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Evaluating the Prevalence of Human T-cell Lymphotropic Virus in Blood Donors and Thalassemia and Hemophilia Patients in Rafsanjan and Jiroft Cities of Kerman Province, Iran

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ABSTRACT

Human T-cell leukemia virus (HTLV) is a member of the retroviridae family that can be transmitted through infected lymphocytes**,** sex, blood transfusion, and organ transplantation as well as from mother to child by breastfeeding. It is estimated that nearly 15-20 million people have been infected with the virus worldwide. HTLV can cause malignant diseases such as adult T-cell leukemia (ATL), inflammatory diseases such as uveitis, HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP), alveolitis, and infectious dermatitis. Iran is considered to be the country most infected with HTLV in Asia after Japan. This study aimed to evaluate the serological prevalence of HTLV 1/2 infection in blood donors and thalassemia major and hemophilic patients in Rafsanjan and Jiroft cities of Kerman province. Sera were collected from 100 blood donors, 60 thalassemia major patients, and six hemophilic patients and tested for the presence of HTLV1/2 specific antibody using enzyme-linked immunosorbent assay (ELISA) and confirmed with Western blot and (real-time reverse transcription polymerase chain reaction) qRT-PCR techniques. The results of the ELISA test for Rafsanjan and Jiroft blood donors, as well as thalassemia and hemophilic patients in Rafsanjan, were negative but among thalassemia major patients of Jiroft, there was one positive case. The results of Western blot and qRT-PCR tests were also positive. The number of provirus copies was 3140750 per ml of the sample. Based on the findings of this study, the Kerman province is important in terms of the spread of the HTLV virus. According to the identification of infection in thalassemia patients in this province, it is recommended to investigate the incidence of this infection in other high-risk populations in this region.

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Introduction

The human T-cell leukemia virus type 1 (HTLV-1) is a member of the Delta retrovirus genus from the Oncovirinae subfamily of retroviruses (Gessain and Cassar, 2012), which includes the related viruses HTLV-2, HTLV-3, HTLV-4; the simian viruses STLV-1-4; and bovine leukemia virus (BLV) (Gonçalves *et al.*, 2010). Seven HTLV-1 subtypes are HTLV-1a, Central Africa HTLV-1b, HTLV-1d, HTLV-1e, HTLV-1f, HTLV-1g, and the Australian-Melanesia HTLV-1c. In addition, it has four subgroups as follows: Intercontinental subcategory (TC), Japanese subtype (Ajp), sub-group of West Africa (Awa), and sub-subgroup of North Africa (Ana) (Gessain and Cassar, 2012, Berini *et al.*, 2013, Verdonck *et al.*, 2007). Infection with HTLV-1 has a worldwide spread; it has been estimated that 15-20 million people are infected with the virus in the world (Martinez *et al.*, 2019). Scientists believe that the infection is indigenous in some parts of the world, including southern Japan, some

Caribbean countries, Central Africa, South America, New Guinea, Melanesia, and the Solomon Islands in the Pacific Ocean as well as northeastern Iran. Viral A subtype is found in many indigenous regions of the world, including Iran, and is therefore known as an international variant (Gessain and Cassar, 2012). Northeastern Iran is regarded as an endemic area for HTLV (Abedi *et al*., 2017; Azarpazhooh *et al*., 2012; Farid *et al*., 1999, Pirayeshfard *et al*., 2018; Rafatpanah *et al*., 2016). The first report on the seroepidemiology of HTLV in Iran has been published in 1993. Rafatpanah and colleagues revealed that the prevalence of HTLV-1 was estimated to be 2.12% in northeastern Iran (Mashhad). However, it appears that HTLV-1 has been observed in residents of certain areas of Iran (Karimi *et al*., 2017). According to available evidence, most people are asymptomatic carriers for their whole life, while a small fraction of carriers is infected with HTLV-1-related diseases. HTLV-1 can cause malignant diseases such as ATL, inflammatory diseases like uveitis, HAM/TSP, and infective dermatitis (Gessain and Cassar, 2012; Gonçalves *et al*., 2010). HTLV-1 is mostly transmitted through cell-to-cell contact because cell-free HTLV-1 particles are not effective in the infection of target cells. T-cells infected with HTLV-1 bind uninfected cells, forming a virological synapse (VS) that consists of viral and cellular molecules at the contact area. Because HTLV-1 can infect several kinds of cells and its receptor is believed to be a frequently expressed molecule. Nevertheless, three molecules, namely Glucose transporter 1 (GLUT1), heparin sulfate proteoglycan (HSPG), and neuropilin-1 were recognized to be essential for the interaction of HTLV-1 envelope and the cell membrane, as well as infiltration of the virus into cells (Hedayati, 2013). Ways of transmitting the virus include breastfeeding (Carneiro-Proietti *et al*., 2014), sexual intercourse (Richardson *et al*., 1990), and blood transfusion (Gonçalves *et al*., 2010; Nagai *et al*., 2001). HTLV-1 can also be transmitted through transfusion of contaminated blood components, as well as liver, kidney, and lung transplantation (Karimi *et al*., 2017). However, intravenous exposure to blood is the most effective route of HTLV-1 transmission. Previously, this occurred chiefly through transfusion of blood not screened for HTLV-1. According to evidence, blood transfusion is the main risk factor for HTLV-1 seropositivity and the highest risk is related to the transfusion of packed red cells. However, the transmission risk by plasma products and cold storage of blood was reduced apparently due to the death of HTLV-1- infected lymphocytes (Gonçalves *et al*., 2010). Despite studies on the prevalence of HTLV-1 in northeastern Iran, there is still no detailed information on the outbreak of the disease in other parts of Iran, including Kerman province. Moreover, there is no information about carriers and patients in this province. Therefore, considering the importance of blood transfusion in the prevalence and transmission of HTLV in the community, we aimed to evaluate the prevalence of the virus in blood donors as well as thalassemia and hemophilia patients in Rafsanjan and Jiroft cities of Kerman province.

