

Original Article



Prevalence and Associated Risk Factors of HTLV-1 and Co-infections of Blood-Borne Viruses in Birjand, Iran's Eastern Border

Masoud Yousefi, PhD¹; Gholam Reza Sharifzadeh, MSc¹; Azadeh Ebrahimzadeh, MD¹; Zohreh Azarkar, MD¹; Mohammad Hasan Namaei, PhD¹; Ghedsiyeh Azarkar, MD¹; Sanaz Ahmadi Ghezeldasht, MSc^{2,3}; Rahim Rezaee, PhD³; Najmeh Valizadeh Zare, PhD⁴; Arman Mosavat, PhD²; Masood Ziaee, MD^{1*}

¹Birjand Infectious Disease Research Center, Birjand University of Medical Sciences, Birjand, Iran

²Blood Borne Infections Research Center, Academic Center for Education, Culture and Research (ACECR), Razavi Khorasan Branch, Mashhad, Iran

³Immunology Research Center, Inflammation and Inflammatory Diseases Division, Mashhad University of Medical Sciences, Mashhad, Iran

⁴Nursing and Midwifery Care Research Center, Mashhad University of Medical Sciences, Mashhad, Iran

Abstract

Background: Blood-borne viruses (BBVs) are one of the most important public health concerns. South Khorasan has a long border with Afghanistan and concern has risen there about blood-borne oncogenic viral infections. The aim of the present study was to evaluate the prevalence and associated risk factors of human T-lymphotropic virus 1 (HTLV-1) and co-infections of BBVs in Birjand, Iran's eastern border.

Methods: In this cross-sectional study, 3441 subjects were tested for sero-prevalence of HTLV-1 by ELISA. The data on demographic features, HTLV-1-related risk factors and other characteristics of the population were analyzed by Pearson chi-square and logistic regression tests. Finally, the co-infection of BBVs was evaluated in the study.

Results: The prevalence of HTLV-1 was 0.3% (95% CI: 0.12–0.48). Notably, the sero-prevalence of HIV, hepatitis B virus (HBV), hepatitis D virus (HDV), and hepatitis C virus (HCV) in our previous studies was reported at 0%, 0.2%, 1.2% and 1.6%, respectively. The results indicated that the occurrence of HTLV-1 infection was associated only with the history of hospitalization (odds ratio [OR]: 0.27, 95% CI: 0.07–0.97, with $P = 0.04$). The co-infection of HBV with HCV was the most common (2.35%), while a co-infection rate of 1.17% was found for both HBV/HTLV-1 and HBV/HDV.

Conclusion: Although a higher prevalence of the viruses was expected, it was close to the overall Iranian population. With respect to close relationship with an HTLV-1 endemic area (Mashhad and Neyshabour), the prevalence is very low; however, more attention is needed. Our findings reinforce the importance of increasing knowledge about BBV-related health risk behaviors to prevent the emergence of new cases, especially in low-risk populations.

Keywords: Blood-borne pathogens, Epidemiology, Human T-lymphotropic virus 1, Iran, Risk factors

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Introduction

Hepatitis B virus (HBV), hepatitis D virus (HDV), hepatitis C virus (HCV) and human T-lymphotropic virus 1 (HTLV-1) are the most significant blood-borne viruses (BBVs) that can be associated with human cancers. These infections are major public health concerns since they are responsible for millions of deaths annually.^{1,2}

HBV and HCV infections represent a significant public health concern; HTLV-1, as an oncogenic virus, is moving progressively towards becoming a threatening health issue. Except HTLV-1, the prevalence of these infections is greater in some eastern provinces of Iran such as South Khorasan due to their closer ties to Afghanistan.³⁻⁵ On the other hand, although co-infections of these viruses are not exactly common, they occur in some circumstances.⁶

The sero-prevalence rate of HBV/HCV co-infection among individuals with seropositive hepatitis B surface antigen (HBsAg), chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma in Asia and Western countries is estimated at 10%–15%.⁷ This condition is the most common in injecting drug users, HIV-infected patients and people who need hemodialysis or blood transfusions.⁸ Co-infection with hepatitis viruses (hepatotropic viruses) are known to influence progression and management as well as the outcome of retroviruses infections, such as HIV and HTLV-1.⁹

