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Original Article

Phylogenetic and Mutational Analysis of the *Tax* Gene in the Human T-Lymphotropic Virus 1 (*HTLV-1*) in Three Provinces of Iran

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Abstract

Background: Human T-lymphotropic virus type 1 (*HTLV-1*) is considered a health issue in Iran. However, its genetic diversity and molecular epidemiologic phylogeny remain poorly characterized.

Methods: The *Tax* gene of 9 asymptomatic individuals across Alborz, Gilan, and Ardabil provinces of Iran was sequenced and analyzed phylogenetically using MEGA-X.

Results: All strains clustered within the Cosmopolitan subtype a, showing high genetic similarity to Japanese and Chinese references. Positive selection (dN/dS > 1) was observed in all samples. Strikingly, the Alborz ISO32 strain exhibited 10 unique nonsynonymous mutations, suggesting regional evolutionary divergence.

Conclusion: This study, as the first multi-provincial study in Iran, reveals the essential requirement for systematic tracking of *HTLV-1* genetic diversity and designing prevention programs tailored to each region.

Keywords: *Tax;* Phylogenetic; Mutation, Genetics; Iran

Introduction

HTLV-1 is a member of the delta retrovirus genus (1). *HTLV-1* is the cause of cancers such as Adult T cell leukemia/lymphoma (ATL) (2). It is also linked to inflammatory diseases like *HTLV-1-associated* myelopathy/Tropical spastic Paraparesis (HAM/TSP) (3). *HTLV-1* affects roughly 10-20 million people globally, mainly in tropical and subtropical areas (4, 5). The virus is common in

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several countries, including Japan, the Caribbean, South America, Africa, and parts of Asia (4-6). It spreads through breastfeeding, mother-to-child transmission, contaminated blood products, and sexual contact (7, 8).

The virus has a single-stranded RNA genome. The *HTLV-1* genome consists of approximately 9 kb of RNA, which is flanked by long terminal repeats (*LTRs*) (6). According to phylogenetic analysis based on the *LTR* and *Tax* sequences, seven subtypes (a-g) of *HTLV-1* have been identified. Subtypes include cosmopolitan (a), African (b, d, e, f), and Melanesian (c) (8, 9). Interestingly, no clear link has been found between specific subtypes and disease severity, such as ATL or HAM/TSP (10).

The *Tax* gene encodes a nuclear phosphoprotein (*Tax*) that plays a pivotal role in viral replication and pathogenesis. Through its interaction with cellular transcription factors and signaling pathways, *Tax* initiates viral gene transcription (11). Moreover, it regulates the expression of host genes linked to cell growth, programmed cell death, and immune reactions, propelling *HTLV-1*-driven cancer development and inflammation (11). With its pivotal role, the *Tax* gene stands as a top focus for phylogenetic and mutational research. Despite its importance, most phylogenetic studies in Iran have focused on the *LTR* region, with limited data available on the *Tax* gene. This gap is particularly notable in studies of asymptomatic carriers, who constitute a critical reservoir for HTLV-1 transmission. Probing the *Tax* gene in these carriers might yield an understanding of viral evolution, selection pressures, and potential immune evasion strategies.

In Iran, *HTLV-1* presents unique epidemiological challenges; the prevalence of *HTLV-1* varies significantly across different regions. For instance, the seroprevalence in Mashhad, an endemic area, is 2.12%, while in Alborz Province, it is as low as 0.13% (12, 13). These regional differences highlight the need for detailed investigations to elucidate *HTLV-1's* epidemiological patterns in Iran. Notably, phylogenetic analyses of Iranian strains reveal that subtype a predominates and exhibits strong genetic ties to isolates from Japan and China, suggesting that *HTLV-1* likely arrived in Iran through ancient trade routes like the Silk Road (13-16).

