

Relative Frequency of HTLV-I Infection in Blood Donors in Ahvaz, Southwest of Iran

Roohangize Nashibi^{1*}, Manoochehr makvandi², Fateme Ahmadi¹, Bijan Helli³, Zahra Lotfi⁴, Mehdi Parsanahad²

1. Infectious and Tropical Diseases Research Center, Health Research Institute, Ahvaz Jundishapur University of Medical sciences, Ahvaz, Iran

2. Virology Department, School of Medicine, Ahvaz Jundishapur University of medical sciences, Ahvaz, Iran

3. Nutrition and Metabolic Diseases Research Center, Ahvaz Jundishapur University of medical sciences, Ahvaz, Iran

4. Ahvaz Jundishapur University of medical sciences, Ahvaz, Iran

* Correspondence: Roohangizenashibi@yahoo.com

Infectious and tropical diseases research center, health research institute, Ahvaz Jundishapur University of medical sciences, Ahvaz, Iran

Roohangizenashibi@yahoo.com

manoochehrmakvandi299@gmail.com

Ahmadi031@yahoo.com

Bizhanhelli@yahoo.com

Zahra_lotfi_60@yahoo.com

mehdiparsanahad@gmail.com

Abstract

Human T-lymphotropic virus type I (HTLV-I) is recognized as the inaugural human retrovirus. It constitutes a significant health concern in endemic areas, with HTLV-1 primarily disseminated through infected biological fluids, such as blood, breast milk, and semen. Contributory risk factors encompass unprotected sexual intercourse, the use of intravenous drugs, tissue transplantation, as well as blood and blood component donation. HTLV-I is implicated in two critical pathologies, namely adult T-cell leukemia and tropical spastic paraparesis. The geographic prevalence of HTLV-I exhibits variability globally, including within the context of Iran. This investigation was undertaken to assess the prevalence of the HTLV-I virus among blood donors in Ahvaz. In this cross-sectional analysis, a total of 777 blood donors were enrolled, comprising 771 (99.2%) males and 6 (0.8%) females, with a mean age of 38.08 ± 8.42 years. All blood specimens were systematically evaluated for HTLV-I antibodies utilizing enzyme-linked

immunosorbent assay (ELISA) in accordance with the manufacturer's protocols. Positive cases were identified through polymerase chain reaction (PCR) analysis utilizing peripheral blood mononuclear cells (PBMC) derived from the positive ELISA blood specimen. Among the population of 777 blood donors, a singular case (0.1%) tested positive for the HTLV-I antibody. The individual in question was a 35-year-old male, married, with no documented history of blood transfusions, surgical procedures, dialysis, intravenous drug use, phlebotomy, or engagement in high-risk sexual behaviors, either personally or through his spouse. The PCR analysis yielded a negative result. The findings of this investigation indicate that HTLV-I infection is not endemic among blood donors in Ahvaz, and thus, the screening of blood samples for HTLV-I infection in this region does not seem warranted.

Keyword: HTLV-I; Blood donors; Ahvaz; Endemic

1. Introduction

Human T-cell lymphotropic virus (HTLV-I) is recognized as the inaugural human retrovirus, first identified by Poiesz et al. in the year 1979. This virus is classified within the Retroviridae family and falls under the Delta Virus group. The mechanisms of transmission of this virus are primarily categorized into three significant pathways: maternal transmission to offspring, sexual intercourse, and through blood transfusions. The maternal transmission to offspring predominantly takes place during breastfeeding and occurs when lymphocytes are compromised by the virus (1). The efficacy of virus transmission has been substantiated through the administration of products enriched with white blood cells, including whole blood, platelets, and packed red blood cells. The incidence of transmission via unprotected sexual intercourse has been reported at a rate of 0.9% per 100 individuals annually (2). Approximately 98% of individuals infected with HTLV-I remain asymptomatic throughout their lifespan. The clinical manifestations associated with HTLV-I are infrequently observed. HTLV-I is linked to several significant pathologies, including adult T-cell leukemia (ATL) and HTLV-I-associated tropical spastic paraparesis (HAM/TSP). The likelihood of disease advancement is contingent upon factors such as age, the mode of infection, and the host's immunological status (3). The geographic distribution of HTLV-I exhibits considerable variability. A majority of ATL cases have been documented in Japan, followed by instances in India, Africa, South America, and certain regions of Iran. The occurrence of the disease is uncommon in Europe and North America, where it has only been reported on rare occasions (4). According to contemporary research, the global incidence of HTLV-I is approximated to range from 5 to 10 million individuals. HTLV-I infection is notably endemic in Central Africa, exhibiting a seroprevalence rate between 0.5-5.5%, as well as in the southwestern region of Japan, the Caribbean islands, and South America (5). In addition, HTLV-I infection demonstrates endemic characteristics in the northeastern region of Iran, particularly within the urban centers of Mashhad, Sabzevar, and Neishabour. The rates of infection in Mashhad, Neyshabur, and Sabzevar are recorded at 2.2%, 3%, and 1.6% respectively (6). Conversely, other regions of Iran, including Urmia (0.34%) in the northwest, Chahar Mahall-Bakhtiari (0.62%) in the southwest, and Mazandaran (0.08%) in the northern area, do not qualify as endemic territories for the virus (7). Currently, the management of this viral infection is obstructing the most efficacious approaches to address the secondary complications that have arisen. In light of the widespread nature of this viral

