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Research Article

Sero-molecular epidemiology of blood donors infected with Hepatitis C virus (HCV) in Peshawar, Pakistan

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Abstract

The aim of the current study was to find the risk of HCV through blood transfusion by sero prevalence via use of molecular tools. Blood Samples were randomly (n= 318) obtained from healthy blood donors who came to the Hayat Abad Medical Blood Bank for blood donation. Permission was garnered on all blood donors and data were analyzed via a questionnaire. Sero-molecular prevalence was assessed using the Immuno Chromatographic Technique (ICT), the Enzyme Linked Immunosorbent Assay (ELISA) and the Real-time Polymerase Chain Reaction (Rt-PCR) test. Out of 318 samples only 7 (2.2%) samples were positive of hepatitis C out of 318 samples. Among them, married people had been significantly affected (3.21 percent). No positive case had been found in unmarried people. 26-30 Years of age were more vulnerable than other age ranges. Popular risk factors for HCV transmission have been found in intravenous drug users (IDUs), alcohol addiction, surgical patients and dentists. The current study will help the patients and healthy people the before the blood transfusion the blood should to be checked properly through advanced diagnostic tools as well as the accidently finding of the people who suffering from this silent killer. So each person should have to check the HCV through PCR, an advanced diagnostic tool. Beside it

the current study will helpful too in the finding a large number of HCV infected people in district Peshawar who have to do some other necessary test like the genotyping of HCV etc.

Keywords: Age; Blood donors; Cirrhosis; Hepatitis C; Prevalence

Introduction

Hepatitis C has affected 3% of the global population and has demonstrated an immense importance of this disease [1]. 3 to 4 million people worldwide are affected annually by this disease according to World Health Organization. It is a serious public health issue, which often leads to liver cirrhosis and hepatocellular carcinoma in grave conditions. In developing countries such as Pakistan, about 5% of HCV is prevalent [2]. Many associated determinants such as intravenous drug administration, alcohol, re-use of blood syringes, age, social status of individual, use of unsterile medical equipment's and metabolic syndromes have been found to be critical risk factors attributed to the transmission of disease from infected to healthy persons [3]. Delayed diagnosis of HCV is the key cause of death in Pakistan because without treatment, most acute infections can evolve into chronic infections such as hepatocellular carcinoma and liver cirrhosis [2].

HCV belongs to the family Flaviviridae and genus Hepacivirus. Being an icosahedron with a diameter of 56-65 nm, it is an enveloped positive RNA virus. The length of the HCV genome comprises approximately 9600 nucleotides [4]. Two highly preserved untranslated zones have been identified, such as 3'-UTR and 5'-UTR. In addition, HCV has been categorized into seven genotypes and several subtypes. Pathogenesis depends on the type of genotypes present in that region [5]. The United States of America, Europe and Australia have the lowest HCV prevalence relative to African and Asian countries where the prevalence rate is quite high [6]. The highest prevalence was observed for HCV genotypes 1, i.e., 44 per cent compared with genotype 3, 4 (25 per cent and 15 per cent) worldwide. HCV comprises seven non-structural proteins (NS4B, p7, NS2, NS5B, NS4A, NS5A, and NS3) and three structural proteins (E2, E1 and Core) and [4]. HCV is primarily spread by blood and blood products. Iatrogenic transmission of HCV has been observed by contaminated medical procedures such as blood transfusion, organ transplantation, wound surgery, and continuous re-use of tainted needles, syringes, intravenous sets, catheters. The sexual transmission method is controversial, but sexually transmitted infections increase the chances of infection with HCV. The mother-to-child transmission rate of HCV was previously observed to be 4.3% worldwide. However, 22.1% prevalence rate was reported when prevalence was tested amongst HIV (Human Immunodeficiency Virus) patients [7].

