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ORIGINAL ARTICLE

Molecular epidemiology and phylogenetic analysis of human T-lymphotropic virus type 1 in the *tax* gene and it association with adult t-cell leukemia/lymphoma disorders

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ABSTRACT

Background and Objectives: Human T-lymphotropic virus type-1 (HTLV-1) belongs to retrovirus family that causes the neurological disorder HTLV-1 adult T-cell leukemia/lymphoma (ATLL). Since 1980, seven subtypes of the virus have been recognized. HTLV-1 is prevalent and endemic in some regions, such as Africa, Japan, South America and Iran as the endemic regions of the HTLV-1 in the Middle East. To study HTLV-1 subtypes and routes of virus spread in Iran, phylogenetic and phylodynamic analyses were performed and for as much as no previous phylogenetic studies were conducted in Tehran, we do this survey. To this purpose, the Tax region of HTLV-1 was used.

Materials and Methods: In this study 100 samples were collected from blood donors in Tehran. All samples were screened for anti-HTLV-I antibodies by ELISA. Then, genomic DNA was extracted from all positive samples (10 people), and for confirmation of infection, ordinary PCR was performed for both the HBZ and LTR regions. Moreover, the Tax region was amplified and purified PCR products were sequenced and analyzed, and finally, a phylogenetic tree was constructed using Mega X software.

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Results: Phylogenetic analysis confirmed that isolates from Iran, Japan, Brazil, and Africa are located within the extensive ''transcontinental'' subgroup A clade of HTLV-1 Cosmopolitan subtype a. The Japanese sequences are the closest to the Iranian sequences and have the most genetic similarity with them.

Conclusion: Through phylogenetic and phylodynamic analyses HTLV-1 strain in Tehran were characterized in Iran. The appearance of HTLV-1 in Iran was probably happened by the ancient Silk Road which linked China to Antioch.

Keywords: Human T-lymphotropic virus type-1; Adult T cell leukemia lymphoma; Phylogenetic; Phylodynamic; Iran

INTRODUCTION

Human T-cell lymphotropic virus Type 1 (HTLV-1) is a widespread virus that discovered in the nineteen century and infects about 15 to 25 million people, that will develop adult T-cell leukemia (1, 3). It belongs to the Delta retrovirus genus of the Retroviridae family (2) and has been related with neoplastic diseases such as adult T-cell leukemia/lymphoma (ATLL), HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP) and some opportunistic infections as uveitis, infective dermatitis, Hashimoto thyroiditis, Pulmonary alveolitis, and rheumatoid arthritis (4, 5). HTLV-1 has a simian and zoonotic origin and humans acquired virus by infected monkeys in ancient time (Africa, Europe and Asia). In some African regions is still zoonotic transmission (6). The HTLV-1 high endemic areas are Southwestern Japan, sub-Saharan Africa, parts of South America, Australia-Melanesia, some foci in the Middle East (such as the Mashhad region in Iran), and the Caribbean region (8, 9). There are four main transmission mechanisms for HTLV-1: a vertical transmission from mother-to-child, prolonged breast-feeding (longer than six months); a horizontally through sexual contact, (mainly from male to female and women were more vulnerable to HTLV-1 infection), contamination with blood products (7). Virus transmission occurs from an infected mother in offspring and ATLL is associated with the greatest risk. Some factors associated with transmission as follows presence of proviral load in breast milk (10), high antibodies titers, prolonged breastfeeding (at least > 6 months) (11, 12) and concordance of HLA class-I antigens between mother and child (13, 14). Regions and populations affected rate of prevalence (16).

HTLV-1 has a diploid RNA genome that is revers-transcribed into double-stranded DNA and integrates into the host genome as a provirus (15, 18). The genome of HTLV-1 contains gag, pol, and env genes alongside long terminal repeat (LTR) sequences at both the 5′ and 3′ ends (17). The LTR region is more informative for phylogenetic analysis (20, 22). According to phylogenetic analysis of the LTR sequences, HTLV-1 is classified into seven genetic subtypes (a–g) , the cosmopolitan subtype a, the Australia-Melanesian subtype c and the Central African Subtypes b, d, e, f and g, based on long terminal repeat (LTR) regions (19, 21). For understanding the transmission chains, natural history and evolution of HTLV-1 infection, genotypes analysis are crucial (23). The HTLV-1 has an unusual molecular feature that is an extraordinary genetic stability (24). The reason for this remarkable genetic stability is probably clonal expansion of viral amplification in infected cells. HTLV-1 can be used as a molecular tool to survey origin (24), models of transmission and their hosts, because the low sequence variation of this virus (24, 25). The specific geographical origin of the patients is revealed by few nucleotide substitutions among virus strain (26). ATK prototype is the first HTLV-1 complete sequence was obtained and it originated from a Japanese patient with ATLL (27). After this achievement, other sequences with low genetic variability were made and studies not revealed a specific mutation associated with ATLL (27). Among HTLV-1 genotypes, the Cosmopolitan a-genotype is the most commonly conveyed clade and is dispersed worldwide (29). Genotype mainly can be more separated into subgroups based geographical character (28, 29). This fact showed that migration of ancient infected populations caused viruses spread and virus existence for thousands of years (31, 32). In genetic variation of retroviruses, both recombination and point mutation contribute (30), but for HTLV-1, no recombination had been identified (30, 31). Since that no super infection revealed at the cellular level, so no recombination in this viruses was supported (33). The main genetic evolution mechanism for these viruses would be point mutations (31). The virus mutations during proliferation in infected cells caused genetic diversity (32). However, HTLV-1 is present all over the world, and endemic distributions of this infection have been reported in many countries (34).