Materials and Methods

Study population and sample collection

In this study, blood samples were taken from healthy blood donors, as well as patients with thalassemia and hemophilia who voluntarily cooperated with this study. The samples were collected from 90 and 10 volunteers donating blood in Rafsanjan and Jiroft, respectively. Additionally, 26 Thalassemia and hemophilia patients in Rafsanjan and 40 in Jiroft gave their blood samples. It should be noted that before carrying out this research, the ethics code IR.kmu.REC.1396.27 was received from the Research Ethics Committee of Kerman University of Medical Sciences. Besides the blood samples, a questionnaire was compiled, investigating subjects' demographic information on transfusion history, previous medical history, injecting drugs, and high-risk sexual contact.

Serological assay and confirmation tests

To diagnose HTLV-1/2 serology, specific antibodies were found in the sera of volunteers using an enzyme-linked immunosorbent assay x (ELISA) kit (Dia. Pro Diagnostic Bioprobes s.r.l, Italy). Then, the repeatedly positive samples were confirmed through the Western blot kit (HTLV blot 2.4, MP Diagnostics, Singapore). In addition, the number of virus copies was determined using Quantitative Real-Time PCR assay with TaqMan chemistry for positive samples. The genomic DNA was extracted from blood cells by DNA/RNA

extraction kit (Primerdesign Genesig Kit, United Kingdom) according to the manufacturer's instructions. Quantitative Real-time PCR was performed using Human T-lymphotropic Virus 1 Advanced Kit (Primerdesign Genesig Kit, England) based on the instructions. In this study, the authors used two positive controls and a negative control for the assessment of HTLV-1 markers. DNA (5µl) was added to a 15 µl master mix reaction presenting a final volume of 20 µl. The reactions were performed under the following cycle conditions: 2 min at 50°C and 10 min at 95°C, followed by 50 cycles of 15 s at 95°C and 1 min at 60°C (Andrade *et al.,* 2010). It should be noted that all tests were repeated three times.

Results

In this research, a total of 100 volunteer blood donors were recruited, of whom 90 subjects were from Rafsanjan and 10 from Jiroft cities. Table 1 demonstrates the demographic information of the subjects. According to the results, no HTLV-1 positive was found among the blood donors. Meanwhile, the authors studied 66 thalassemia and hemophilia patients, of whom 26 participants were from Rafsanjan and 40 from Jiroft (Table 2).

Table 1. Demographic Information of Blood Donors.

City	Rafsanjan	Jiroft
Total samples	90	10
The age range	19-62	22-41
Male	99.7%	100%
Female	2.22%	0%
married	87.77%	80%
Single	12.22%	20%
History of specific disease	2.2%	0%
History of injection of blood products	2.2%	0%
History of surgery	38.88%	40%
History of hospitalization	51.11%	40%
History of invasive procedure	53.33%	20%
History of travel to endemic areas	87.77%	30%
History of drug injection	6.66%	0%
History of high-risk sexual contact	7.77%	0%

Table 2. Demographic Information of Special Patients.