The pathogenesis of HCV begins after attaching the envelope protein to the surface of two host cell receptors (such as scavenger receptor B1 (SR-B1) and CD81 [8]. The exact mechanism of infection with hepatocytes caused by HCV is still unclear. However, key damage is thought to have been found in predominantly immunocompromised patients. HCV induces two forms of human illness, e.g., acute and chronic hepatitis. More than 90% of patients displayed no signs and symptoms in the acute phase of the disease. However, the high rate of serum transaminases has been noted [6]. In about 20% of cases, HCV has been cleared in the body due to the rapid action of innate and adaptive immune action and viral RNA, which cannot be identified in blood samples within 3-4 months of infection [1]. Serum transaminase levels may be normal or moderately elevated in the chronic period [9]. Many factors, such as > 40 years of age, HIV co-infection, gender, alcoholism, fatty liver, all increase the risk of liver cirrhosis [7]. Sero-molecular tests, such as ELISA, RT-PCR, have been used to diagnose HCV

infection. Serological screening of patients is not sufficient during the acute phase since anti-HCV antibodies are developed after infection [5]. Additional nucleic acid base HCV tests are also used to confirm infection with HCV. The treatment of HCV has improved substantially since the last two decades. Antiviral drugs such as Sofosbuvir, ribavirin, daclatasvir, interferon alpha, telaprevir and boceprevir have been successfully used to treat human HCV infections [10].

Materials and Methods

Study area

This study was carried out at Hayat Abad Medical Complex, Hospital Khyber Pakhtunkhwa, Pakistan, to determine the prevalence of HCV amongst the local population between the months of January and June, 2016.

Ethical consideration

Ethical clearance was confirmed according to ethical guidelines of the Hayat Abad Medical Complex and Institutional Bioethics Committee University of Agriculture, Faisalabad (UAF), before conducting the study. Signed informed consent was obtained from all donors before they made their blood donations.

Collection of samples

A total of 318 blood samples were collected from symptomatic or asymptomatic people living in district Peshawar. Venous phlebotomy was performed for the collection of 3 ml blood in adequately labelled vacutainers. These tubes were stored at -20ºC prior to further processing.

Evaluation of risk assessment

Risk assessment of HCV was conducted with the help of Questionnaire. Questionnaire contained different risk factors such as residence, age, blood groups, sex, history of a recent surgery, organ transplantation, hemodialysis, dental visit, blood transfusion, ear pinning or tattooing, recent intravenous drug use (IDUs), alcoholism.

Serological identification of HCV

Serum was separated from the whole blood sampled from each individual, who was included into the study. Donors aged between 16 and 50 years. The serum samples were subsequently tested by ICT strip (CTK-Biotech®, San Diego, United States), ELISA (BIOKIT™, S.A. SPAIN).

Molecular identification of HCV

Qualitative-RT PCR (Bosphore™ Quantification Kit) was used to confirmation of HCV.

Statistical analysis

All data was statistically analyzed by GraphPad Prism Software version 8.4.3, by using Chi-square test whereby odds ratios were computed with the help of Fisher's exact test.

Results

218 blood donors out of 318 males that were sampled were found to be married whereas 100 of these individuals were unmarried. A greater percentage of married men were found positive for HCV i.e., 3.21%. Highest percentage of donors aged between 26-30 years (48.43%) (Table. 1).

The data also showed few parameters in which 0.9% (1 person), who did surgery, while one person visited to dentist 2 (100%) person were of IDUs, 1 (100%) person who used to drinking alcohol, similarly 2 persons (0.9%) used to get shaved by street barbers as shown in (Table. 2).

In the present study the three methods ICT, ELISA and PCR were also compared for result accuracy for the detection of HCV. A total of 5 (1.57%) samples were positive by ICT, 7 (2.2%) samples were detected positive by ELISA and PCR as shown in (Table. 3).

A PCR graph illustrating positive and negative detection of HCV from collected samples has been included in the manuscript as well (Fig. 1).

Table 1. Personal factors like age, sex and marital status of blood donors

Variable		HCV infection				P value
		Negative		Positive		
		No.	%	No.	%	
Age	16-20	13	100	0	0	0.065
	21-25	87	97.75	2	2.25	
	26-30	152	98.70	2	1.3	
	31-35	47	95.92	1	2.08	
	36-40	6	85.71	1	14.29	
	41-50	6	85.71	1	14.29	
Sex	Male	311	97.80	7	2.20	0.000
Marital status	Married	211	96.79	7	3.21	0.347
	Single	100	100	0	0	