Iran is an endemic region that has the prevalence of infection approximately 4% in some of northeast cities but that increases in other cities (33). The most abundant HTLV-1 sequence is LTR that is available in databases and covering wide range of countries. Unfortunately, few studies explore the HTLV-1 genetic diversity based on Tax region in the Iran population. Tax protein is vital for effective virus expression and plays an important role for activation of cellular genes, such as cytokine genes and protooncogenes (31, 33). According to the recent results, there were a low number of Tax sequences from Iran and this could be the result of a lack of data of the actual infected Iran population. Pervious phylogenetic studies in Iran revealed that HTLV-1 genotype belongs to the subtype a (31). In the present study, HTLV-1 risk factors such as age, gender, and socioeconomic variables were studied in Tehran. In addition, a phylogenetic analysis of the Tax sequence was performed to determine the type of virus in this city.

MATERIALS AND METHODS

Population study and serologic confirmatory analysis. Blood samples were collected from people referring to Imam Hussein, Shariati and Imam Khomeini hospitals in Tehran, Iran. Subjects' agreements were obtained for participating in the study. The study was approved by the Ethics Committee of Tehran University of Medical Sciences. All sera from 100 suspected ATLL patients were first screened for the presence of HTLV-1 antibodies by an Enzyme-linked immunosorbent assay (ELISA, Dia.Pro-Italy). Positive sera patients were confirmed by PCR technique. The Research Ethics Committee of Tehran University of Medical Sciences (TUMS) approved this study (Number: 1398.095).

DNA extraction. DNA was extracted from Peripheral blood mononuclear cells (PBMCs) using an available commercial Kit (Roje,Iran). The isolated DNA was stored at -20°C until the PCR analysis started.

Amplification of the LTR and HBZ regions. We used a segment of LTR and HBZ genes to confirm HTLV-1 infection and then were amplified, using primers: LTR forward primer (GGCTCGCATCTCCCCTTCAC), LTR reverse primer (GAGCAAGCAGGGTCAGGCAA), HBZ forward primer (ACGTCGCCCGGAGAAAACA) and HBZ reverse primer (CTCCACCTCGCCTTCCAACT). PCR amplification was performed in a 25 µL reaction containing 20 mM Tris-HCl (pH 8.4), 100 mM KCl, 2 mM $MgCl_2$, 0.2 Mm dNTPs, 0.5U Taq DNA polymerase, 2 µl the genomic DNA (50-100 ng), and 10pmol/ml of each specific primer. A T100 thermocycler (Biorad, USA) were used to amplify LTR region with the following amplification program: 94°C for 4 min; 45 cycles of 95°C for 40 sec, 62°C for 40 sec, 72°C for 30 sec, and a final extension of 72°C for 5 min and HBZ region with program: 94°C for 4 min, 95°C for 40 sec for 45 cycles, 60°C for 40 sec, 72°C for 30 sec and a final extension of 72°C for 5 min. The products were analyzed on 1.5% agarose gel, stained with safe stain, and visualized under UV light.

DNA sequencing and phylogenetic analysis. The TAX region 1200 was amplified with the following primers: TAX forward (GGATAGCAAACCGTCAAGCAC) and TAX anti forward (GGTGAGGGGTTGTCGTCAA). PCR amplification was carried out in a 25 µL reaction containing 20mM Tris-HCl (pH 8.4), 100 mM KCl, 2 mM $MgCl_2$, 0.2 mM dNTPs, 0.5U Taq DNA polymerase, and 10 mmol/l of each specific primer and 2 µl the genomic DNA. Each PCR cycle consisted of denaturation at 95°C for 40 sec, annealing at 58°C for 35 sec, extension at 72°C for 80 sec, and final extension at 72°C for 5 min. All samples were sequenced in full TAX region. Sequencing runs were performed on ABI 3730 sequencers with 50 cm capillaries. Double stranded sequencing was performed. At first, sequenced results were edited with Chromas DNA Sequencer software. Later, sequences were analyzed with the NCBI BLAST software, and subsequently online software (http://www.ebi.ac.uk/tools) was used to identify the genotypes. Further- more, sequences were compared with each other and aligned by Clustal W using the BioEdit software.