City	Rafsanjan	Jiroft
Total samples	26	40
Thalassemic patients	20	40
Hemophilic patients	6	0
The age range	6-36	1-35
Male	42.30%	65%
Female	57.69%	35%
Married	15.88%	7.5%
Single	84.6%	92.5%
History of surgery	23.07%	15%
History of hospitalization	50%	62.5%
History of invasive procedure	30.76%	47.5%
History of travel to endemic areas	53.84%	50%
History of drug injection	3.84%	2.5%
History of high-risk sexual contact	0%	2.5%

The serological results showed one subject with HTLV-1 (Fig. 1), which was confirmed by the western blot test. The results revealed the presence of viral proteins Rgp 46-I, P36, P32, P28, P26, P24, P19, and GD21, and confirmed the presence of HTLV type 1 (Fig. 2).

On the other hand, a quantitative Real-Time PCR assay was performed for final approval of HTLV-1 positive and also to determine the number of virus copies in the sample. According to the results, not only HTLV-1 positive was confirmed but also the number of provirus copies was determined. Accordingly, the number of provirus copies was reported at 3140750 per ml.

Discussion

Infection with HTLV is widespread worldwide. It is currently estimated that approximately 15-20 million people have been infected with this virus in the world (Olière *et al.,* 2011; Lairmore *et al.,* 2011). The infection is endemic in some parts of the world, including southern Japan, some Caribbean countries, Central Africa, South America, New Guinea, Melanesian, and Solomon Islands as well as northeastern parts of Iran (Gessain and Cassar*,* 2012; Gonçalves *et al.,* 2010). Most infections in Iran presumably occurred following the Mongols attack and virus transmission to the country were further facilitated by traveling through the Silk Road. According to the researchers, Mashhad, the capital of Razavi Khorasan province and the Neishabour are currently endemic for HTLV-1 in the country, However, the virus is also prevalent in other parts of Iran (Hedayati-Moghaddam *et al.*, 2022).

However, there is little information about the incidence of the disease in Kerman province. Given that blood transfusion and contaminated blood products are important transmission routes of HTLV-1, the authors of the current research sought to investigate the possible presence of this virus in blood donor volunteers as well as patients with thalassemia and hemophilia. In this study, sampling was performed on 90 volunteer blood donors in Rafsanjan city. Depending on the frequency of infection in the general population and donors as well as the window period, it takes 41- 65 days for the infection to appear and this period can sometimes be longer. In studies on the prevalence of HTLV among donors in some countries of the world, the prevalence rate was

0.001 - 4.3% in different countries. However, in a group of natives from northern Australia, the prevalence of infection is up to 14% (Karimi *et al.,* 2017; Gonçalves *et al.,* 2010).

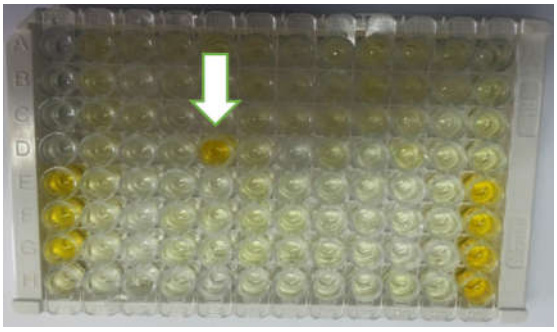

Fig. 1. The results of the ELISA test in thalassemia and hemophilia patients: Serological test (ELISA test), ▼ showed the HTLV-1 positive.

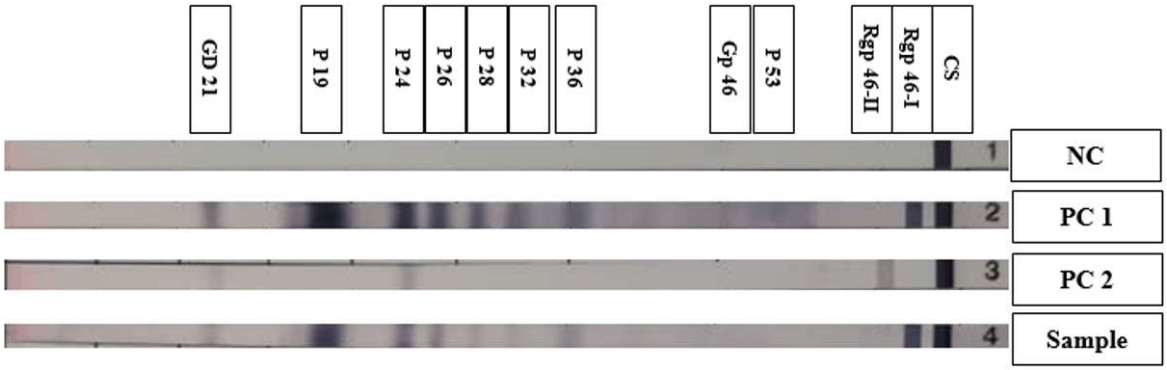

Fig. 2. . The western blot tests. The results of these tests, confirmed the presence of viral proteins, and the presence of HTLV type1.

