicine, Peshawar Medical College, Peshawar, Peshawar, Khyber-Pakhtunkhwa, Pakistan

³Department of Medicine, Fuji Foundation Hospital, Peshawar, Peshawar, Khyber-Pakhtunkhwa, Pakistan

⁴Centre of Biotechnology and Microbiology, University of Peshawar, Peshawar, Khyber-Pakhtunkhwa, Pakistan

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Abstract

Hepatitis C is one of the most common blood-borne disease causing significant morbidity and mortality globally. About 3% of the world population is infected by this virus. HCV infection is a leading reason for liver cirrhosis and therefore a major source of hepatocellular carcinoma. This study was designed to determine anti-HCV prevalence based on ELISA (Enzyme link immune sorbent assay), among blood donors in Khyber Teaching Hospital (KTH), Peshawar, Khyber-Pakhtunkhwa province of Pakistan. A total of 8634 blood samples were collected during June 2017 to November 2017 in the blood bank of KTH, Peshawar. All samples were tested for anti-HCV antibodies using ELISA. Analysis of all 8634 blood donors indicated that 97 individuals (1.12%) had anti-HCV antibodies in their sera. According to our study the prevalence of HCV infection is even lower (1.12%) than the common HCV prevalence recorded in the case of all previous studies in our subject areas. The decreasing movement of HCV infection indicates the development in health care facilities and awareness among the common population over the past few years.

* Corresponding Author: Sajid Ali ✉ Sajid@awkum.edu.pk

Introduction

Hepatitis causes inflammation of the liver. The condition can be self-limiting or can progress to fibrosis (scarring), cirrhosis or liver cancer. Hepatitis is mostly caused by hepatitis viruses but other infections, toxic substances (e.g. alcohol, certain drugs), and autoimmune diseases can also cause hepatitis. From the time of its discovery in 1989, hepatitis C virus (HCV) has been known worldwide as the main reason for chronic liver disease (Shepard *et al.*, 2005). Globally, an estimated 130–170 million persons (2%–3% of the world's population) are living with HCV infection (WHO, 2004). This infection, particularly in its chronic form, is related with sizable morbidity and mortality. More than 350 000 deaths are attributed to HCV infection each year, most of which are caused by liver cirrhosis and hepatocellular carcinoma (HCC) (Perz *et al.*, 2006). HCC is a common cancer worldwide and accounts for ~5.6% of all cancers. It is the fifth common cancer in the world and the third common cause of cancer death (Bosch *et al.*, 2004; Sherman, 2010).

HCV is mostly dangerous among all hepatitis strains because its morbidity rate is high (Karkiet *et al.*, 2008). HCV is enveloped, small circular, positive-sense and single stranded ribonucleic acid (RNA) virus from genus *Hepacivirus*, family *Flaviviridae* with a diameter of 50 nm (Bostan *et al.*, 2010). The total length of RNA genome is about 9.6 kb with one open reading frame (ORF) and 5' and 3' untranslated regions (UTRs) at both edges (Simmonds *et al.*, 2004). HCV has six main genotypes (1–6). Genotyping is most important for organization of HCV treatment and helps to cure HCV infections (Islam *et al.*, 2015). HCV G-1, HCV G-2 and HCV G-3 are distributed worldwide, whereas HCV G-4, HCV G-5 and HCV G-6 are present in certain areas of the globe (Rockstroh *et al.*, 2012). Genotype 1 (G1) is the most predominant in the world (~83.4 million people) followed by G3 (~54.3 million), then G2, G4 and G6 (~15.6 million), and G5 (~1.4 million) (Messina *et al.*, 2015). Transmission of HCV infection is mainly by exposure to infected devices and tools

despite rigid hygienic control, infected blood or blood products, hemodialysis, intravenous (IV) drug abuse, and organ transplantation (Alavian *et al.*, 2008). In hemodialysis centers, HCV infection remains a major concern. Blood transfusions as well as nosocomial infection continue to play important roles in the transmission of HCV (Norris *et al.*, 2013).

Prevalence of HCV is different in different region of the world. Like Western Europe, the Americas and Australia are considered regions of low HCV prevalence (<2%) (Lavanchy D, 2009). In Egypt, the prevalence of HCV is greater than 14%, the highest of any country in world (Guerra *et al.*, 2012). Mongolia has also higher HCV prevalence (above 10%), followed by Uzbekistan and Pakistan where, according to some reports, around 6% of the total population is infected with HCV (Lavanchy D, 2011).

The aim of the study was to determine anti-HCV prevalence in the blood donors, coming to the blood bank of KTH. Although previous studies exist that have revealed HCV prevalence in different time frames (Ali *et al.*, 2013) but as prevalence differs in respect to time, that's why to report the recent HCV prevalence in Peshawar regions of KPK, We have conducted this study.

Material and method

To find out HCV prevalence based on anti-HCV, among the blood donors, patients' data were collected from June to November 2017. ELISA technique was used to screen the subjects.

ELISA

The blood samples were screened for anti-HCV antibodies through ELISA (Monolisa Anti-HCV, Plus version 3) according to the manufacturer's instructions. The following procedure was followed for conducting ELISA.

Procedure:

We used positive and negative controls sera for each test in order to validate the test quality, following good laboratory practices.

Carefully, established the sample distraction and identification plane. Prepared the diluted washing solution R2. The well from the pack were taken and added the following in the respective wells of the plate in the following order.