Phylogenetic analysis was done using reference sequences from subtype a (subgroups A, B, C, D, and E), b, c, d, e. The Cladogram was constructed in Mega software (version X), with Maximum Likelihood method in Kimura-two parameter substitution model with 1,000 bootstrapping value.

Similarity sequence analysis. Simplot software version 3.5.1 was used to test potential of sequences similarity. Furthermore, the sequences were used to

investigate possible DNA nucleotide changes in the strains from Iran and other countries.

Statistical analysis. Demographic characteristics, medical history, and related risk factors were analyzed by SPSS software version 22. Chi square test and the Fisher exact test were used to test possible associations between risk factors and the positive HTLV-I Infection. A P value <=0.05 was considered statistically significant.

RESULTS

Serological and molecular studies for HTLV-1. In this study, 10 out of the 100 samples taken from individuals clinically diagnosed with ATLL during January 2018 to November 2020 were selected. Eight patients were male (80%) and two (20%) were female with average age of 52.6 ± 16.83 years (range 32-65). The mean age of females was 42.7 ± 1.56 years and for males was 53.4 ± 2.13 years.

One female and one male in the 30-39 years age group, one males and one female in 40-49 years age group, and six males in the ≥ 50 years age group were HTLV-1 positive. HTLV-1 infection rate for males was 80% (8/10) and for females was 20% (2/10). No significant difference was observed in HTLV-1 sero-reactivity between males and females (P value = 0.096). The Socio demographic and clinical factors related to HTLV-1 infection in the participated population of study are summarized in Table 1. HTLV-1 prevalence was associated with age and established a significant increase among those older than 50 years (P<0.05), (Table 1).

The high rate of infection is associated with age, other variables such as marital status, breastfeeding, literacy; history of any kind of surgery and history of hospitalization are risk factors for HTLV-I transmission. However the place of birth, ethnic background, had no important effect on the rate of HTLVI infection.

All collected blood samples were analyzed for anti-HTLV1 antibodies. Sera positive samples were checked by PCR that confirm HTLV-1 infection in LTR and HBZ genes based specific primers, of which whole ten patients were positive (eight males and two females). The PCR products running on gel and then bands revealed by sizes respectively HBZ (152 bp) and LTR (221 bp).

Afterwards, the DNA extracted samples were amplifies for Tax in the expected region with specific primers. The PCR products showed Tax amplified Bands (1200 bp).

Similarity sequence analysis. Full Tax sequence (1200 bp) as a reference sequence of the present study showed maximum homology with HTLV-1 subtype A. Simplot analysis revealed the same sequence in HTLV1 cosmopolitan subtype. The Simplot homology graph is shown in Fig 1. Each curve is a comparison between full Tax sequence (1200 bp) being analyzed and considered sequence is similar to cosmopolitan Aa sequences.

The DNA sequence of a HTLV-I fragment of 1200 base pairs (bp), around the Tax region, was obtained from ten patients affected by ATLL, respectively. The analysis of the viral DNA sequence exposed nucleotide changes that distinguish this Iranian HTLV-I from other HTLVI subtypes (Fig. 2). However, the rareness of nucleotide changes indicated a high degree of similarity between the Tehran Iranian HTLV-I and the cosmopolitan HTLV-I (isolates from the Africa, Europe, and the South Americas). To better explain the relationship of these Iranian isolates with the remaining HTLV-I, we constructed a phylogenetic tree, using the program Clustal. Fig. 3 shows obviously that the Iranian viruses clustered together within the larger cosmopolitan HTLV cluster, and that the Melanesian and some other HTLV clustered separately as previously verified.

Phylogenetic results. The phylogenetic analysis of 10 Iranian Tax sequences using 25 reference sequences representing all HTLV-1 subtypes and one Tax sequences of HTLV-2 subtype as the out group, obviously confirmed that all Iranian isolates belong to the Transcontinental subgroup (A) of the Cosmopolitan subtype (1a). The tree with the highest log likelihood (-2176.17) is shown in Fig. 3.