HTLV-1 infection, as a neglected oncovirus, is becoming a major threat for public health because of stigmatic beliefs about it and its associated life-threatening diseases. Furthermore, it is a growing problem, which is

*Corresponding Author: Masood Ziaee, MD; Shahid Ayatollah Ghaffari St., Infectious Diseases Research Center, Birjand University of Medical Sciences, Birjand, 9717853577, Iran. Tel: +98-91-51613942; Email: dr.m.ziaee@gmail.com

associated with increased risk of spreading through organ transplantations, blood transfusion and human behavioral changes.¹⁰ The North and Razavi Khorasan provinces, in close relationship with South Khorasan, constitute the main endemic area in Iran for HTLV-1 at 2%–7%.¹¹ Even a segregated population in the Razavi Khorasan, close to South Khorasan, is endemic (1.25%) for this virus.¹²

Given the importance of global growth of HTLV-1 along with the danger of its co-infections with other common blood-borne oncogenic viruses, it could provide new insights into the world of microbial pathogenicity. Therefore, in this study, the incidence of HTLV-1 infection and co-infections of BBVs in Birjand, Iran's eastern border, were evaluated.

Materials and Methods

Study Design and Population

This cross-sectional study was conducted on 3441 subjects in urban areas of Birjand city, Iran with an age range of 14 to 70 years, who were chosen by simple random sampling using a random numbers table from a comprehensive study including 5235 subjects, which was performed in Birjand, South Khorasan, Iran, during 2013-2014.⁵ The checklist of socio-demographic characteristics and behavioral risk factors were based on a review of the literature.

Sample Collection

After obtaining informed consent and completing the demographic data, 8 mL venous blood was collected by venipuncture from each of the participants in sterile tubes containing EDTA. The samples were stored at -30°C until further analysis.

Serological Assays

The serum samples were screened in this study for the presence of anti-HTLV-1 using Enzyme-linked Immunosorbent Assay (ELISA) according to the manufacturers' recommendations (Dia.pro, Italy). Furthermore, anti-HIV, anti-HCV, anti-HDV and anti-HBc antibodies as well as hepatitis B virus surface antigen (HBsAg) were evaluated by ELISA in our previous studies.^{5,13-15} It is noteworthy that the blood serum of patients with positive HBsAg was used in order to determine the prevalence of HDV infection. Finally, the positive anti-HCV serological results were confirmed using specific primers for HCV by polymerase chain reaction (PCR).¹³

Statistical Analysis

The data on demographic features, HTLV-1-related risk factors and other characteristics of the population were analyzed with the Pearson chi-square and Fisher's exact tests. The association of HTLV-1 with sociodemographic characteristics and behavioral risk factors was analyzed using a univariate logistic regression model, by estimating

the odds ratio (OR) and 95% confidence interval (CI). Data were analyzed using SPSS version 21. A *P* value less than 0.05 was considered statistically significant.

Results

Demographic Characteristics

This study was conducted on 3441 subjects in urban areas of Birjand, eastern Iran. The study population involved 1611 (46.8%) males and 1830 (53.2%) females. The mean age of participants was 38.67 ± 14.49 (range: 14 to 70) years. The data showed that 81.4% and 16.4% of the population were married and single, respectively; the others (2.2%) were widowed or divorced.

Sero-Prevalence of Viruses

In the present study and according to the serology tests, the sero-prevalence of HTLV-1 was 0.3% (95% CI: 0.12-0.48). It is noteworthy that in this study, six participants (0.2%) were reported as suspected of having HTLV-1 infection. Furthermore, the sero-prevalence of anti-HIV, anti-HCV, anti-HBc and HBsAg, as reported in our previous studies, were 0% (95% CI: 0-0.001), 0.2% (95% CI: 0-0.036), 15% (95% CI: 0.086-0.235) and 1.6% (95% CI: 0.002-0.07), respectively (5, 13, 15). Also, the results of the PCR confirmed 7 cases out of 10 people with anti-HCV positive, and so the actual prevalence of HCV was reported at 0.14%. Amongst HBsAg-positive patients, HDV infection sero-prevalence was 1.2% (1 out of 85 patients) with 95% CI, 0-0.054 (14). The sero-prevalence of blood-borne oncogenic viruses (HCV, HBV, HDV, HIV and HTLV-1 infections) is shown in Figure 1.