Table 2. HCV infections in different related risk factors

Variable	HCV				P-value
	Negative		Positive		
	No.	%	No.	%	
Surgery					0.171
Yes	110	34.59	1	0.9	
No	207	65.09	0	0	
Dentist Visit					0.344
Yes	167	52.83	1	0.6	
No	150	47.17	0	0	
Organ Transplantation					0.000
Yes	0	0	0	0	
No	318	100	0		
Haemodialysis					0.000
Yes	0	0	0	0	
No	318	100	0	0	
IDUs					0.000
Yes	0	0	2	100	
No	316	100	0	0	
Alcohol Addicts					0.000
Yes	0	0	1	100	
No	317	99.69	0	0	
Shaved by Streets Barbers					0.347
Yes	219	68.87	2	0.9	
No	97	30.50	0	0	

Table 3. Comparison of HCV positive and negative results of ICT, ELISA and PCR

HCV	ICT		ELISA		PCR	
	No.	%	No.	%	No.	%
Negative	313	98.43	311	97.80	311	97.80
Positive	5	1.57	7	2.20	7	2.20
Total	318	100	318	100	318	100

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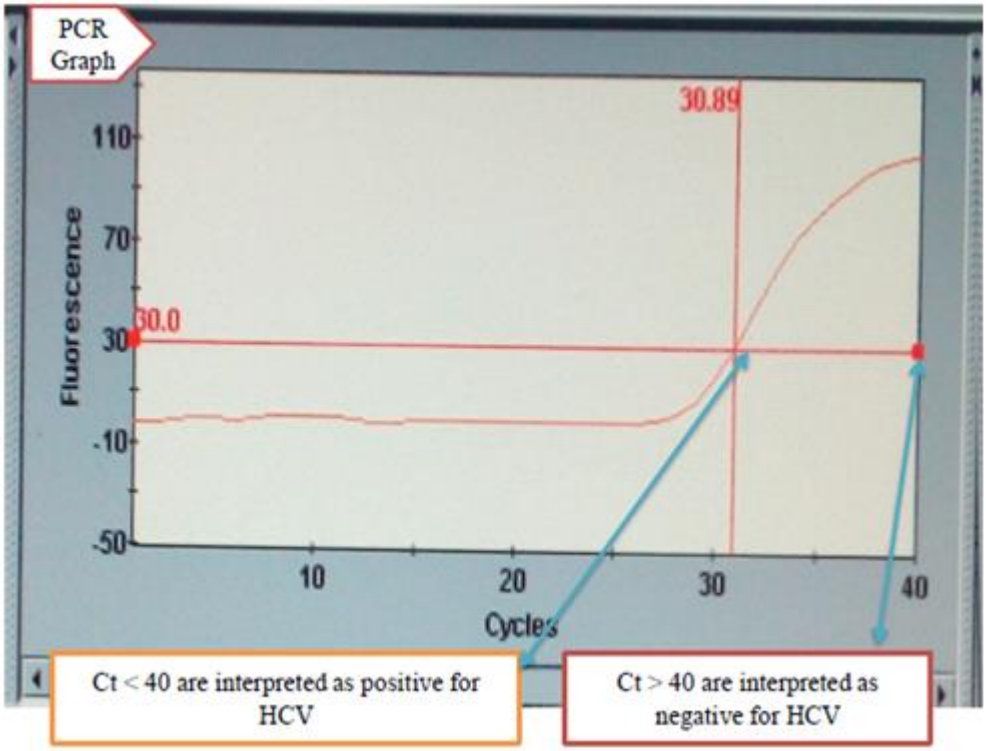

Figure 1. Qualitative RT PCR for detection of Hepatitis C Virus both positive and negative

Discussion

A wide range of infectious and non-infectious agents could be attributed to the etiology of Hepatitis [6]. Prior findings that suggested only 2-3% viral loads amongst the population of Peshawar only included clinical cases which were presented with cirrhosis [11]. It has been estimated that chronic liver infections were accountable for about 350000-500000 annual deaths due to liver failure. It has been demonstrated in various clinical studies that HCV was present in most body fluids including genital secretions, plasma, amniotic fluid, cerebro-spinal fluid peritoneal and pleural effusions [2]. Several researchers have postulated that HIV infection in several instances exasperates the risk of HCV infection. A total of 5894 male volunteer prisoner donors were screened and 857 (14.5%) were found to be HCV positive. Most of the HIV positive donors were initially excluded but later found to be positive for HCV as well when screened for it [12]. Similarly, 1498.1 positive cases for blood borne infections, per 100,000 donations were reported in Ukraine during a period of 2010-12 [13]. Therefore, present study endeavored to highlight the significance of HCV amongst blood donors and their risk factors associated with its transmission.