Despite these insights, four key limitations emerge in current *HTLV-1* research. First, inadequate characterization of asymptomatic carriers' epidemiological role. Second, insufficient *Tax* gene sequence data despite its regulatory importance, and third, lack of comprehensive studies linking genetic variations to phenotypic consequences in viral pathogenicity and transmission and Notably, previous studies, except those in Alborz and Tehran (13, 14) have not examine positive and negative selection pressures – a gap our study fills by being the first to systematically assess these evolutionary forces across multiple provinces. Fourth, given the clonal growth of *HTLV-1*, different regions and provinces may exhibit distinct viral attributes and mutations. Broad sampling across multiple Iranian provinces is essential to address this.

While Babakhani et al. compared asymptomatic carriers with HAM/TSP patients (14), our study was designed to focus exclusively on asymptomatic individuals, who represent the majority of *HTLV-1*–infected cases and the main source of silent transmission. In addition, our samples were collected from three provinces of Iran, which allowed us to capture regional genetic diversity and identify a unique Alborz ISO32 strain. Moreover, whereas recent Babakhani's publication entitled Phylogenetic and mutational analysis of the *Tax Gene* in the Human T-Lymphotropic Virus 1-Associated HAM/TSP in comparison with asymptomatic carriers (14) emphasized clinical comparisons, our study advances the molecular epidemiology of asymptomatic carriers across multiple regions, providing complementary and novel insights.

Besides, past studies have focused narrowly on areas like Alborz Province or Mashhad city, leaving a notable knowledge gap (13-15). We aimed to tackle this gap by studying samples from Alborz, Gilan, and Ardabil provinces.

Materials and Methods

Ethics Statement

The protocol of this study was approved by the institutional review board (IRB) of the Tehran University of Medical Sciences, Tehran, Iran (Ethics code No: IR. TUMs. SPH. REC. 1401.285).

Blood sample collection

In 2022, the blood samples of 75 people were collected from healthy individuals who were carriers of the human T-lymphotropic Type 1 virus (*HTLV-1*). The samples were collected from people who were referred for blood donation to the Tehran Blood Transfusion Organization. All enrolled subjects were rigorously screened and found to be asymptomatic for both ATL and HAM/TSP, with negative results on all clinical diagnostic measures. Primarily, the blood samples were collected by the Tehran Blood Transfusion Organization and screened to confirm that they are ACs (asymptomatic carriers). *HTLV-1* infection in all collected blood samples was confirmed by Enzyme-linked immunosorbent assay (ELISA, DiaPro-Italy).

PBMCs Isolation and DNA Extraction:

PBMCs (Peripheral blood mononuclear cells) were isolated from all samples using Ficoll gradient medium. DNA was extracted from PBMCs using a commercial kit (ROJE Technologies, Iran). After extraction, the DNA was stored in a freezer at -20 °C until the PCR test started. We used Primers for Partial *LTR* (P*LTR*) and Partial *HBZ* (P*HBZ*) for infection confirmation, and Full *Tax* (F*Tax*) for sequencing (14). The P*LTR* and P*HBZ* regions were amplified using a T100 thermocycler (Biorad, USA). For the P*LTR* region, the amplification protocol began with an initial denaturation at 94 °C for 4 min, followed by 45 cycles consisting of denaturation at 95 °C for 40 seconds, annealing at 62 °C for 40 seconds, and extension at 72 °C for 30 seconds. A final extension step was performed at 72 °C for 5 min, after which the samples were held at 4 °C for 2 min.

For the P*HBZ* region amplification, similar conditions were applied with slight modifications: the annealing temperature was reduced to 60 °C, and the extension time was shortened to 30 seconds during each cycle, while maintaining the same number of cycles and other parameters as the P*LTR* protocol. The final extension and holding conditions remained identical to those used for P*LTR* amplification.

The 1062 bp *Tax* region was amplified using specific primers (14). The PCR conditions involved an initial denaturation at 94 °C for 4 min, followed by 45 cycles of denaturation (95 °C for 40 seconds), annealing (58 °C for 35 seconds), and extension (72 °C for 80 seconds), with a final extension at 72 °C for 5 min and storage at 4 °C for 2 min.