Risk Factors for HTLV-1 Infection

As shown in Table 1, there was no evidence of a significant relationship between HTLV-1 infection and demographic features of patients including age, sex and education level ($P > 0.05$). The associations of HTLV-1 infection with the behavioral risk factors are shown in Table 1. The occurrence of HTLV-1 infection was associated only with history of hospitalization ($P = 0.04$). HTLV-1 seropositivity was

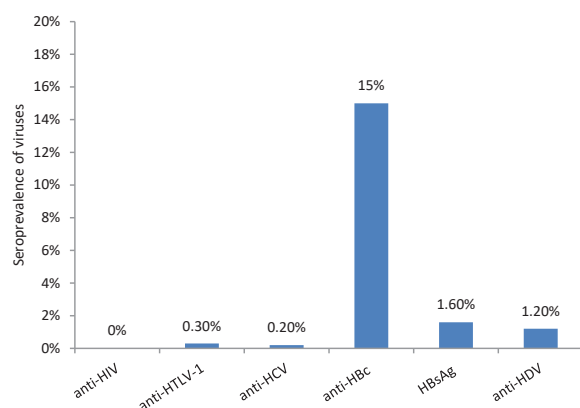


Figure 1. The Sero-Prevalence of Blood-Borne Oncogenic Viruses (HCV, HBV, HDV, HTLV-1 and HIV Infections).

reported in 0.6% of cases with a history of hospitalization vs. 0.2% in those with no history. The results of univariate logistic regression analysis showed that the risk of HTLV-1 infection in patients with no history of hospitalization was 73% lower than those with a history of previous hospital

Table 1. Associations of HTLV-1 Infection with the Socio-demographic and Behavioral Risk Factors

Item/Status	Positive (%)	Negative (%)	P value
Age group (y)			
14–24	1 (0.1)	682 (99.9)	0.15
25–34	5 (0.6)	807 (99.4)	
35–44	1 (0.1)	683 (99.9)	
45–54	1 (0.1)	674 (99.9)	
55–64	4 (0.9)	455 (99.1)	
65–70	0 (0)	128 (100)	
Gender			
Male	4 (0.3)	1607 (99.7)	0.34
Female	8 (0.5)	1822 (99.5)	
Education			
Illiterate	0 (0)	375 (100)	0.41
Primary	4 (0.6)	654 (99.4)	
Pre-high school	0 (0)	362 (100)	
High school	4 (0.4)	1016 (99.6)	
University	4 (0.4)	1022 (99.6)	
Blood transfusion			
Yes	0 (0)	138 (100)	0.47
No	12 (0.4)	3291 (99.6)	
Hospitalization			
Yes	9 (0.6)	1495 (99.4)	0.04
No	3 (0.2)	1934 (99.8)	
Cupping			
Yes	2 (0.4)	525 (99.6)	0.94
No	10 (0.3)	2904 (99.7)	
Tattoo			
Yes	0 (0)	53 (100)	0.67
No	12 (0.4)	3376 (99.6)	
Needle stick			
Yes	0 (0)	24 (100)	0.75
No	12 (0.4)	3405 (99.6)	
Dental service			
Yes	3 (0.4)	704 (99.6)	0.52
No	9 (0.3)	2725 (99.7)	
Major surgery			
Yes	4 (0.5)	808 (99.5)	0.48
No	8 (0.3)	2621 (99.7)	
Minor surgery			
Yes	1 (0.1)	865 (99.9)	0.13
No	11 (0.4)	2564 (99.6)	
Alcohol drinking			
Yes	0 (0)	21 (100)	0.77
No	12 (0.4)	3408 (99.6)	
IV drug abuse			
Yes	0 (0)	58 (100)	0.89
No	12 (0.4)	3371 (99.6)	
Non-IV drug abuse			
Yes	0 (0)	97 (100)	0.55
No	12 (0.4)	3332 (99.6)	
Illegal sexual contact			
Yes	0 (0)	25 (100)	0.78
No	12 (0.4)	3404 (99.6)	

admission (OR 0.27, 95% CI 0.07-0.97) (Table 2). It is noteworthy that since the prevalence of HTLV-1 was low at some levels of the factors studied, ORs were reported only for those variables that could be fitted to the model correctly.