DISCUSSION

Asymptomatic HTLV-1 carriers regularly live in really endemic regions where the rate of infection is closely ranged from 3% to 5% in the Caribbean islands and to above 10% in the same areas of South Japan (10, 17). Regions of the Middle East (Iran and Kuwait) have been found endemic for HTLV-1 (7). HTLV-1 infection rate has been informed almost

Table 1. Sociodemographic and Clinical Factors related to HTLV-I Infection in participation.

Variable	ATLL N=10 N (%)		P-Value
	Female 2 (20%)	Male 8 (80%)	
Age (years)			
18-29	0	0	
30-39	1 (50%)	1 (12.5%)	
40-49	1 (50%)	1 (12.5%)	
≥ 50	0	6 (75%)	0.018
Marital status			
Single	0	4 (50%)	
Married	2 (100%)	4 (50%)	0.345
Literacy			
Illiterate	0	4 (50%)	
Primary school	0	3 (37.5%)	
Secondary school	0	0	
High school	0	0	
Academic	2(100%)	1 (12.5%)	0.752
Ethnic background			
North Khorasan	0	1 (12.5%)	
South Khorasan	0	1 (12.5%)	
Razavi Khorasan	0	1 (12.5%)	
Tehran	1 (50%)	2 (25%)	
Alborz	1 (50%)	2 (25%)	
Ardabil	0	0	
West Azerbaijan	0	1 (12.5%)	0.110
Twin birth			
Yes	0	1 (12.5%)	
No	2 (100%)	7 (87.5%)	0.254
Breast feeding			
Yes	2 (100%)	7 (87.5%)	
No	0	1 (12.5%)	0.192
History of surgery			
Yes	2 (100%)	5 (62.5%)	
No	0	3 (37.5%)	0.004
History of dentistry procedure			
Yes	2 (100%)	8(100%)	
No	0	0	0.780
History of traditional cupping			
Yes	1 (50%)	3 (37.5%)	
No	1 (50%)	5 (62.5%)	0.850
History of tattooing			
Yes	1 (50%)	5 (62.5%)	
No	1 (50%)	3 (37.5%)	1.000
History of imprisonment			
Yes	0	0	
No	2 (100%)	8 (100%)	0.013
History of drug abuse			
Yes	0	0	
No	2 (100%)	8 (100%)	0.013
History of hospitalization			
Yes	0	7 (87.5%)	
No	2 (100%)	1 (12.5%)	0.005

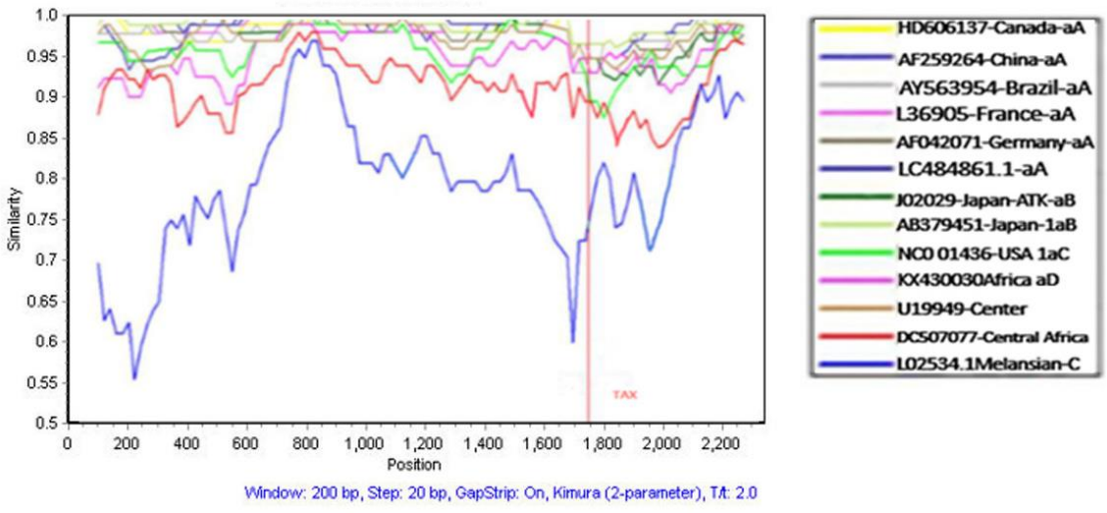

Fig. 1. Plot of similarity (generated by Simplot) of a set of reference sequences to the Tax sequence.