Following amplification, the *Tax* gene products were submitted to the Codon Institute for automated Sanger sequencing. The obtained sequences underwent verification through NCBI BLAST analysis. For sequence analysis, we utilized the BioEdit software package.

Population selection for sequencing and phylogenetic step

A total of 75 asymptomatic *HTLV-1* carriers from 3 endemic regions of Iran (Ardabil, Gilan, Alborz, Tabriz, and Shiraz) were included. Eventually, 9 samples were enrolled in this study for sequencing and phylogenetic analysis and were compared to 17 others from different regions and subtypes around the world. 5 samples from Gilan province and 2 samples from each Ardabil and Alborz provinces alone. Three of them were female (33.33%), and the mean age of all was 44.42 years (Table 1).

Table 1: Demographic characteristics of *HTLV-1* isolates, including province of origin, sex, and age of the carriers (Ardabil, Gilan, and Alborz provinces)

Isolate Name	Province	Sex	Age(yr)
2	Ardabil	Male	47
4	Gilan	Male	44
14	Gilan	Female	58
17	Gilan	Male	20
19	Gilan	Female	52
27	Alborz	Male	55
32	Alborz	Male	37
38	Gilan	Male	29
A1	Ardabil	Female	38

Bioinformatics & Phylogenetic Analysis

Forward and reverse primers binding and multiple sequence alignment were performed using the ClustalW algorithm in BioEdit V7.2.9, followed by manual refinement to ensure accurate positioning of indels and conserved regions relative to the Japanese reference strain (AB979451). Phylogenetic trees were constructed using MEGA-X version 10.0.5 with the Maximum Likelihood (ML) method under the Kimura two-parameter substitution model. Bootstrap analysis with 1000 replicates was conducted to assess nodal support.

Mutational analysis

For evolutionary analysis, a multiple sequence alignment was generated using ClustalW implemented in BioEdit. Nucleotide and amino acid substitutions were identified relative to the Japanese prototype strain (AB979451). Selection pressure analysis was performed by calculating the ratio of nonsynonymous to synonymous substitutions (dN/dS). Positive and negative selection were determined with statistical significance thresholds of $P < 0.05$.

Results

Genetic Diversity

Comparative genomic analysis revealed 38 variable positions, of which 21 exhibited amino acid-altering (nonsynonymous) substitutions (Table 2).

Table 2: Mutation profile of *HTLV-1* isolates from Ardabil, Gilan, and Alborz provinces compared with the NC_001436.1 USA reference genome. All isolates showed amino acid substitutions without synonymous mutations, and all tested positive

NC 001436.1 USA	Total Mutation	S.M	A.S.M	A/S	Pos/Neg
Iso 2(2) Ardabil	8	0	8	8	Pos
Iso 4(4) Gilan	7	0	7	7	Pos
Iso 14(10) Gilan	10	0	10	10	Pos
Iso 17(12) Gilan	7	0	7	7	Pos
Iso 19(13) Gilan	8	0	8	8	Pos
Iso 27(21) Alborz	7	0	7	7	Pos
Iso 32(25) Alborz	15	0	15	15	Pos
Iso 38(30) Gilan	6	0	6	6	Pos
Iso A1(1) Ardabil	7	0	7	7	Pos
Total	75	0	75	75	Pos

Phylogenetic Analysis

As depicted in the cladogram Fig. 1, all Iranian strains were clustered into a subtype of the cosmopolitan clade. Pronouncedly, the entire sequence collection established a clearly independent subclade grouping with a Japanese strain. Furthermore, there was not any specific difference between individual samples from different regions. It is noteworthy that Substantial genetic and evolutionary divergence was observed between Iranian and African sequences.

