EPIDEMIOLOGICAL STUDIES OF HCV INFECTION IN THE VOLUNTEER BLOOD DONORS AT DISTRICT BAJAUR, KHYBER PAKHTUNKHWA, PAKISTAN

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ABSTRACT

Hepatitis C is one of the most common blood born disease caused by the Hepatitis C Virus (HCV). HCV infection is the leading cause of liver cirrhosis and may leads to hepatocellular carcinoma (HCC). An about 3% of the overall population of the world is infected by HCV. This study was designed to determine anti-HCV prevalence based on ICT (Immune-Chromatographic Test) and ELISA (Enzyme linked immune sorbent assay) among the blood donors of DHQ hospital Bajaur, Khyber Pakhtunkhwa (KPKP) province of Pakistan. During January 2016 to December 2018, a total of 7,309 blood samples were collected in the blood bank of DHQ hospital Bajaur. For the detection of anti-HCV antibodies all the samples were tasted with ICT, followed by ELISA. Among the total, only 23 (0.291%) were positive on ICT and among total positive samples, only 17 (0.232%) were positive on ELISA. According to our study, HCV prevalence is even lower than that of any districts of KPK and this lower prevalence might be due to the green and clean environment of district Bajaur and might be due to development of the health care centers in district Bajaur, and possibly the awareness level among the common population.

Key words: HCV, ELISA, Bajaur, KPK, Pakistan.

INTRODUCTION

Hepatitis C is one of the most common blood-borne diseases caused by the Hepatitis C Virus (HCV). HCV infection is the leading cause of liver cirrhosis and may lead to hepatocellular carcinoma (HCC). About 3% of the overall population of the world is infected by HCV (Ali *et al*., 2018). It has been predicted that 40-80% of people having hepatitis C may lead to chronic infection. In Pakistan, about 10 million people are affected by hepatitis C infection. (Hamid *et al*., 2004). Chronic Hepatitis C infection has high morbidity and mortality ratio and about 350000 deaths happen due to HCV infection annually (Perz *et al*., 2006).

HCV is an enveloped virus with positive-sense RNA genome, belongs to family Flaviviridae and has a diameter of 50 nm (Bostan and Mahmood, 2010). HCV has major six genotypes and genotypes have important role in management and treatment of HCV infection (Islam *et al*., 2015). The main source of transmission of HCV is through contaminated equipments and also with infected blood and drinking unhygienic water and eating spoil food. Beside this, organ donation is the main reason for the transmission of HCV from one individual to another (Alavian *et al*., 2008). Epidemiological study of Hepatitis C infection varies from country to country like USA, Europe and Australia are considered areas of low HCV prevalence (<2%) (Lavanchy, 2011). Among all, Egypt have high prevalence ratio of HCV infection which is greater than 14% (Guerra *et al.*, 2012) and the prevalence ratio of HCV infection in Mongolia is also high which is more than 10% and some studies showed that the prevalence in Uzbekistan and Pakistan is about 6% it mean 6% of the total individuals are infected by HCV (Lavanchy, 2011).

The aim of our study was to find out anti-HCV prevalence in the blood donors, coming to the blood bank of DHQ hospital, Bajaur, KPK province of Pakistan. Although previous studies have revealed HCV prevalence in different time frames (Ali *et al*., 2013) but as Bajaur districts is far away from capital of KPK, no more concentration was given, no study was conducted in either aspect. Hence, we for the first time aimed to determine HCV prevalence based on ELISA.

MATERIALS AND METHODS

Approval from Ethical committee

The study was approved by the Ethical Committee of the Department of Biotechnology, Abdul Wali Khan University Mardan KPK, Pakistan

The main aim of this study was to find out prevalence of anti-HCV antibodies among the volunteer blood donors of district Bajaur from January 2016 to December 2018. The ICT and ELISA technique were used to screen the subjects.

Blood donors selection

Only blood was taken from those subjects who voluntarily wanted to donate the blood. Blood samples were taken from the blood donors with informed consents and screened in the blood bank of DHQ hospital Khar district Bajaur, KPK Pakistan. A total of 7309 volunteer blood donor samples were collected from January 2016 to December 2018.

