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Immunochromatographic Test and Third Generation ELISA for Elucidating the Existence and Risk Factors of Anti-HCV Antibodies among Blood Donors from Swabi Pakistan

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Abstract

This cross sectional study was designed to investigate the frequency and potential risk factors of anti-HCV among blood donors as well as to elucidate the most sensitive screening method for routine practice in Bacha Khan Medical Complex (BKMC) Shahmansoor and District Head Quarter (DHQ) Hospital Swabi, Khyber Pakhtunkhwa Pakistan. A total of 3390 male volunteer blood donors with age range 18-55 years were screened for HCV-Ab through an immuno-chromatographic test (ICT) and 3rd generation-enzyme-linked immunosorbent assay (ELISA). The sensitivity of ICT and ELISA was also evaluated by comparing their results. Among 3390 donors, 59 (1.74 %) were found positive for HCV-Ab through ICT and 62 (1.82 %) were HCV-Ab positive by ELISA assay. The highest frequency rate (40.3 %) was observed among donors within the age group of 18-30 years followed by (27.4 %) among 31-40 years, (17.7 %) among 41-50 years and the lowest frequency (14.5 %) was found among 51-55 years. Dental treatment (37.0 %) was significantly associated with anti-HCV seropositivity followed by blood transfusion (20.9 %), major surgery (14.5 %), travel abroad and unknown reason (11.2 %) for each and the lowest anti-HCV association was observed among donors who shaved by the barbers (4.8 %). The results from ICT and ELISA indicated that ELISA is a more sensitive, and reliable technique for routine screening of blood donors to control infectious diseases particularly HCV infection.

Keywords: HCV-Ab, Frequency, Risk factors, Blood donors, ICT, ELISA

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1. INTRODUCTION

Hepatitis C virus (HCV) is an infective liver agent that belongs to the Flaviviridae family, comprised of about 9.6 kb single-stranded RNA genome[1]. This virus is responsible for causing acute and chronic hepatitis in individuals that are characteristically progressing with cirrhosis, and hepatocellular carcinoma ultimately leads to death[2]. Previously it has been anticipated that 50 to 80 % of the people having HCV infection leads

to chronic hepatitis[3]. The annual mortality ratio of HCV infection is more than 3.5 million and the majority of the people are dying due to liver cirrhosis and hepatocellular carcinoma (HCC)[4]. Among all the infectious hepatitis viruses, the morbidity ratio of HCV is high; hence it considering riskier than other viral hepatitis infections[5]. According to the 2017 world health organization (WHO) report, about 170 million (3 %) of the world's people are infected with HCV in which the maximum frequency rate is reported from Africa[6].

In Pakistan, the scenario is worse than in the advanced countries, where about 170 million individuals are living with low educational levels and poor health conditions. According to the human development index of the United Nations, Pakistan is at the 134th position among 174 countries[7]. Among viral hepatitis infections, HCV is the primary public health concern that is rising rapidly and up till now infected about 10 million of the country's population[8]. The frequency of HCV reported from different parts of the country is highly variable and goes through the same for diverse groups of the same area[9]. According to previous studies, 5.31 % prevalence of HCV was reported in Islamabad[10] 0.4-31.9 % in different parts of Punjab[11,12], 1.1-9 % was observed in Khyber Pakhtunkhwa[13], 4-6% was perceived in Sindh province[14], 1.5 % in Quetta[15] and 25.7 % prevalence was reported in Gilgit Baltistan[16].

HCV is a blood-borne pathogen that has several transmissions routes[17]. The most imperative routes of transmission of this virus are through infected surgical instruments, contaminated blood transfusion, reuse of infected syringes, organ transplantation, dental surgery, drug abuses, sexual contacts, sharing of household stuff such as toothbrushes, shaving in the barbershop, razors, and use of unhygienic food and water[17-19]. Recently, several health care procedures such as dental treatment and surgery have been identified as risk factors for acute HCV infection[20]. In Pakistan, still, blood transfusion is considered the primary cause of HCV transmission that might be due to inadequate policy implementation, lack of resources, weak infrastructure, frequent power breakdown, poorly trained staff and unproductive screening of blood donors for HCV-Ab[21].

Volunteer's blood donors are usually considered as a healthier part of a country's population. All the blood banks in the public and private health sectors have standard selection criteria for blood donation that helps to identify and as a result, only healthy donors are permitted to donate blood. The percentage of HCV-Ab positive blood donors and risk factors associated with HCV-Ab positive situations among healthy individuals may reflect the extent of HCV infection in the general population[22].

Keeping in view the infectivity, transmission routes and sensitivity of the screening tests used for the detection of anti-HCV antibodies, this study was aimed to elucidate the most sensitive techniques for routine practice as well as to investigate the frequency and associated risk factors of anti-HCV among volunteer blood donors who visited blood banks of Bacha Khan Medical Complex Shahmansoor and DHQ Hospital Swabi Khyber Pakhtunkhwa Pakistan.

