



Relative Frequency of Blood-Borne Viruses in Hemodialysis-Dependent and Kidney Transplant Recipients in Iran

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Abstract

Background: Hemodialysis (HD) patients and kidney transplant (KT) recipients are exposed to be infected by blood-borne viruses (BBVs). Current study was conducted to evaluate the prevalence of BBVs in HD and KT patients in the whole Iranian population.

Methods: From Jan 2016 to Dec 2017, 174 hemodialysis and 139 kidney transplant recipients enrolled in this survey. After blood sampling, serum samples were detected for HBV, HCV, HCMV, HIV and HTLV antibodies. Seropositive samples confirmed by Polymerase chain reaction (PCR) method.

Results: Overall, 6 (3.44%) and 3 (2.15%) of hemodialysis-dependent and transplantation patients had evidence of HCV infection, whereas no patients were HIV and HBV positive, two cases (1.14%) of hemodialysis and one case (0.71%) of transplantation patients demonstrated the HTLV-1 infection. 52 (37.4%) of patients received graft were positive for HCMV antibody. In addition, our study showed a co-infection of HCMV with HCV (3 patients, 2.15%) in transplantation patients.

Conclusion: Prevalence of BBVs infection was lower in comparison to the previous studies. The current strict infection control practices in Iran appear to be effective in limiting dialysis and related infections after transplantation. Because BBVs infections constantly occur especially in dialysis and after transplantation units, our data will be useful to build a new strategic plan for the elimination of BBVs infection in kidney therapy centers.

Keywords: Hepatitis B virus (HBV); Hepatitis C virus (HCV); Human Cytomegalovirus (HCMV); Kidney transplant

Introduction

Blood-borne diseases are the group of disorders mainly caused by infectious agents transmitted by direct blood transfusion or direct blood contact from one individual to another from injured skin or a mucous membrane. These infections could also be transmitted through blood doping, drug abuse and via sexual contact (1). The main blood-borne viruses (BBVs) are Hepatitis B Virus (HBV), Hepatitis C Virus (HCV), Human Cytomegalovirus (HCMV), Human Immunodeficiency Virus (HIV) and Human T-cell Lymphotropic Virus type 1 (HTLV-1) (1-5).

Organ transplantation and blood transfusion are two main ways of viral transmission. Therefore, BBVs are important risks for patients and staff in hemodialysis units (2, 6), and those undergoing transplantation surgery. Survival in patients with chronic renal failure has significantly increased by accessibility to dialysis. During hemodialysis, the patient's blood flows through a filter in a dialysis machine. Although this method can be efficient to treat renal failure, it also put hemodialysis patients at risk of infectious complications of hemodialysis include infections caused by contaminated water or equipment, and blood-borne viruses (primarily the hepatitis B and C viruses) and it may also lead to the transmission of some blood-borne infections, such as HIV, HTLV and HCMV (7, 8).

Solid-organ transplantation (SOT) is considered as an accepted therapy for end-stage disease of the kidneys, liver, heart and lungs for nearly 30 years. Blood-borne viruses make vital complications for surgical and technical obstacles combined with the impact of immune suppression, predisposes recipients of SOT to clinically important infectious consequences (9, 10).

To the best of our knowledge, previous studies in Iran have investigated the prevalence of BBVs in high risk or healthy individuals but these studies are limited by their focus on some provinces. Therefore, for the first time we sought to reveal a national reporting of BBVs seroprevalence with the focus on the HTLV-1 not studied in most of the provinces in Iran.

We aimed to estimate the relative frequency of BBVs in these patients. The present study could be useful in determining the epidemiology of the major blood-borne viral infections and it could help authorities to improve the quality of the patient's care. In addition, these results may be considered to prevent disease transmission to healthy individuals.

Materials and Methods

Literature search

Studies related to our survey were selected from PubMed and Institute for Scientific Information (ISI) databases using keywords: BBV, HBV, HCV, HCMV, HIV, HTLV-1, Iran, Hemodialysis, organ transplantation, etc. After excluding the unrelated studies, 16 eligible studies were remained, for additional investigation their titles, abstracts and full texts were scanned in our literature search.

Study Population

The study population consisted of two groups of hemodialysis-dependent patients with 174 participants who received dialysis 2 times a week, and 139 kidney transplant patients. All the participants collected from Hasheminezhad Hospital which is a referral center in Tehran, Iran.

The principles of current study were described in the Declaration of Helsinki for all human or animal experimental investigations.