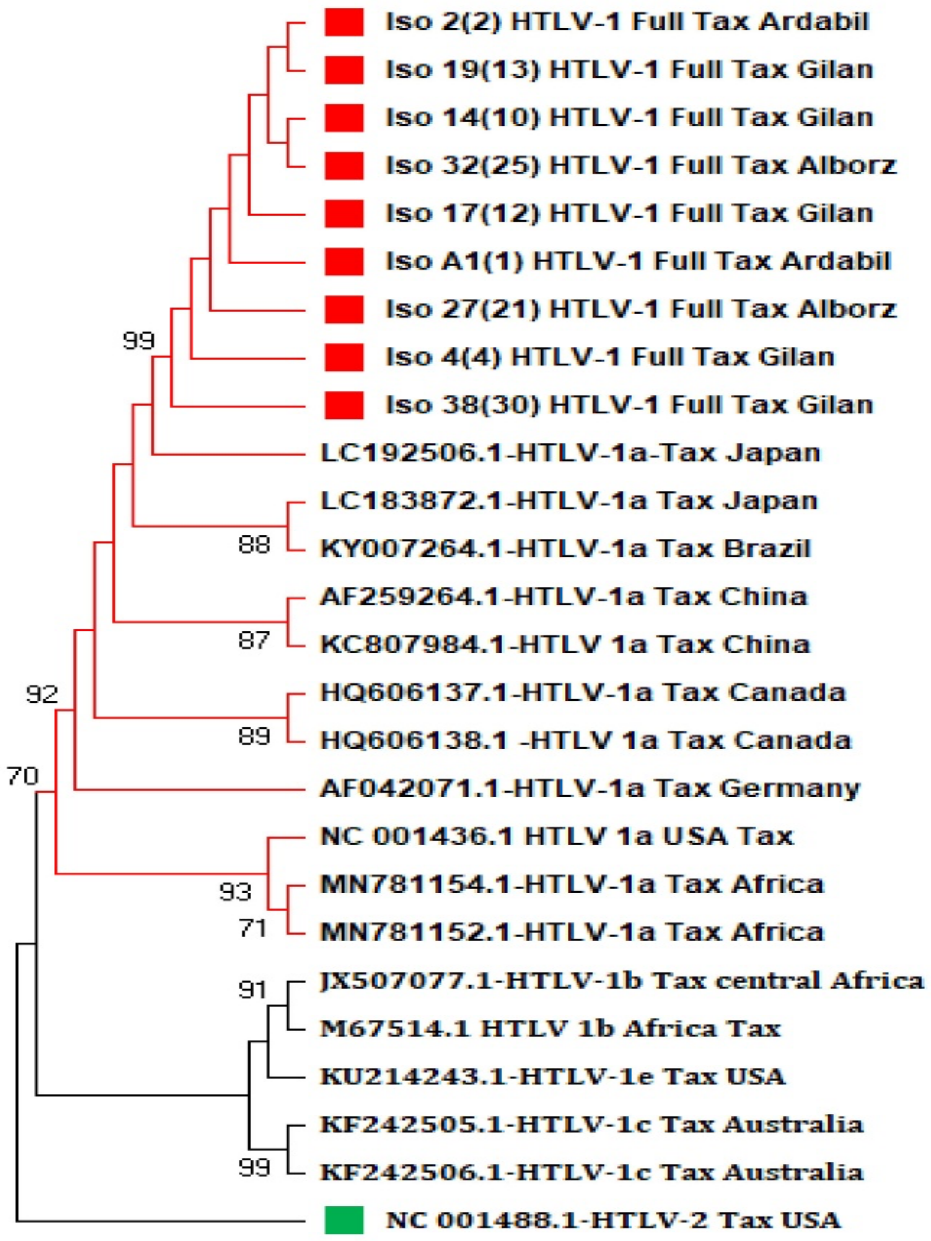

Fig. 1: Maximum likelihood phylogenetic tree of HTLV-1 full Tax sequences isolated from different provinces of Iran (Ardabil, Gilan, and Alborz) compared with reference sequences from Japan, Brazil, China, Canada, Germany, USA, Africa, and Australia. Bootstrap values (≥70%) are shown at the nodes. Iranian isolates are highlighted in red, while reference sequences from other regions are indicated in black; HTLV-2 is included as an outgroup (green)

