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Research Paper

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

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ABSTRACT

Introduction: Human T-lymphotropic virus type I (HTLV-I) is recognized as the first 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, intravenous drugs use, tissue transplantation, as well as blood and blood component donation. HTLV-I is implicated in two critical pathologies: Adult T-cell leukemia and tropical spastic paraparesis. The geographic prevalence of HTLV-I exhibits global variability, including within the context of Iran. This investigation was undertaken to assess the prevalence of the HTLV-I virus among blood donors in Ahvaz.

Materials & Methods: 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.

Results: 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 777 blood donors, a single 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.

Conclusion: 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 to be warranted.

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1. Introduction

Human T-cell lymphotropic virus (HTLV-I) is recognized as the inaugural human retrovirus, first identified by Poiez et al. in the year 1979 [1]. 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. 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; 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 prevalence of HTLV-I is estimated 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 focuses on 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, such an investigation 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 among a group of blood donor at the Ahvaz Blood Transfusion Center. According to the consulting doctor, participants were confirmed to have no specific disease or risk behaviors. Inclusion criteria included no history of tattoos, blood transfusions, cupping, or surgical procedures in the last year and no fever during the donation.

2.2. Sample collection and processing

Blood samples were collected after obtaining written consent and recording the donor information (gender, age, marital status, place of residence, place of birth, number of households, occupation, history of surgery, history of blood product transfusion, etc). Approximately 5 mL of peripheral blood was collected from each donor in the sterile tube, the serum was then separated by centrifugation and stored at -20 ˚C until use.

2.2. Serological test and polymerase chain reaction (PCR)

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 [7]. Antibodies tested against Gag proteins instructions. Peripheral blood mononuclear cells (PBMC) of the ELISA- positive 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 Revert Aid First Strand cDNA Synthesis Kit (Thermo, USA)

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

Characteristics		No. (%)
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)

according to the manufacturer's instructions [7]. 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 $MgCl_2$, 0.5 mM dNTPs, and 3 U of Taq DNA -Polymerase. 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 visualized directly under ultraviolet (UV) light.

2.3. Statistical analysis

Descriptive and analytical statistical methods, including frequency distribution tables, frequency plots, and central propensity index and appropriate dispersion, were used to analyze the data. All analyses were analyzed using SPSS software, version 22.

3. Results

Of the 777 participants recruited at the 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 observed 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 ELISA, one subject (0.1%) was positive for HTLV-I antibodies. The positive sample was tested for HTLV-I proviral DNA in human PBMCs using a PCR test and was negative. He was a 35-year-old married man 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. He had a single tattoo from 25 years ago and no specific medical history.

4. Discussion

In general, the northeastern region of Iran is considered endemic region for HTLV-I infection. Other provinces such as Fars, Tehran, and Qom, there is a relatively high prevalence of this virus; therefore, the 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, ranging from 18 to 63 years. The highest frequency was in the age group of 25 to 35 years (36.6%) and 35 to 45 years (36.2%). In a study conducted by Baradaran et al. in Urmia, 2,046 blood donors and 114 dialysis patients were examined; in their blood donor group, 1910(93.4%) were male and 136(6.6%) were female, with an average age of 45 years [8]. The gender composition of individuals in these two studies

was similar to that in our study. In a study by Ghaffari et al., involving 1200 patients at Sari Hospital reported that 65% were female and 35% were male [9]. Furthermore, a study by Miller et al. focusing on Mashhad's Jews in Palestine, there were more women (893 women out of 1679) [10]. It is possible that women are less likely to donate 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 [11], Uganda [12], Brazil [13], China [14], and Nigeria [15] reported prevalence rates of 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 [16], Mashhad [17], Ilam [18], and Babol [19]. 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 Neyshabur (3.6%) [9, 20, 21]. 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 [22]. In another study, HIV-positive patients did not show anti-HTLV antibodies in serum [23]. In thalassemia and hemodialysis patients [24], 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 individuals in the community with lower behavioral risks, the low prevalence compared to the general population is expected. Based on these interpretations, it appears that evaluation of this virus in high-risk groups and immunocompromised individuals remains necessary.

5. Conclusion

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 recommended.

Study 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 with no concern about cost because it can focus more on the results

2. It could determine the prevalence of HTLV-1- associated disorders for example lymphoma, ATL and HAM/TSP.

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Compliance with ethical guidelines

This study was approved by the Research Ethics Committee of Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran (Code: IR.AJUMS.REC.1393.159).

Data availability

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

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This study was done as research project (Project No.: 6108) in Virology Department of Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.

Authors' contributions

Conceptualization and study design: Roohangize Nashibi, Manoochehr Makvandi, Fateme Ahmadi, and Zahra Lotfi; Data acquisition: Mehdi Parsanahad; Experiments and data interpretation: Roohangize Nashibi, Zahra Lotfi, and Mehdi Parsanahad; Statistical analysis: Roohangize Nashibi and Mehdi Parsanahad; Project administration, technical, and material support: Bijan

Helli and Zahra Lotfi; Supervision and writing: Roohangize Nashibi.

Conflict of interest

The authors declared no conflict of interest.

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