Serological assays

From Jan 2016 to Dec 2017, 10 ml of blood samples were obtained from each individual. Serum was separated through centrifugation and was stored at -20°C . Serum samples were screened for HBV, HCV, HCMV, HIV and HTLV-1 antibodies using Enzyme-linked immunosorbent assay (ELISA; Dia Pro Diagnostic bio probe, Italy) according to the manufacturer's instruction. In order to confirm the presence of these infections in seropositive samples, PCR

method was performed with specific primers (11-13).

Peripheral Blood Mononuclear Cells (PBMCs) were isolated from blood samples of positive serums using Ficoll density gradient centrifugation (Lympholyte H, Cederlane, CANADA) according to the manufacturer's instructions, and genomic DNA was extracted (Blood mini kit, Qiagen, Germany) for confirmation of infection with PCR amplification using specific primers (Table

1) for each mentioned viruses (11, 14). PCR amplification started with initial denaturation at 94 °C for 4 min, followed by 35 cycles of 94 °C for 50 sec, 60 °C for 50 sec, 72 °C for one minute and final extension at 72 °C for five minutes. PCR products were examined by electrophoresis using 2% agarose gel. The sizes of PCR products were estimated according to the migration pattern of a 100 bp DNA ladder.

Table 1: Specific primers sequences for amplification of blood-borne viruses (BBVs)

<i>HBV Forward primer</i>	<i>5'-GGAGTGTGGATTTCGCACT-3'</i>
<i>HBV Reverse primer</i>	<i>5'-TGAGATCTTCTGCGACGC-3'</i>
HCV Forward primer	5'-CGTCCAGGCACCTCGATTAG-3'
HCV Reverse primer	5'-CCTCCAGGGTCAAGGAAGGC-3'
HCMV Forward primer	5'-CATGAAGGTCTTTGCCAGTAC-3'
HCMV Reverse primer	5'-GGCCAAAGTGTAGGCTACAATAG-3'
HIV Forward primer	5'-GAGGAAGCTGGAGAATGGG-3'
HIV Reverse primer	5'-ATGATGCAGAGAGGCAATTT-3'
HTLV-1 (TAX) Forward primer	5'-AGGGTTTGGACAGAGTCTT-3'
HTLV-1 (TAX) Reverse primer	5'-AAGGACCTTGAGGGTCTTA-3'
HTLV-1 (LTR) Forward primer	5'-CATAAGCTCAGACCTCCGGG-3'
HTLV-1 (LTR) Reverse primer	5'-GATGGCGGCCTCAGGTAGG-3'

Statistical analysis

Descriptive data were summarized as mean, Standard Deviation (SD) and/or percent and were analyzed by GraphPad Prism.

Ethical approval

Present study was accepted by the Medical Ethics Committee from Tehran University of Medical Sciences. Moreover, informed consent has been obtained from all involved human subjects.

Results

Clinical data

Table 2 demonstrates a summary of Iranian surveys that are related to blood-borne viruses in hemodialysis and kidney transplantation groups. The authors, year, region, study population, sample size, infection, confirm case and prevalence were extracted from the selected studies for more

considerations. In this study, all of patients were indigenous of Iran with a slight predominance of male subjects. Hemodialysis-dependent patients included 99 males and 75 females, and 89 males and 50 females participated in the transplantation group. According to the age, the participants were divided into five age groups of >20, 20-35, 35-50, 50-65 and <65 yr old, most of them were 50-65 yr old and their average age was 56 yr (range 17-86) for hemodialysis-dependent patients, and 44 yr (range 17-74) for transplantation patients.

Demographic data were presented in Tables 3 and 4. In our survey, all patients in both groups of hemodialysis and transplant were negative for HIV and HBV; however, six cases (two male and four female) in hemodialysis group and three cases (two male and one female) in transplant group involved with HCV infection.

Table 2: literature search of blood-borne viruses in hemodialysis patients and kidney transplant patient in Iran