Selection Pressure

The amino acid sequences of the *Tax* gene showed marked adaptive evolution, with all Iranian isolates undergoing significant positive selection relative to US counterparts (NC_001436.1 USA). Table 2 enumerates both silent (synonymous) and replacement (nonsynonymous) mutations. Our analysis identified seven Iran-specific nonsynonymous mutations when compared to the American reference strain (Fig. 2). Notably, sample iso32 from Alborz province exhibited the highest mutation burden (15 total mutations), including ten unique variants. Interestingly, this isolate lacked two of the characteristic Iran-specific mutations observed in other samples.

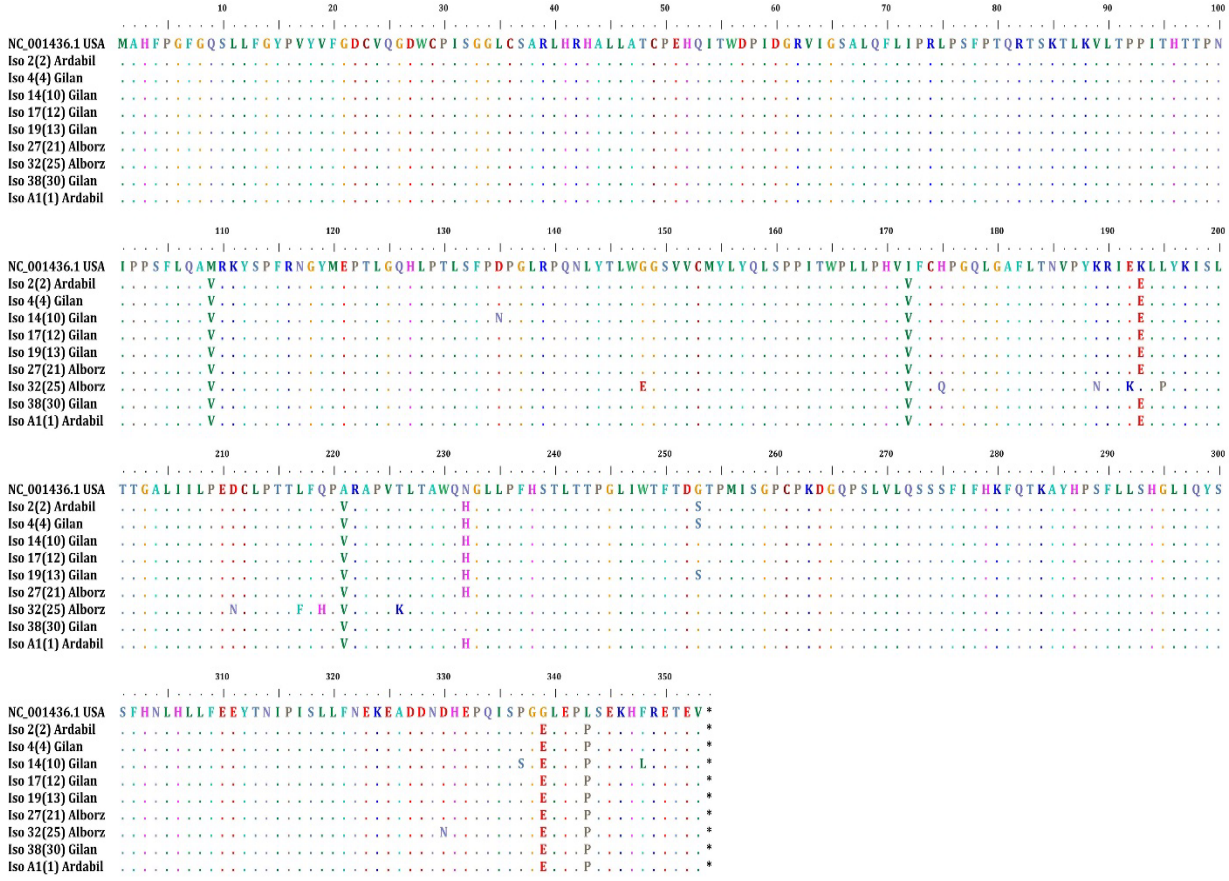

Fig. 2: Alignment of *HTLV-1* isolates from Ardabil, Gilan, and Alborz provinces against the NC_001436.1 USA reference. Amino acid substitutions are highlighted, showing variation across isolates with no synonymous mutations detected.

Discussion

This study provides novel insights into the molecular epidemiology of *HTLV-1* in Iran, establishing that Cosmopolitan subtype a is widely present, with marked evolutionary adaptations in the *Tax* gene and unique mutations in the Alborz ISO32 strain. Here, we discuss how these findings relate to regional infection patterns, evolutionary shifts, genetic ties, and their potential impact on public health planning.

Two Studies (16) and (13) in Tehran and Alborz, combined with our work in Gilan and Ardabil, consistently find Cosmopolitan subtype A strains

closely linked to those from Japan and China (13, 14, 16). Our results support the hypothesis of historical transmission of this virus along the Silk Road—a crucial route for trade and the spread of pathogens such as *HTLV-1* (17). The connection to Mongol and Afghan invasions before the 15th century, particularly through Iran's northeastern borders, is in favor of this issue (18). Although the African slave trade has been suggested as another route, our phylogenetic data show clear genetic differences between Iranian and African strains, rendering this hypothesis less plausible (19).