infection, it is imperative to establish effective screening protocols and to evaluate high-risk populations. By determining the seroprevalence of this virus within the general populace, a benchmark for comparison among high-risk groups will be established. Variability in infection rates has been observed across different regions of Iran, and considering the absence of data regarding the seroprevalence of HTLV-I in the area's general population, it becomes essential. Consequently, an investigation into the prevalence of HTLV-I among blood donors, representing a segment of the general population in this city, can furnish us with extensive insights into the epidemiological status of this disease in the region.

2. Materials and methods

2.1. Study population

A cross-sectional study was conducted in 2017 in a blood donor group related to the Ahvaz blood transfusion center. According to the consulting doctor, it was found that the individuals did not have any specific disease or risk behavior and had no tattoos, no blood transfusions, no cupping, no surgical procedures in the last year and did not have a fever during the donation. 2.2. Sample collection and processing Blood samples after obtaining written consent and filling in the information required from the donor (gender, age, marital status, place of residence, place of birth, number of households, occupation, history of surgery, history of blood transfusion, etc. Blood products) was taken. Approximately 5 ml of peripheral blood was collected from each donor in the sterile tube, then the serum was removed by centrifugation and stored at -20°C until use.

2.2. Serological test and PCR

The serum samples of each blood donor were tested for anti-HTLV-I IgM and IgG using the enzyme-linked immunosorbent assay (ELISA) method (Dia Pro Diagnostic Bio Probe, Italy) according to the manufacturer's instructions. Antibodies tested against Gag proteins instructions. Peripheral blood mononuclear cells (PBMC) of the positive ELISA blood sample were isolated by density gradient centrifugation using Ficoll (Sigma, USA). Total RNA was extracted using the RNeasy Mini Kit (Qiagen, Germany) and cDNA was prepared using the RevertAid First Strand cDNA Synthesis Kit (Thermo, USA) according to the manufacturer's instructions. PCR was performed to confirm HTLV-I infection using the following specific primers: forward primer, LTR-sense (5'-CATAAGCTCAGACCTCCGGG-3') and LTR-anti-sense (5'-GGATGGCGGCCTCAGGTAGG-3'). The PCR reaction was performed in a 25 µL volume containing 100 ng of genomic DNA, 10 pmol of each primer, 2.5 µL of 10x PCR buffer, 2 mM MgCl₂, 0.5 mM dNTPs, and 3 U of Taq DNA -Polymerase contained. Thermal PCR conditions were as follows: 1 cycle at 95°C for 5 minutes, 35 cycles including denaturation at 95°C for 60 seconds, annealing at 61°C for 45 seconds, and extension at 72°C for 45 seconds; and a final extension at 72°C for 5 minutes. The PCR product was loaded onto a 2% agarose gel and observed directly under ultraviolet (UV) light.

2.3. Statistical analysis

Descriptive and analytical statistical methods such as frequency distribution tables, frequency plots, central propensity index and appropriate dispersion were used to analyze the data. Data were analyzed using SPSS v.22.

3. Results

Of the 777 patients referred to Ahvaz Blood Transfusion Center, 771 (99.2%) were male and 6 (0.8%) were female. The mean age of the cases was 38.08 ± 8.42 years, the highest age was 63 and the lowest was 18 years. The highest frequency was in the age group of 25 to 35 years (36.6%) and 35 to 45 years (36.2%). Of these blood donors, 708 (91.1%) were married and 69 (8.9%) were single. The demographic characteristics of blood donors are shown in Table 1. At primary screening using enzyme-linked immunosorbent assay, one subject (0.1%) was positive for HTLV-I antibodies. The positive sample was tested for HTLV-I proviral DNA in PBMCs using a PCR test and was negative. He was a 35-year-old man, married and living in Ahvaz. The history of blood transfusions, surgery, dialysis, drug injections, cupping, and high-risk sexual behavior among themselves and their spouses was not mentioned. A tattoo from 25 years ago. He had no specific medical history.