Table 2. Analysis of Risk Factors Related to HTLV-1 infection in the Study Subjects

Item/Status	Beta	OR	P value	95% CI
Age group (y)				
14–24	-0.56	0.57	1	—
25–34	15.47	—	0.99	—
35–44	13.94	—	0.99	—
45–54	14.01	—	0.99	—
55–64	15.88	—	0.99	—
65–70*	Ref.	Ref.	Ref.	Ref.
Gender				
Male*	0.30	1.35	0.64	0.37–4.96
Female	—	—	—	—
Education				
Illiterate	-15.77	—	0.99	—
Primary	0.42	1.53	0.62	0.27–8.68
Pre-high school	-15.15	—	0.99	—
High school	0.292	1.33	0.7	0.28–6.19
University*	Ref.	Ref.	Ref.	Ref.
Blood transfusion				
Yes*	15.81	—	0.99	—
No	—	—	—	—
Hospitalization				
Yes*	-1.41	0.27	0.04	0.07–0.97
No	—	—	—	—
Cupping				
Yes*	-0.08	0.917	0.91	0.193–4.36
No	—	—	—	—
Tattoo				
Yes*	15.5	—	0.99	—
No	—	—	—	—
Needle stick				
Yes*	15.71	—	0.99	—
No	—	—	—	—
Dental service				
Yes*	-0.5	0.6	0.47	0.15–2.4
No	—	—	—	—
Major surgery				
Yes*	-0.17	0.84	0.8	0.21–3.29
No	—	—	—	—
Minor surgery				
Yes*	1.95	7.07	0.067	0.87–57.27
No	—	—	—	—
Alcohol drinking				
Yes*	14.82	—	0.99	—
No	—	—	—	—
IV drug abuse				
Yes*	14.98	—	0.99	—
No	—	—	—	—
Non-IV drug abuse				
Yes*	-14.79	—	0.99	—
No	—	—	—	—
Illegal sexual contact				
Yes*	14.12	—	0.99	—
No	—	—	—	—

Beta, Regression coefficient; OR, odds ratio; CI, confidence interval.

*Reference level.

—No estimation due to small number of cases.

Co-infection of Blood Borne Viruses

The occurrence of HTLV-1, HDV and HCV co-infections with HBV among the population are shown in Table 3. The results indicated that simultaneous presence of HBV and HCV was the most common co-infection among 85 HBsAg positive patients (2.35% with 95% CI, 0.002–0.072). It is noteworthy that no co-infection was detected except for the simultaneous presence of HBV and other viruses (HTLV-1, HDV and HCV).

Discussion

BBVs (BBVs) are still one of the most important global public health concerns. In addition to HBV and HCV, the Khorasan provinces are an endemic region for HTLV-1, which is the most significant blood-borne oncogenic virus.^{2,10} In the North and Razavi Khorasan provinces, the prevalence of HTLV-1 has been reported at 2-3%¹¹; however, the prevalence of this virus in South Khorasan is yet to be evaluated.

In the present study, the sero-prevalence of HTLV-1 was evaluated among a population in South Khorasan, eastern Iran with a long border with the Afghanistan. According to the serology tests, anti-HTLV-1 was 0.3%. Furthermore, the sero-prevalence of anti-HIV, anti-HCV, anti-HDV, anti-HBc and HBsAg in our previous studies was reported at 0%, 0.2%, 1.2%, 15% and 1.6%, respectively.^{5,13-15} The results of our studies show that the prevalence of blood-borne viral infection in South Khorasan is close to that observed in the overall Iranian population.¹⁶⁻¹⁸ However, the prevalence of HTLV-1 was 10 times lower than North and Razavi Khorasan provinces (0.3% vs. 2-3%),¹¹ and even 4 times lower than a segregated population in this region close to South Khorasan.¹² On the other hand, in other studies on subjects at risk of these viruses in northeastern Iran,^{1,19-21} the sero-prevalence of blood-borne oncogenic viruses (HCV, HBV, HTLV-1 and KSHV infections) was reported to be considerably higher than our study. With respect to the present study, it is more likely that the source of HTLV-1 infection in Iran, as an archetypic virus, is in Razavi Khorasan, Neyshabour or Mashhad.