2. MATERIALS AND METHODS

2.1 Study area and setting

This cross-sectional study was designed and conducted at the Bacha Khan Medical Complex (BKMC) Shahmansoor and DHQ Hospital Swabi. The ethical endorsement for study conduction was approved by the Institutional Research Ethical Committee (IREC) of BKMC and DHQ Hospital Swabi.

2.2 Study population

We included a total of 3390 volunteer blood donors who visited the two respective hospitals' blood banks for volunteer blood donation (2255 blood donors from BKMC Shahmansoor and 1135 from DHQ Hospital Swabi). The study duration was from 1st January 2018 until 25th October 2019. The nurses were assigned to interview the participants and recorded the risk factors data according to the pre-structured questionnaire.

2.3 Sample collection

Five cc of blood was collected from each donor under the aseptic condition and was kept at room temperature for 20 min to facilitate clotting. The serum was alienated in a centrifuged machine at 5000 rpm for 5 min. The serum samples were reserved in the refrigerator at -20 ◦C until transferred to the biochemistry laboratory for serologic screening.

2.4 Serologic Assay

For the preliminary qualitative detection of HCV-Ab, ICT strips (Acon USA) were used. The sensitivity and specificity of the strip are above 98 %. The samples were further screened through 3rd generation enzyme-linked immunosorbent assay (ELISA) (EASE BN-96 TMB, Taiwan) as previously described to evaluate the specificity and sensitivity of both ICT and ELISA assays[23].

2.5 HCV-Ab detection through ICT

Anti-HCV antibodies were detected through ICT strips (Acon USA) following the company instructions. The strip was detached from the foil pouch and was placed on a hygienic, dried surface. Then 5 µL of serum was decanted in the strip and was dispensed with two drops of a buffer. After 15 min, the results were interpreted according to the appearance of color bands. To check the validity of the test strip, a control group was also run. In both test and control bands, a purplish-red color appeared on the membrane of the strip which confirmed a positive result. One red line appears in the layer of the strip in the control region (C). The appearance of no red line in the test area indicated a negative result.

2.6 HCV-Ab detection through ELISA

Anti-HCV antibodies were detected through third-generation ELISA (EASE BN-96 TMB, Taiwan) as per company instructions. Three wells pre-coated with anti-HCV antigens were taken and kept in a holder. 50 µL of specimens, positive control and negative control were dispensed in their specific wells. Then 50 µL of horse-reddish peroxidase conjugate (HRP-conjugate) was added to each well except the blank and was mixed by pattering the plate smoothly. Enclosed the plate with glue slip and was incubated at 37 °C for one hour. After incubation, the glue slip was detached from each well and washed five times with a diluted buffer. 50 µL of chromogenic solution A and 50 µL of chromogenic solution B were dispensed into each well including the blank and were mixed by pattering the plate smoothly for 15 seconds. The plate was then incubated at 37 °C in the dark for 15 min without shaking. 50 µL of stop-solution was added to stop the reaction. The absorbance of specimens and controls was determined within 15 min by spectrophotometer at 492 nm. The enzymatic reaction between the HRP-conjugate and chromogenic solutions forms a blue color in HCV-Ab positive sample wells and positive control well before the addition of the stop solution. After adding the stop solution, the blue color in HCV-Ab positive wells and positive control well altered to yellow color; Negative samples have a clear water-like appearance before and after the dispensing of the stop solution. The sample with absorbance value greater than or equal to the cut-off value i.e. (2.00) was considered reactive for HCV-Ab while the sample with absorbance value less than the cut-off value was considered HCV-Ab negative[24].

3. RESULTS AND DISCUSSIONS

In this cross-sectional study, we investigated the frequency and risk factors of anti-HCV among voluntarily blood donors as well as elucidated the most sensitive screening method for routine practice in District Head Quarter Hospital Swabi and Bacha Khan Medical Complex Shahmansoor from the surrounding urban and rural areas of Swabi region, Khyber Pakhtunkhwa Pakistan. An overall 3390 blood donors participated in the study setup with an age range of 18-55 years. The frequency of HCV revealed from ICT results was (1.74 %), while it was augmented to (1.82 %) by ELISA assay run on the same serum samples. The highest frequency rate (40.3 %) was found among donors within the age group of 18-30 years followed by (27.4 %) among 31-40 years, (17.7 %) among 41-50 years and the lowest frequency (14.5 %) was observed among 51-55 years (Table 2). Dental treatment (37.0 %) was significantly associated with anti-HCV seropositivity followed by blood transfusion (20.9 %), major surgery (15.5 %), travel abroad and unknown reason (11.2 %) for each and shaving by the barbers (4.8 %) (Table 3).

The donors included in this setup were all males because the majority of women in Khyber Pakhtunkhwa do not donate blood either to women or men due to social and cultural restrictions. Usually, men of young age or middle age are voluntarily donating their blood[23].