Screening of blood samples through Immune-Chromatographic Test (ICT)

Initially all the collected blood samples of the volunteer blood donors were tested through Immune-chromatographic test (ICT) and each sample with anti-HCV antibodies was tested twice. The samples positive for ICT were tested and confirmed through ELISA Technique.

Enzyme-linked Immunosorbent Assay (ELISA)

According to the manufacturer's (Monolisa Anti-HCV, Plus version 3) instructions, the following procedure was followed.

Procedure of ELISA

In order to validate the test quality, we used positive and negative controls sera for each test. Following good laboratory practices. Carefully, established the sample distraction and identification plane. Washing solution is prepared according to manufacturer's instructions. The wells from the pack were taken and added the followings 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

And then 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 37Ċ. Removed the adhesive film followed by aspiration of the contents of all the wells in a liquid waste container and added a minimum of 0.370 ml 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 37Ċ. Washing was 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 color was pink for negative sample and blue for positive sample. Fades from the wells, which become colorless for negative sample or yellow for positive sample after adding stopping 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.

Statistical analysis: All analysis was done using SPSS version 16.

RESULTS

Initially, screening of total 7,309 voluntary blood donors was done through ICT. Among the total, 23 samples were positive (0.291%) and then the ELISA technique was used to confirm the positivity in the samples. Out of the total positive samples, only 17 (0.232%) were positive for anti-HCV antibodies. Table 1 shows the year wise prevalence of HCV in 2016, 2017 and 2018, respectively,

Table 1. Year wise prevalence of HCV in 2016, 2017 and 2018.

S. No	year	Total Samples	HCV (+)	HCV(-)	HCV %
01	2016	1404	07	1397	0.4980
02	2017	3714	04	3710	0.1077
03	2018	2191	06	2185	0.2730
Total	03 years	7309	17	7292	0.2312

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. Due to poor health care facilities, unhygienic conditions and lack of awareness in most parts of the country, HCV is increasing with passage of time in some of the unhygienic and less developed areas of Pakistan. In KPK and mostly in the tribal areas where the health care services are badly prepared with essential for screening and sterilization, HCV has become an economic burden over population (Ali *et al*., 2013). Earlier studies done in Pakistan used different methods of selection of subjects supported the presences of high HCV percentage in Pakistan ranging from 3.3% to 5.3% (Qureshi *et al*., 2010).

The purpose of the present study was to determine the prevalence of HCV in the volunteer blood donors in District Bajaur. The screening of the blood through ICT and ELISA technique revealed that 17 (0.232%) blood donors were positive for anti-HCV antibodies (Table 1). Previous studies conducted to explore HCV prevalence in different subjects of population (Karim *et al*., 2016; Ahmad *et al*., 2006; Ali *et al*., 2011; Raza *et al*., 2012). According to these studies, prevalence of HCV was 1.05% 1.89% 2.23% and 4.5%, respectively. While study conducted in other parts of the country like in Kurram Agency (Bangash *et al*., 2009), Interior Sindh (Mujeeb *et al*., 2008) and Hyderabad (Tunio *et al*., 2013) has shown prevalence of 1.1%, 5.7%, 3.45%, respectively. This means that prevalence of HCV is different in different areas of the country and is more than our study prevalence.

In comparison to all of the above studies conducted in different parts of KPK/ Pakistan, prevalence of HCV is in decreasing order in this particular region of KPK. This might be happened due to the advancement in health care facilities, awareness of the spread of infection or it might be due to adaptation of proper hygienic condition and migration of refuges back to Afghanistan from this area of the study. Out of the total numbers of volunteers, only 17 (0.232%) subjects were positive for anti-HCV antibodies (Table 1). Moreover HCV prevalence was lower than the other areas of the KPK province of Pakistan.

Conclusion

HCV prevalence in the blood donors of District Bajaur of KPK province of Pakistan was lower (0.232%) than the other Districts of the KPK province.

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(*Accepted for publication June 2020*)