- 100 µl of sample diluents in each well then.
- 50 µl of negative control in one well.
- 50 µl of positive control in second well.
- 50 µl of sample in the next wells.

Homogenized the mixture with at least 3 aspirations or with a micro-plate shaker for 5 seconds. After the sample distribution, the well containing sample or controls turned purple to blue. The plate was covered with adhesive film and incubated the micro plate for 60 minutes at 37°C. Removed the adhesive film followed by aspiration of the contents of all the wells in a liquid waste container and add a minimum of 0.370ml of washing solution into each well aspirated again and repeated the washing. Dried the strips by turning them upside down on absorbent paper. Distributed quickly 100µl of the conjugate solution into each well within the plate. Covered again with a new film and incubated for 30 minutes at 37°C. Washing is done five times. Enzymatic solutions were added to each well and incubated for 30 minutes in dark. Added 100µl of the stopping solution using the same sequence and same rate of distribution as for the development solution.

The substrate colour pink for negative sample or blue for positive sample. Fades from the wells, which become colourless for negative sample or yellow for positive sample after adding stoping solution. Carefully wiped each plate bottom. Waited at least 4 minutes after the stopping solution addition and within 30 minute of stopping the reaction read the optical density at 450/620-700nm using a plate reader. Checked for agreement between the spectrophotometric and visual reading against the plate and sample distribution and identification plan.

Result

A total of 8634 voluntary blood donors were initially screened for anti HCV antibody by ELISA, which indicated that out of the total number of volunteers,

97 subjects (1.2%) were positive for anti-HCV antibodies (Table 1). Moreover, HCV prevalence was more in month of October 19 (1.38%) (Figure 1).

Table 1. Month wise HCV prevalence.

Month	Donor	Anti-hcv (elisa)+	Percentage
June	1103	12	1.09%
July	1681	17	1.01%
August	1793	18	1.00%
September	1482	18	1.21%
October	1370	19	1.38%
November	1205	13	1.07%
Total	8634	97	1.12%

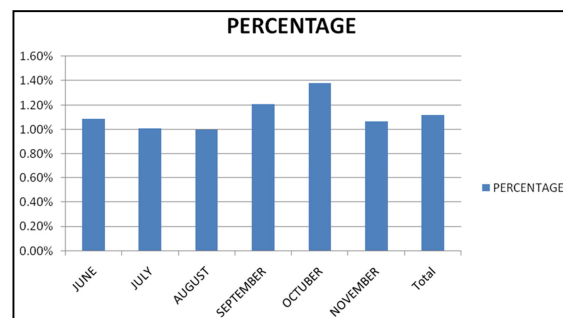


Fig. 1. Comparative HCV prevalence in different months.

Discussion

Hepatitis c is extremely attentive problem for the public health worldwide. Pakistan has the second highest number of HCV infection in the world after Egypt. Both HCV and hepatitis B virus (HBV) are associated with increasing ratio of morbidity and mortality. Hepatitis C infection is common in some parts of the country due to poor health care conditions. In KPK, where the health care services are badly prepared with essentials for screening and sterilization, HCV has become an economic burden over population with considerable number of people living below the deficiency line (Ali S *et al.*, 2013).

Earlier studies done in Pakistan used different methods of selection of the subjects. The studies supported the presence of high HCV prevalence in Pakistan and is ranged from 3.3 to 5.3%, (Qureshi H *et al.*, 2010). Donation records and serum of all donors reporting to the KTH Peshawar, Pakistan from June 2017 to November 2017 were analyzed.

Mean of the present study was to determine the prevalence of HCV in the volunteer blood donors, through Elisa technique in KPK, Peshawar. In this study, screening of blood by Elisa technique revealed that 1.12% of the blood donors were positive for anti-HCV which is well in the range of previously reported anti-HCV prevalence like studies conducted by karim, Ahmad and Najibullah (Karim F *et al.*, 2016; Ahmad *et al.*, 2006; Najib U Khan *et al.*, 2011 ; Arshad *et al.*, 2012).

According to these the prevalence rates of HCV in blood donors was 1.05% 1.89% 2.23%.and 4.5%, respectively. Studies conducted in other parts of the country including Kurram Agency (Bangash *et al.*, 2009) Interior Sindh (Mujeeb *et al.*, 2008) and Hyderabad (Tunio *et al.*, 2013) has shown prevalence rates of 1.1%, 5.7%, 3.45% respectively, but in this study we analyzed all the donor blood samples by Elisa which indicated that 1.12% of the blood donors had antibodies against HCV [Table. 1]. Looking to HCV prevalence in different regions of KPK province, it is obvious that prevalence of HCV is variable and mostly is more than our study prevalence.

This might be due to difference in the subjects' involvement, technique used for screening, area of involvement and possibly seasons of study. In comparison to all of the above studies conducted either in KPK or in Pakistan, the tendency of HCV prevalence is in descending order that is it is almost lower than the previous.

This might be due to improvement in the health care facilities adopted by the patients or adopted for the patients in different hospitals, or might be due to improved awareness regarding spread of infection and possibly due to migration of refugees that had came from Afghanistan. Comparatively the percentages of HCV positive cases were more in October which was 1.38% [Table.2]. This might be due an effect of the season burden as in summer the chance of getting HCV infection is more, so patients after getting infection have to screen themselves.

Conclusion

HCV prevalence in the volunteers blood donors was lower (1.12%) than the previous studies conducted in Khyber Pkhtunkhwa.

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