Our results align with Babakhani et al.'s findings (14), but differ from Safavi et al.'s report, which observed positive selection in just 3 of 19 *Tax* sequences (13). This contrast might suggest that *Tax* develops to escape from the host immune system, while negative selection maintains the *LTR* sequence.

The Alborz ISO32 isolate exhibited distinct characteristics, revealing 10 unique nonsynonymous mutations, and missing two mutations typically seen in other Iranian strains. Still, phylogenetic analysis groups it with other Iranian isolates, suggesting elevated mutation rates in the *Tax* gene. These observations stress the need for continued surveillance and detailed molecular studies of *HTLV-1* strains across various Iranian populations.

HTLV-1 exhibits clear geographical distribution patterns, with asymptomatic carriers primarily located in endemic regions. Infection rates vary from 3–5% in the Caribbean to over 10% in southern Japan (20). The Middle East, especially Iran and Kuwait, stands as a significant endemic hub (15, 21). In northeastern Iran, prevalence reaches about 4% (15), whereas other provinces show much lower rates: Ardabil (0.01%) (22), Alborz (0.057%), and Gilan (0.024%) (23). This variation in prevalence reinforces the clonal expansion pattern typical of *HTLV-1*.

The *Tax* oncoprotein is crucial to HTLV-1 pathogenesis, driving processes like NF-*κ*B activation, cell cycle interference, genomic instability, and immune modulation (24). Examining *Tax* sequences through phylogenetic analysis offers valuable perspectives on viral evolution and leukemogenic risks, especially in asymptomatic carriers. Furukawa et al. (2000) showed a key classification system by grouping *Tax* sequences into subgroups A and B, observing that *Tax* A is more common among HAM/TSP patients than asymptomatic carriers (25). This observation indicates a potential association between certain *Tax* mutations and disease progression. Further studies are needed to confirm this initial observation.