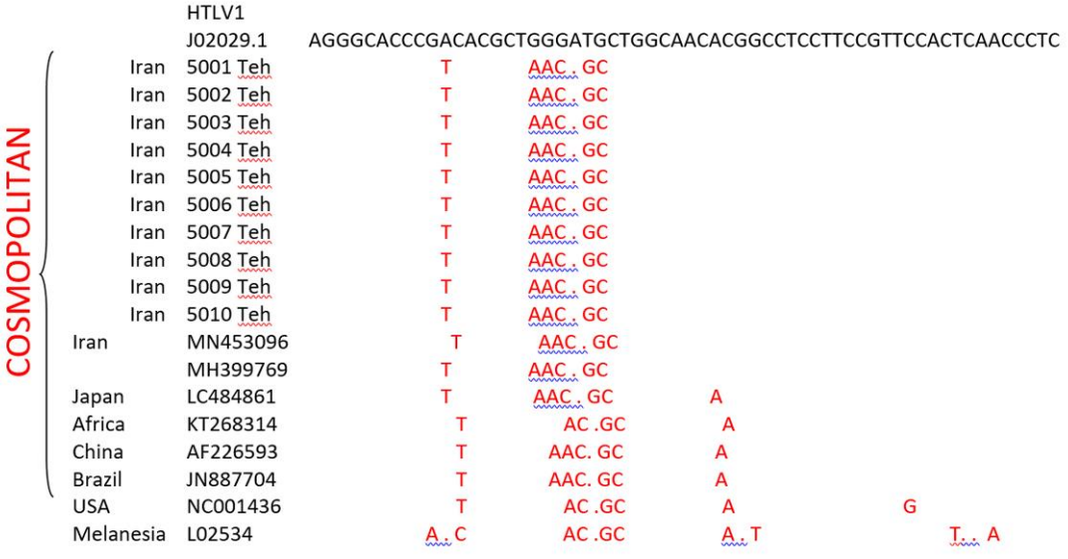

Fig. 2. Summary of DNA nucleotide changes in the strains of Iran and other countries. The sequences at the top represent reference to the HTLV-I ATK sequence.

4% in some areas of northeastern Iran. The HTLV-1 may have arrived through one of the following way: trade and Silk Road, an invading group such as the Mongols, pilgrims to the holy Muslim shrine, and African slaves (18, 21). Slavery looks more likely to have introduced the virus to Iran cities, because it has been generally experienced even before the growth of Islam in these cities (19). Silk Road trade not only played an important role in the development of economic relations between countries but also in the spread of diseases such as plague, smallpox and HTLV-1 (25). According to Bayesian analysis, the origin date of HTLV-1 is estimated to be about 700 years ago and also observed rapid exponential growth rates in the effective number of infections, early eighteenth-century. It has been proposed that the presence of the virus depends on variable factors such as environmental, social, behavioral, and cultural (29). In spite of the usual origin and similarity of the virus in the region, different prevalence of HTLV-I have been reported in some countries near Iran such as Turkmenistan, Kuwait, Saudi Arabia, and Turkey so that infection rate in these countries were 0.27%, 0.016%, 0.006%, and 0% respectively, which indicates a lower prevalence than in Iran (16). Moreover, the social announcement among countries such