Previous studies have also shown that different risk factors can be involved in the incidence of BBV infections. Therefore, knowledge on these factors can help in prevention and control of infections caused by BBVs. Our study indicated that the occurrence of HTLV-1 infection was associated only with history of hospitalization, such that the risk of HTLV-1 infection in patients with no history of hospitalization was 73% lower than those with a history of previous hospital admission. Accordingly, the authorities should pay more attention to HTLV-1 screening related to blood banking and surgical activities, as this region has a close relationship with the Razavi Khorasan, an endemic area for this virus. In a study in Khorasan Razavi province, the main risk factors for HTLV-1 infection were hospitalization, traditional cupping, age,

Table 3. HTLV-1, HDV and HCV Co-infections with HBV among HBsAg Positive Patients

Co-infections	No. (%)	95% CI
HBV, HTLV-1	1 (1.17)	0–0.056
HBV, HDV	1 (1.17)	0–0.056
HBV, HCV	2 (2.35)	0.002–0.072

marital status, history of blood transfusion and surgery. However, occupational infection has not been reported so far for HTLV-1 in a cohort study of 10 years.²⁴ In a previous study conducted among Peruvian women, history of blood transfusion, chronic scabies, having a relative with leukemia, age ≥ 38 years, young age at first intercourse, having more than four lifetime partners, educational status and having more than four pregnancies were significantly associated with HTLV-1 infection.²⁵ It seems that the risk factors for BBV infections such as HTLV-1 can be different depending on the study population and the cultural, educational, behavioral, socio-economic, and geographical variables.

Finally, in the present study, HBV-HTLV-1, HBV-HDV and HBV-HCV co-infections were found at 1.17%, 1.17% and 2.35%, respectively. In a previous study,²⁶ a co-infection rate of 0.5% was found for HBV/HCV, while a rate of 1.1% was reported for HBV/HTLV-1 co-infection, which is nearly the same as our study. On the other hand, in a survey conducted in Australia in 2017,²⁷ the sero-prevalence of HTLV-1 among HBV-infected patients was 14.4%, which is much higher than the 1.17% found in our study. These discrepancies may be the result of heterogeneity in patient populations studied in different geographical regions. Taken together, many studies suggest that physiological, iatrogenic and acquired immune-compromise along with environmental changes are the main factors for emerging and re-emerging microbial co-infections. However, host ecology and virus-virus interactions are important drivers of the occurrence and spread of viral co-infection.²⁸

A limitation of the present study was the impossibility of Generalized Estimating Equation (GEE) analysis due to lack of identification of clusters at the time of data collection. In addition, given the rarity of the infection and lack of a sufficient number of positive cases in the age subgroups, it was not possible for us to use a direct standardization method for adjusting the incidence rate.

In conclusion, apart from HTLV-1, which had a lower prevalence compared to the neighboring provinces, the prevalence of other blood-borne oncogenic viruses in South Khorasan is close to that observed in the overall Iranian population. Our results suggest that the most important risk factor for HTLV-1 infection was history of hospitalization. Consequently, our findings reinforce the importance of improving our knowledge on BBV risk of hospitalization and behavioral intention, particularly in

general populations to prevent the emergence of new cases.

Authors' Contribution

GRS, AE and ZA collected the data and provided the first reports. MZ designed and supervised the research. MY and SAGH interpreted the analysis and wrote the final manuscript. MHN, GHA, RR, NVZ and AM contributed to the discussion and critical revision of the manuscript.

Conflict of Interest Disclosures

None declared.

Ethical Statement

The study was approved by the Bio-medical Ethics Committee of Birjand University of Medical Sciences (IR.BUMS.REC.1394.381).


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