In Iran, the prevalence of HTLV virus was investigated for the first time in 1995 in 21 provinces of the country. According to the reports, the prevalence of infection was 0.29%. In subsequent studies, the incidence of infection in different cities was as follows: Mashhad (1.16%, 0.77%, and 0.45%), Bushehr (0.013%), Neishabour (1.15%), and Urmia (0.344), Chaharmahal and Bakhtiari (0.62%), Ilam (0.208%), Hormozgan (0.18%), South Khorasan (0.042%), Karaj (0.11%), Fars (15.15%), Kermanshah (0.5%), Tehran (0.42%), and Isfahan (0%) (Karimi *et al.,* 2017; Hedayati, 2013). However, there was no accurate report on the prevalence of this virus in Kerman province. Given that no positive cases of HTLV virus were found in blood donors of Rafsanjan or Jiroft in the present study, the probability of infection in these cities could not be considered equal to zero. The lack of a positive case of HTLV infection in this research could be due to the small number of volunteers participating in the study. Therefore, further investigations with a larger population of blood donors in these cities, the use of diagnostic methods with high sensitivity, as well as a genetic study on specific races living in Jiroft are recommended. On the other hand, due to frequent blood transfusions in patients with thalassemia and hemophilia, this group of patients is considered to be among the high-risk categories in terms of HTLV infection. In a study on the incidence of HTLV infection in people with thalassemia in some countries, the prevalence of HTLV-1 and HTLV-2 in Italy was 0.23% and 0.88%, respectively. Also in Greece, this rate is 1.1% and in India, no positive case has been reported. However, there is no study on the prevalence of viral infections among thalassemia patients in the Middle East (Keshvari *et al.,* 2014). In a study conducted in 1993 in Iran on the prevalence of HTLV infection in patients with hemophilia and thalassemia, the frequency of infection was reported as follows: Fars (2.5%), Tehran (2.97 - 6.29%), Zahedan and Zabol (1.6%), Bushehr

(2.18%), Golestan and Gorgan (4.42%), Urmia (1.05%), Birjand (1.25%), Hormozgan (2.38%), and Mazandaran (1.39%) (Hedayati, 2013). Moreover, in a study conducted in 2013, the incidence of HTLV infection in this group of patients was as follows: Mazandaran (6.9%), Golestan (4.4%), Tehran (6.3%), Khorasan Razavi (6.1%), Kurdistan (2.2%), Kermanshah (3.4%), Chaharmahal and Bakhtiari (7.2%), Isfahan (3.3%), Fars (3%), Bushehr (1.1-3%), Hormozgan (3.1%), and Sistan and Baluchestan (1.6%) (Keshvari *et al.,* 2014). However, based on available reports, Kerman province was not investigated.

According to the present study, a patient with thalassemia was positive for HTLV-1, and the results of a qualitative qRT-PCR test showed 3140750 copies of the provirus per milliliter. Therefore, based on the obtained results, the prevalence of HTLV-1 infection is estimated to be 2.5% in thalassemia patients living in Jiroft and, in general, according to the results and considering all the investigated groups, the prevalence of infection in 166 cases investigated in this study is 0.6%. Of course, to achieve a more accurate statistical result concerning the incidence of HTLV-1 infection in patients from Kerman province, especially in the city of Jiroft, it is necessary to study a larger statistical population.

Conclusions

The incidence of HTLV-1 infection among thalassemia and hemophilia patients in the southeast of Iran was remarkably higher compared to the healthy population and blood donors. It seems that more special preventive actions are required to control the infection. In addition, investigating the incidence of this infection in other high-risk populations in this region is highly recommended.

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Ethics approval

The ethics code IR.kmu.REC. 1396.27 was received from the Research Ethics Committee of Kerman University of Medical Sciences.

Conflict of interests

The authors declare no conflict of interest.

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