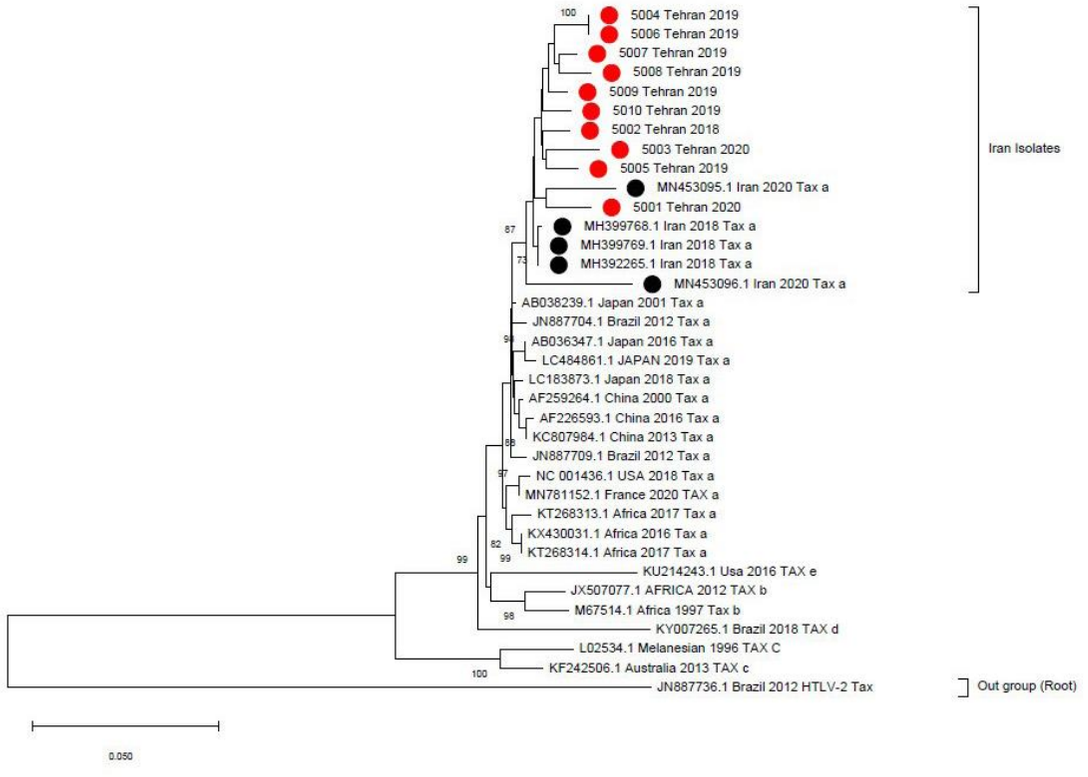

Fig. 3. Phylogenic analysis for the Tax region of HTLV-I provirus isolated from a sample of patients in Tehran, Iran. Phylogenetic analysis was performed by MEGA software (version X), with the maximum likelihood method in Kimura-two parameter substitution model. Bootstrap value is 1,000. All sequences belong to subtype a, subgroup A (Cosmopolitan) and were in the subcategory of Iran strains

as international trade, tourism, and pilgrimage looks to be playing a critical role in virus distribution (17).

ELISA method was mostly used as a screening method due to its availability, simplicity, and cost effectiveness, but this method has some disadvantages. One of the most important ones is false positive results as a confirmation method. PCR methods were used in the study because of specificity and sensitivity of this method was 99.4% so this method is more efficient at identifying the virus type and can be used for phylogenetic studies (21).

Our results exposed that HTLV-I infection grows with age, mainly amongst those older than 50 years; this is similar to the previous cross-sectional study in Mashhad, which revealed that HTLV-I prevalence increases among those older age. It has been exposed that HTLV-I seroprevalence rises with age and is higher among subjects more than 50 years than in those less than 30 years. Sexual contact is more probable to be the primary way of virus transmission after age 30 years and breastfeeding during infancy has a major role in HTLV-I transmission.

Although the high rate of infection is associated with age, other variables such as marital status, breastfeeding, literacy, History of any kind of surgery and History of hospitalization are risk factors for HTLV-I transmission. In this study, a greater prevalence of infection was associated with hospitalization, and a history of surgery, which is dependable with previous studies that reported that the virus is transmitted through contaminated needles and syringes following surgery.

However the place of birth had no important result on the rate of HTLV-I infection. The best way to study HTLV-1 strains were phylogenetic and phylodynamic analysis of strain from the endemic peoples of different regions of the Iran. However no previous phylogenetic studies were conducted in Tehran. We selected the Tax area for phylogenetic analysis of HTLV-I virus , because phylogenetic subtypes are important in the Tax HTLV-I gene, and in subtype tax a, nucleotide mutations in the Tax gene al-

ter amino acid function (positions nt 7959 and 8208) and may affect the function of the tax protein. The viral tax protein appears to play a major role in the process leading to ATL. Tax increases the expression of many viral and cellular genes through pathways associated with CREB / ATF, SRF- and NF-kappaB. In addition, Tax activates CBP / p300 and p / CAF to enable full transcription of each of these paths.

Tax also affects the function of other regulatory proteins by direct protein / protein interactions. Through these activities, Tax induces infected T cells to undergo uncontrolled continuous replication, destabilizing their genomes by interfering with telomerase and topoisomerase-I function and inhibiting DNA repair. Tax has the potential to regulate the overexpression of various cellular genes , including cell proliferation, such as interleukin-2 (IL-2), IL-2 receptors, IL-6, GMCSF, and c-fos. It can be thought that tax plays a key role in the progression of the disease and malignancy. In addition , Tax prevents cell cycle arrest and apoptosis and as a result of the accumulation of mutations, they can contribute to the process of adult T cell leukemia lymphoma. Overall these activities make Tax very carcinogenic (23). However, the tax sequence did not change among the HTLV-1 sequences in our study patients, But there is a need for broader phylogenetic studies based on the Tax gene, with a larger statistical population in other parts of Iran. In this study, all the detected strains of Tehran belonged to HTLV-1 cosmopolitan subtype (1a) transcontinental subgroup (A), that is reliable with other studies from Iran. Affording to phylogenetic tree, isolates from Iran, Japan, Brazil, and Africa are located in a clade. The Japanese sequences are the closest to the sequences from Iran and have the most genetic similarity with them. This confirms the hypothesis of the Milky Way trade and the Mongol invasion is strengthened.