Table 1. Results distribution and percentages of anti-HCV infection among blood donors using ICT and ELISA techniques

Parameter	Total donors	Negative cases (%)	Positive cases (%)
Anti-HCV (ICT)	3390	3331 (98.25)	59 (1.74)
Anti-HCV (ELISA)	3390	3328 (98.17)	62 (1.82)

The overall frequency of HCV in our study was (1.82 %) indicating an intermediate endemic frequency which is consistent with the findings of the previously reported studies from Karachi, Peshawar, Quetta and Mardan. According to these reports, the prevalence of HCV among blood donors was 1.80 %[25], 1.85 %[26], 1.87 %[27] and 1.89 %[28] respectively. This slight decrease in HCV frequency among blood donors might be due to increasing public awareness of the disease with the virus, leading to a decline in new cases, adopting sensitive screening techniques such as ELISA, an accomplishment of strict donor selection and self-adjournment by high-risk persons. Comparatively, the prevalence of HCV in our study was higher than the previously described frequency of (0.07 %) among blood donors from Liaquetpur[29]. Likewise, an earlier HCV prevalence of (7.94 %) among blood donors was very high compared to our study[2]. This variation in results among the studies might be due to an unhygienic environment, lack of awareness, low socioeconomic conditions, and differences in the geographical distribution among the countries.

This study evaluated the frequency distribution of anti-HCV among different age groups ranging from 18 to 55 years. The highest frequency rate (40.3 %) was found among donors within the age group of 18-30 years followed by (27.4 %) among 31-40 years, (17.7 %) among 41-50 years and the lowest frequency (14.5 %) was observed among 51-55 years (Table 2). Consistent findings were reported by studies in India[30,31].

Table 2. Frequency distribution of Anti-HCV among different age groups of blood donors

Age groups (Years)	Positive cases	Positive percentage (%)
18-30	25	40.3
31-40	17	27.4
41-50	11	17.7
51-55	9	14.5

Our study investigated the most common risk factors including dental treatment (37.0 %), followed by blood transfusion (20.9 %), major surgery (14.5 %), travel abroad and unknown reasons (11.2 %) for each and shaving by the barber (4.8 %) respectively. Similar risk factors were also previously reported by Rehman et al. 2018 in Peshawar[26]. According to them, history of dental treatment (50 %) was the most significant associated risk factor of anti-HCV followed by travel abroad (23.07 %), major surgery (11.53 %) blood transfusion and unknown reasons (7.69 %) for each respectively. In our study, there were 13 (20.9 %) blood donors who had a previous history of blood transfusion. Among them, three of the donors were declared free of any disease during a blood transfusion. This might be due to the low sensitivity of the operated tests or low efficiency of the test procedure. Comparable findings were reported from four other studies: 19 %[32], 7.69 %[26], 3.2 %[33], and 2.7 %[34] of anti-HCV infected blood donors had a history of blood transfusion. These findings indicate the severity of the problem and raise several questions on the sensitivity of the screening test. With these facts in mind, all the samples were screened on both ICT and ELISA to evaluate their sensitivities. Our results indicated that ICT utilized for the detection of anti-HCV was less sensitive as compared to ELISA because three additional donor samples were detected by ELISA assay (Table 1). These false-negative results by ICT might be due to a short incubation period of ICT because usually short incubation screening tests do not detect a low concentration of antibodies in the serum and as a result more chances of false-negative outcomes in a nutshell incubation test. It was observed in our

study that the ELISA assay is more sensitive and specific than the ICT test for routine donor screening. These results are consistent with the findings of Abou et al.[35]. Khan et al.[36] and Nagi et al[1].

Table 3. Associated risk factors of anti-HCV among blood donors

Risk factors	Positive cases	Positive percentage (%)
Dental treatment	23	37.0
Blood transfusion	13	20.9
Major surgery	9	14.5
Travel abroad	7	11.2
Unknown reason	7	11.2
Shaving by barbers	3	4.8

The potential to evaluate the study quality was limited by the point that several studies failed to offer comprehensive information about chosen blood donors or valid data on significant factors. However, we have an assertion on our outcomes since the involved literature was mostly from multi-resources and had a big sample size, which would decrease publication prejudice to some level.

4. CONCLUSIONS

HCV infection in blood donors was 1.82 % which reflects an intermediary endemicity in the study area. The primary cause of HCV transmission and spreading is due to contaminated blood transfusion. The blood donors with a history of dental treatment and blood transfusion were the significant risk factors associated with anti-HCV seropositivity. It can be concluded from our study that the ELISA technique is more sensitive and reliable than ICT for routine blood donor screening before a blood transfusion to reduce its transmission.

Awareness regarding control measures in health-care settings including proper sterilization procedures of medical instruments and educating barbers about the significance of sterilization of their instruments might reduce the burden of HCV infection in this and similar settings. There is an urgent need to raise relevant guidelines for counselling and management of HCV-Ab positive blood donors. It is also compulsory to implement the strict standard selection criteria to defer and discourage blood donors with certain high-risk factors. As it was observed in our study that ELISA is more sensitive and reliable than ICT, so ELISA should be adopted in routine blood screening to prevent the transmission of HCV infection and to ensure safe blood transfusion.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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