Despite the fact that testing only male donor could not possibly present a representative cross-section of the population, owing to the fact that only male individuals donated blood at the requisite collection point during the allocated time period, researchers had to contend with the available data. This anomalous situation could be rationalized by the fact that there are several cultural dogmas associated with donating blood in sub-continental countries, making it quite difficult to receive consensual female blood donations at collection centers. In a similar Indian research, out of 180,477 sampled

individuals most of them were males (95.86%) [14].

In a Serbian study, out of 27,160 blood donors 52 were found to be anti-HCV-positive and seroprevalence was 0.19%, for HCV [15]. In another similarly designed study, a total of 56,377 donors were serologically screened, whereby 403 were seropositive for HCV (357 men and 46 women) [16]. A blood donor survey conducted at tertiary healthcare facilities in India revealed that amongst the annual donations of 15,322 units, 464 blood donors were found to be sero-reactive for HCV. However, 49 of these sero-reactive cases were found to be actually positive for HCV [17]. Whereas, primary screening was accomplished by employing immuno-chromatographic test strips in our study, and positive cases for HCV were subsequently confirmed by enzyme linked immune-Sorbent assay and polymerase chain reaction tests. Unlike prior investigations conducted at a screening camp in Faisalabad, prevalence of HCV in current study was found to be much lower. A lower prevalence rate in Khyber Pakhtunkhwa province of Pakistan could probably be attributed to a greater adherence to Islamic regulations. Another rationalization could be the relatively rural settings of the area where blood transfusions, organ transplantation and Haemo-dialysis are infrequent [18]. A relatively smaller number of samples (Blood donors) could also be attributed these astonishing figures. Prior investigations in Pakistan have suggested greater odds of HCV amongst married donors as opposed to unmarried ones [19]. Similar trends were observed in our current observations as well. Our findings have corroborated prior recordings pertaining to a greater prevalence of HCV amongst individuals ageing between 35-50 years [19]. However, gender was not reported as a significant risk factor by both prior and current studies [18, 19]. A prevalence study conducted in Brazil that attributed demographics to be salient risk factors for HCV incidence, reported infection rates ranging between 1% to 2% [20].

Most studies have reported inappropriate intravenous injection practices, hemodialysis, organ replacement, infected blood transfusions, and sexual contact as substantial risk factors for HCV [5-7, 15]. Lack of vaccines and authentic post-exposure prophylaxis have been a serious impediment towards formulating a critical control strategy against aforementioned risk factors [14]. HCV epidemiology undertaken within the various population groups in Croatia determined several risk factors underlining the spread of HCV genotype. This study revealed that about 35,000-45,000 Croatians were either chronically diseased or at serious risk of infection. Addicts who abused drugs intravenously were observed to at the greatest risk for HCV infection and consisted about 29 to 65 percent of the total positive cases in Croatian prisons [21]. As the present study was performed amongst volunteers who had participated in blood collection drives, it would be understandable to assume that such drug abusers could neither have been sampled nor identified. Researchers have demonstrated that ELISA test is incapable of differentiating between past and recent infectious exposures. Therefore its implementation as a diagnostic tool is limited to only screening of suspected samples so a more definitive diagnostic tool may be employed at a later instance [7]. Therefore, anti-HCV antibody-positive sample have to be tested for HCV RNA for establishing pre-existing HCV infection.

Conclusion

The incidence of HCV in current study was highest amongst middle aged donors. Concomitantly, a higher prevalence rate was presented amongst drug abusers as well. PCR and ELISA tests were concluded to be more reliable diagnostic technique for HCV

detection than ICT. These findings corroborated prior publications pertaining this subject matter. However, admittedly lower prevalence of HCV in our current findings were subject to limitations in our sampling practices and would not truly be a representative of entire population. Therefore, precautionary and preventive measures pertaining to HCV must not be forsaken. Awareness amongst general public regarding the routes of HCV transmission must be instructed through public service messaging to avoid further spread of the disease in humans.

Authors' contributions

The study was conceived and designed by: S Ali & MI Arshad, Experiments were performed by: Q Ullah & F Sadique, Data was analyzed by: M Shahid, NU Akbar & HM Safwan, Materials were contributed by: M Rauf, Original draft was written by: S Ali, O Naseer & AH Rabbani, Manuscript was revised by: MM Jalees & F Wadood

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