CONCLUSION

Through phylogenetic and phylodynamic analyses HTLV-1 strain in Tehran were determined in Iran. Phylogenetic analysis showed the presence of the Iranian populations with Japan, Brazil, and Africa isolates within the widespread ‘‘transcontinental’’ subgroup A clade of HTLV-1 Cosmopolitan subtype a. Moreover, the hypothesis of a multiple overviews of HTLV-1 into Iran especially before the 18th century and at the same time the Mongol invasion of Iran was suggested. The appearance of HTLV-1 in Iran was probably happened by the ancient Silk Road which linked China to Antioch. Furthermore, HTLV-I screening of patients in endemic cities will decrease the risk of HTLV-I infection and appropriate counseling regarding the risk of HTLV-I transmission can be recommended for those people who are infected by the virus. Educational programs are needed to prevent HTLV-I sexual transmission for those who are of a sexually active age. Since the risks related with HTLV-I infection, similar sero epidemiologic studies are needed in other parts of country to identify the prevalence of the virus in Iran as a first step toward controlling the infection.

REFERENCES

1. Rafatpanah H, Farid R, Golanbar G, Jabbari Azad F. HTLV-I infection: virus structure, immune response to the virus and genetic association studies in HTLV-I-infected individuals. *Iran J Allergy Asthma Immunol* 2006;5: 153-166.
2. Saffar S, Azadmanesh K, Golmohammadi T, Golkar M, Amminian M, Mirshahabi H, et al. Production of recombinant human T lymphotropic virus type 1 Tax protein in Rosetta (DE3) bacterial host. *J Param Sci* 2010;1: 35-42.
3. Ahmadi Ghezeldasht S, Shirdel A, Assarehzadegan MA, Hassannia T, Rahimi H, Miri R, et al. Human T lymphotropic virus type I (HTLV-I) oncogenesis: molecular aspects of virus and host interactions in pathogenesis of adult T cell leukemia/lymphoma (ATL). *Iran J Basic Med Sci* 2013;16: 179-195.
4. Acheson NH (2007). Fundamentals of molecular virology. 2nd ed. Wiley. New York.
5. Fogarty KH, Zhang W, Grigsby IF, Johnson JL, Chen Y, Mueller JD, et al. New insights into HTLV-1 particle structure, assembly, and Gag-Gag interactions in living cells. *Viruses* 2011;3: 770-793.
6. Cook LB, Elemans M, Rowan AG, Asquith B. HTLV-1: persistence and pathogenesis. *Virology* 2013;435: 131-140.
7. Boxus M, Willems L. Mechanisms of HTLV-1 persistence and transformation. *Br J Cancer* 2009;101: 1497-1501.
8. Rafatpanah H, Farid Hosseini R, Pourseyed SH. The impact of immune response on HTLV-I in HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP). *Iran J Basic Med Sci* 2013;16: 235-241.
9. Farid Hosseni R, Jabbari F, Shabestari M, Rezaee SA,

Gharivani Y, Valizadeh N, et al. Human T lymphotropic virus type I (HTLV-I) is a risk factor for coronary artery disease. *Iran J Basic Med Sci* 2013;16: 217-220.