<i>Ref.</i>	<i>Year</i>	<i>Region</i>	<i>Study population</i>	<i>Sample size (n)</i>	<i>Infection</i>	<i>Confirmed cases</i>	<i>Relative frequency</i>
(24)	2016	Bandar abbas	Hemodialysis	149	HCV	5	3.36%
(7)	2016	Mazandaran	Hemodialysis	482	HBV/ HCV/ HIV	10/40/0	2.1%/ 8.27%/ 0%
(25)	2016	Bandar abbas	Hemodialysis	153	HBV/ HIV	9/ 0	5.88% / 0%
(17)	2014	Isfahan	Hemodialysis	499	HBV /HCV	6/26	1.2% / 5.2%
(26)	2014	Tabriz	Hemodialysis	455	HCV	37	8.10%
(20)	2013	Mazandaran	Hemodialysis	160	HTLV	1	0. 6%
(18)	2012	Kerman	Hemodialysis	228	HBV /HCV/ HIV	16/16/0	7% / 7% / 0%
(16)	2012	Guilan	Hemodialysis	514	HBV/ HCV	7/2	1.4%/ 0.38%
(27)	2012	Tabriz	Hemodialysis	412	HBV	13	5.80%
(28)	2011	Qazvin	Hemodialysis	195	HBV/ HCV	6/13	3.1% / 6.7%
(29)	2011	Guilan	Hemodialysis	514	HBV/ HCV	7/ 61	1.4%/ 11.9%
(19)	2010	Tehran	Hemodialysis	289	HBV/HCV/ HIV	8/9/1	2.8%/ 3.1%/ 0.34%
(15)	2010	Northern Iran	Hemodialysis	334	HCV	67	20%
(21)	2013	Southern Iran	Transplant	46	HCV	8	17.40%
(23)	2011		Transplant	91	HCMV	31	34%
(22)	2010	Urmia	Transplant	91	HTLV-1	1	1.09%

Table 3: Hemodialysis patient’s demographics data

<i>Demographics Data</i>	<i>Number of Patients</i>
Age (yr)	
=<20	1
20-35	25
35-50	43
50-65	61
>65	54
Female	75
Male	99
Total	174

The HCV incidence was 3.44% in the hemodialysis group and 2.15% in the transplant group. We found that 52 individuals of transplant patients were HCMV positive (37.4%). Current study demonstrated that two cases of hemodialysis group with the age of <65 yr old infected by HTLV-1 (one male and one female), and one female was found as a HTLV-1 infected individual

Table 4: Kidney Transplant patient’s demographics data

<i>Demographics Data</i>	<i>Number of Patients</i>
Age (yr)	
<20	6
20-35	38
35-50	46
50-65	40
>65	8
Female	50
Male	89
Total	139

with the age of 35-50 yr old in KT group. Incidence of HTLV-1 infection among the hemodialysis patients were 1.14% and 0.71% for transplant group. In KT group, all HCV seropositive individual showed simultaneously infection with HCMV. The study demonstrated that the rate of co-infection with HCV and HCMV in this group was 2.15% (Table 5).

Table 5: Relative frequency of blood-borne viruses in transplant patients and hemodialysis patients

<i>Transplant</i>				
HIV				
Sample size	Confirmed cases	Relative frequency	Gender	Co-infection
139	0	0	-	No
HTLV-1				
Sample size	Confirmed cases	Relative frequency	Gender	Co-infection
139	1	0.71	F	No
HCV				
Sample size	Confirmed cases	Relative frequency	Gender	Co-infection
139	3	2.15	M(2) F(1)	3/HCMV
HBV				
Sample size	Confirmed cases	Relative frequency	Gender	Co-infection
139	0	0	-	No
HCMV				
Sample size	Confirmed cases	Relative frequency	Gender	Co-infection
139	52	37.4	M(35) F(17)	3/ HCV
Hemodialysis				
HIV				
Sample size	Confirmed cases	Relative frequency	Gender	Co-infection
174	0	0	-	0
HTLV-1				
Sample size	Confirmed cases	Relative frequency	Gender	Co-infection
174	2	1.14	M(1) F(1)	0 0
HCV				
Sample size	Confirmed cases	Relative frequency	Gender	Co-infection
174	6	3.44	M(2) F(4)	0 0
HBV				
Sample size	Confirmed cases	Relative frequency	Gender	Co-infection
174	0	0	-	0

Discussion

A review of existing scientific literature showed that northern Iran had the highest prevalence of HCV infection in the HD patients among other nine studies (20%) (15), whereas lowest prevalence was observed in Guilan Province with the 0.38% frequency (16). Mean prevalence of HCV infection in total studies was 7.41%.

Nine studies were investigated HBV infection in HD patients, highest and lowest prevalence of HBV infection goes to Kerman and Isfahan with a frequency of 7% and 1.2% (17, 18). Mean prevalence of these nine studies was 3.4%. Among four surveys that studied on the HIV infection in

HD patients, Tehran was only city that demonstrated the HIV infection in HD patients with a prevalence of 0.34% (19). HTLV-1 infection was evaluated in Mazandaran and it displayed 0.6% frequency (20). Three surveys based on southern Iran and Urmia studied on kidney transplant patients (21-23). Each of them considered one of the HCV, HCMV and HTLV-1. Their results indicated that HCV prevalence in southern Iran was 17.4% (21), HCMV infection had 34% frequency in KT patients (23), while HTLV-1 prevalence of KT patients was 1.09% in Urmia (22). Due to these results, HCMV infection among KT patients may be an important factor in patients who will go under transplantation.