4. Discussion

In general, the northeastern region of Iran is an endemic region for HTLV-I infection and in some other provinces such as Fars, Tehran and Qom, there is a relatively high prevalence of this virus; Therefore, screening of blood donors appears to be necessary. In other parts of the country, people infected with the virus are also noticeable in special groups such as injecting drug users and people with thalassemia. In our study, 771 (99.2%) were male and 6 (0.8%) were female. The mean age was 38.08 ± 8.42 years, the highest age was 63 and the lowest was 18 years. The highest frequency was in the age group of 25 to 35 years (36.6%) and 35 to 45 years (36.2%). In Rostamzadeh et al. In the study in Urmia, 2,046 blood donors and 114 dialysis patients were examined. In the blood donor group, 1910 (93.4%) were male and 136 (6.6%) were female, and the average age was 45 years (7). In the study by Masoudi et al. In Karaj, of 32,985 blood donors, 94.1% of the subjects were male and 5.9% female and were between 17 and 60 years old. The gender composition of individuals in these two studies was similar to that in our study. In a study by Ghaffari and colleagues, of 1200 patients referred to Sari Hospital, 65% were female and 35% were male (8). Also in a study by Miller et al. What was carried out in Mashhad's Jews in Palestine, there were more women (893 women as of 1679) (9). It is possible that women are less likely to give blood for cultural reasons, and some women are not accepted due to anemia. The reason for the number of women in the two studies mentioned above is because the study was conducted in the general population. In our study, one individual (0.1%) had a positive serologic test with a negative PCR test. The reason for these results may be to determine the prevalence of the virus in a low-risk population, since the person may be exposed to the virus and the immune system is stimulated, but it is controlled by the immune system before it integrates into the host genome and produces a provirus. HTLV-I virus was not found in blood donors in our study, which was lower than in other studies. Several studies have been conducted to determine the prevalence of HTLV-I in blood donors, with the prevalence of this virus found in India (10), Uganda (11), Brazil (12), China (13), and Nigeria (14). reported 0.18%, 0.54%, 0.15%, 0.13% and 3.2%, respectively. In Iran, HTLV-I infection has been studied in several cases in blood donors, including Sabzevar (15), Mashhad

(16), Ilam (17), and Babol (18). The infection rates were reported as 0.14%, 0.77%, 0.002% and 0.2%, respectively. HTLV-I infections have been studied in the general population in different regions of Iran, such as Golestan (0.29%), Sari (0.08%), and Neishabur (3.6%) (8, 19, 20). The high prevalence in Neyshabur indicates the endemic nature of this region in Iran. Several studies have examined the prevalence of this virus in high-risk groups in Iran. In a study among drug addicts, an HTLV-I prevalence of 16.33% was reported (21). In another study, HIV-positive patients did not show anti-HTLV antibodies in serum (22). In thalassemia and hemodialysis patients (23), the prevalence of this virus was reported to be 1.4% and 0.6%, respectively. Due to the lack of infection with the virus, it is likely that Ahvaz is a non-endemic region. Therefore, there appears to be no need to screen blood donations for HTLV-I infection in Ahvaz. Of course, since blood donors are considered individuals in the community who are at lower behavioral risk, the low prevalence of this virus in blood donors compared to the general population seems reasonable. Based on these interpretations, it appears that evaluation of this virus in high-risk groups and those with low immunity is necessary. In conclusion, our study showed that the prevalence of HTLV-I infection among blood donors in Ahvaz is 0.1%. Therefore, blood donation screening for HTLV-I infection is not required in Ahvaz. However, further studies on high-risk groups and high-risk individuals are needed.

limitation: Due to reducing the costs of this research, it was considered to make the sample sizes more below.

1. To make the sample sizes larger than this study without any worries about cost because it can focus more on the results
2. It could be determining the prevalence of HTLV-1- associated disorders for example lymphoma, ATL and HAM/TSP

Ethical Statements: This study was approved by the Ethics Committee of Ahvaz Jundishapur University of Medical Sciences under the ethical code of IR.AJUMS.REC.1393.159.

Authors' Contribution: Study concept and design: R.N, M.M, F.A, Z.L. Acquisition of data: M.P. Analysis and interpretation of data: R.N, Z.L, M.P. Drafting of the manuscript: R.N. Critical revision of the manuscript for important intellectual content: R.N. Statistical analysis: R.N, M.P. Administrative, technical, and material support: B.H, Z.L. Study supervision: R.N.

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Conflict of Interests: The authors declare no conflicts of interest.

Data Availability: The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Table 1 - Demographic and clinical characteristics of studied blood donors in Ahvaz

Preprint

<i>Characteristics</i>	<i>Number</i>	<i>Percent</i>
Gender		
Male	771	99.2
Female	6	0.8
Age, y		
15-25	137	17.6
25-35	284	36.6
35-45	281	36.2
≥45	75	9.7
Residence status		
Ahvaz	717	92.3
Other Cities	60	7.7
Marital status		
Single	69	8.9
Married	708	91.1
Education level		
Under high school diploma	205	26.3
High school diploma	257	33.08
Academic education	315	40.54
History		
Surgery	204	26.3
Blood transfusion	10	1.3