10. Shoeibi A, Etemadi M, Moghaddam Ahmadi A, Amini M, Boostani R. "HTLV-I infection" twenty-year research in neurology department of Mashhad university of medical sciences. *Iran J Basic Med Sci* 2013;16: 202-207.
11. Akbarin MM, Rahimi H, Hassannia T, Shoja Razavi G, Sabet F, Shirdel A. Comparison of HTLV-I proviral load in adult T cell leukemia/lymphoma (ATL), HTLV-I-associated myelopathy (HAM-TSP) and healthy carriers. *Iran J Basic Med Sci* 2013;16: 208-212.
12. Hedayati-Moghaddam MR. A systematic review for estimation of HTLV-I infection in the blood donors of Iran. *Iran J Basic Med Sci* 2013;16: 196-201.
13. Kadas J, Boross P, Weber IT, Bagossi P, Matuz K, Tozser J. C-terminal residues of mature human T-lymphotropic virus type 1 protease are critical for dimerization and catalytic activity. *Biochem J* 2008;416: 357-364.
14. Futsch N, Mahieux R, Dutartre H. HTLV-1, the other pathogenic yet neglected human retrovirus: from transmission to therapeutic treatment. *Viruses* 2017;10:1.
15. Stienlauf S, Yahalom V, Schwartz E, Shinar E, Segal G, Sidi Y. Epidemiology of human T-cell lymphotropic virus type 1 infection in blood donors, Israel. *Emerg Infect Dis* 2009;15: 1116-1118.
16. Kchour G, Rezaee R, Farid R, Ghantous A, Rafatpanah H, Tarhini M, et al. The combination of arsenic, interferon-alpha, and zidovudine restores an "immunocompetent-like" cytokine expression profile in patients with adult T-cell leukemia lymphoma. *Retrovirology* 2013;10: 91.
17. Martin JL, Maldonado JO, Mueller JD, Zhang W, Mansky LM. Molecular studies of HTLV-1 replication: an update. *Viruses* 2016;8: 31.
18. Azran I, Schavinsky-Khrapunsky Y, Aboud M. Role of Tax protein in human T-cell leukemia virus type-I leukemogenicity. *Retrovirology* 2004;1: 20.
19. Hall WW, Fujii M. Deregulation of cell-signaling pathways in HTLV-1 infection. *Oncogene* 2005;24: 5965-5975.
20. Matsuoka M, Yasunaga JI. Human T-cell leukemia virus type 1: replication, proliferation and propagation by Tax and HTLV-1 bZIP factor. *Curr Opin Virol* 2013;3: 684-691.
21. Romanelli MG, Diani E, Bergamo E, Casoli C, Ciminale V, Bex F, et al. Highlights on distinctive structural and functional properties of HTLV Tax proteins. *Front Microbiol* 2013;4: 271.
22. Grassmann R, Aboud M, Jeang KT. Molecular mechanisms of cellular transformation by HTLV-1 Tax. *Oncogene* 2005;24: 5976-5985.
23. Stoppa G, Rumiato E, Saggioro D. Ras signaling contributes to survival of human T-cell leukemia/lymphoma virus type 1 (HTLV-1) Tax-positive T-cells. *Apoptosis* 2012;17: 219-228.
24. Ciminale V, Rende F, Bertazzoni U, Romanelli MG. HTLV-1 and HTLV-2: highly similar viruses with distinct oncogenic properties. *Front Microbiol* 2014;5: 398.
25. Fujikawa D, Nakagawa S, Hori M, Kurokawa N, Soejima A, Nakano K, et al. Polycomb-dependent epigenetic landscape in adult T-cell leukemia. *Blood* 2016; 127: 1790-1802.
26. Gligorijevic V, Przulj N. Methods for biological data integration: perspectives and challenges. *J R Soc Interface* 2015;12: 20150571.
27. Kawaguchi A, Orba Y, Kimura T, Iha H, Ogata M, Tsuji T, et al. Inhibition of the SDF-1alpha-CXCR4 axis by the CXCR4 antagonist AMD3100 suppresses the migration of cultured cells from ATL patients and murine lymphoblastoid cells from HTLV-I Tax transgenic mice. *Blood* 2009;114: 2961-2968.
28. Yamagishi M, Nakano K, Miyake A, Yamochi T, Kagami Y, Tsutsumi A, et al. Polycomb-mediated loss of miR-31 activates NIK-dependent NF- κB pathway in adult T cell leukemia and other cancers. *Cancer cell* 2012;21: 121-134.
29. Rahman S, Quann K, Pandya D, Singh S, Khan ZK, Jain P. HTLV-1 Tax mediated downregulation of miRNAs associated with chromatin remodeling factors in T cells with stably integrated viral promoter. *PLoS One* 2012;7(4): e34490.
30. Sfetcu O, Gauci AA (2015). Geographical distribution of areas with a high prevalence of HTLV-1 infection. Stockholm: ECDC.
31. Afonso PV, Cassar O, Gessain A. Molecular epidemiology, genetic variability and evolution of HTLV-1 with special emphasis on African genotypes. *Retrovirology* 2019;16: 39.
32. Pineda M, Bouzas MB, Golemba M, Remesar M, Aulicino P, Mammana L, et al. Molecular epidemiology of HTLV-1 in Argentina. *Retrovirology* 2015;12(Suppl 1): P85.
33. Ngoma AM, Mutombo PB, Omokoko MD, Mvika ES, Nollet KE, Ohto H. Prevalence and molecular epidemiology of human T-lymphotropic virus type 1 among women attending antenatal clinics in Sub-Saharan Africa: a systematic review and meta-analysis. *Am J Trop Med Hyg* 2019;101: 908-915.
34. de Almeida Rego FF, Mota–Miranda A, de Souza Santos E, Galva˜ o–Castro B, Alcantara LC. Seroprevalence and molecular epidemiology of HTLV-1 isolates from HIV-1 co-infected women in Feira de Santana, Bahia, Brazil. *AIDS Res Hum Retroviruses* 2010;26: 1333-1339.