In this study the prevalence rates of HBV and HCV in hemodialysis patients were moderate to low in the Mazandaran province. Current findings indicated that no cases were positive for HBV infection in hemodialysis group and KT patients, this comparison shows an improvement in prevalence of HBV infection among intended groups. Furthermore, among patients receiving hemodialysis, 6 of them were HCV positive patients (3.44%), on the other hand, in transplantation patients 4 of them had HCV infection (2.15%). HCV infection among patients that received kidney graft were decreased in comparison with previous studies (21).

Compared to many previous studies, less prevalence of HCV infection in hemodialysis patients were observed, but its prevalence was more than few studies (17-19, 24, 26, 28-30). Desirable reduction of HCV infection in hemodialysis groups must need strategies that are more practical.

The prevalence rate of HTLV-1 infection in the general population and blood donors varies between provinces such as West Azerbaijan (0.34%), Bushehr (0.13%), and Chaharmahal and Bakhtiari (0.62%) (14, 20, 31).

HTLV-1 is endemic in some areas of the world. It is also endemic in Mashhad, Northeast Iran, the capital of Razavi Khorasan Province, and prevalence of HTLV-1 infection in 2011 was estimated to be 2.1% in the general population and 0.44% in blood donors with no significant difference between males and females ($P=0.093$) (32-34). In our study, samples were investigated using ELISA verified by PCR. Findings showed two reactive cases for HTLV antibodies (one male and one female).

Overall, 6 (3.44%) and 3 (2.15%) of hemodialysis-dependent and KT patients had evidence of HCV infection. Due to the studies on hemodialysis patients and KT patients done to evaluate the prevalence of infection with HTLV-1, Mazandaran had 0.6% frequency of HTLV-1 infection in hemodialysis patients and this infection was 1.09% in KT patients in Urmia (20, 22).

Whereas many previous studies showed remarkable prevalence of HIV infection, in general population of some provinces of Iran, our results

demonstrated no patients were HIV positive in hemodialysis and KT patients (35).

About 34% of patients who received kidney grafts had HCMV infection (23). However, in this study, HCMV positive patients were detected in 52 (37.4%) of KT patients which is slightly more. Generally, patients required to go under SOT should be routinely checked for incidence of positive pre-transplant HCMV IgM test; however, those who are in urgent for operating despite positive pre-transplant HCMV IgM. In addition, in the present study, the frequency of hemodialysis dependent patients was in individuals older than 50 yr old with slight tendency toward men. Indeed, among 174 patients receiving hemodialysis, 99 of them were men and 75 were women and frequency of KT patients was in individuals between 35-50 yr, among them 89 were men and 50 were women. Nevertheless, this study provides new evidence on the prevalence of BBVs among hemodialysis-dependent and kidney transplant patients in all geographic regions of Iran from Hasheminezhad referral hospital.

Screening practices can now be adjusted based on this epidemiological information to enable the care of hemodialysis-dependent and KT patients to be optimized as well as continuing to maintain low levels of blood-borne viruses seroconversions across dialysis units and despite of significant role of infections in morbidity and mortality after transplantation, improved prophylactic, diagnostic and treatment strategies have decreased the negative effect of infection on transplant outcomes. Albeit, transmission of HTLV-1 via organ transplantation is rare, the prevalence of HTLV-1 needs more attention before solid organ transplantation, also in low prevalence countries. Ongoing attention to infection prevention beginning before transplantation, as well as improved surveillance for infections, should be maintained in all patients being considered for transplantation in Iran.

The main limitation of this study is that our findings might not be fully generalizable to the whole population of Iran because our study population

comprised hemodialysis-dependent and transplantation patients.

Conclusion

The probable cause of low prevalence of BBVs infection in our survey in these groups compared to the previous studies might be current strict infection control practices that appear to be effective in limiting dialysis and after transplantation related infections.

Because BBVs infections constantly occur especially in dialysis and after transplantation units, our data will be useful to build a new strategic plan for the elimination of BBVs.

Ethical considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

Conflict of interest

The authors declare that there is no conflict of interest.

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