The major challenge and limitation we faced in this survey was access to biological samples. After multiple formal communications, we were able to obtain the required samples from the Tehran Blood Transfusion Organization. However, the number of available samples was limited, which imposed significant constraints on the experimental procedures. To address this, all laboratory tests were performed with maximum care to ensure that no samples were wasted and that reliable results could still be obtained. However, we recommend further study with desirable samples to access more reliable information in this field.

Conclusion

This study provides important insights about the molecular epidemiology of *HTLV-1* in Iran by phylogenetic study based on *Tax* gene mutations in Acs and is the first conducted in multiple provinces. Results confirm that Cosmopolitan subtype a is dominant in the studied provinces. All samples were under positive selection pressure which suggests adaptive evolution of the virus under the pressure of the host immune system. Strikingly, the sample ISO32 from Alborz province had 10 distinct mutations and lacked two prevalent mutations which shows its different evolutionary pattern. It demonstrates the importance of persistent observation of *HTLV-1* genetic diversity in different parts of Iran to identify new mutations and to design prevention programs proportional to the diversity in Iran. Despite of the small sample size, this study is a

stride to understand the genetic diversity of *HTLV-1* in Iran.

Journalism Ethics considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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Conflict of Interest

The authors declare that there is no conflict of interest.

References

1. Poiesz BJ, Ruscetti FW, Gazdar AF, et al (1980). Detection and isolation of type C retrovirus particles from fresh and cultured lymphocytes of a patient with cutaneous T-cell lymphoma. *Proc Natl Acad Sci U S A,* 77(12):7415-9.
2. Bangham CR, Ratner L (2015). How does HTLV-1 cause adult T-cell leukaemia/lymphoma (ATL)? Current opinion in virology. *Curr Opin Virol*, 14:93-100.
3. Yamano Y, Sato T (2012). Clinical pathophysiology of human T-lymphotropic virus-type 1-associated myelopathy/tropical spastic paraparesis. *Front Microbiol*, 3:389.
4. Gessain A, Cassar O (2012). Epidemiological aspects and world distribution of HTLV-1 infection. *Front Microbiol*, 3:388.
5. Gessain A, Ramassamy J-L, Afonso PV, et al (2023). Geographic distribution, clinical epidemiology and genetic diversity of the human oncogenic retrovirus HTLV-1 in Africa, the world's largest endemic area. *Front Immunol*, 14:1043600.
6. Verdonck K, González E, Van Dooren S, et al (2007). Human T-lymphotropic virus 1: recent knowledge about an ancient infection. *Lancet Infect Dis*, 7(4):266-81.
7. Millen S, Thoma-Kress AK (2022). Milk Transmission of HTLV-1 and the need for innovative prevention strategies. *Front Med (Lausanne)*, 9:867147.
8. Paiva A, Casseb J (2014) . Sexual transmission of human T-cell lymphotropic virus type 1. *Rev Soc Bras Med Trop*, 47(3):265-74.
9. Berini CA, Delfino C, Torres O, et al (2013). HTLV-1 cosmopolitan and HTLV-2 subtype b among pregnant women of non-endemic areas of Argentina. *Sex Transm Infect,* 89(4):333-5.
10. Proietti FA, Carneiro-Proietti ABF, Catalan-Soares BC, et al (2005). Global epidemiology of HTLV-I infection and associated diseases. *Oncogene*, 24(39):6058-68.
11. Currer R, Van Duyne R, Jaworski E, et al (2012). HTLV tax: a fascinating multifunctional co-regulator of viral and cellular pathways. *Front Microbiol*, 3:406.
12. Rafatpanah H, Hedayati-Moghaddam MR, Fathimoghadam F, et al (2011). High prevalence of HTLV-I infection in Mashhad, Northeast Iran: a population-based seroepidemiology survey. *J Clin Virol,* 52(3):172-6.
13. Safavi M, Habibian-Sezavar F, Letafati A, et al (2024). Determination of molecular epidemiologic pattern of human T-lymphotropic virus type 1 (HTLV-1) in Alborz province, Iran. *Virus Genes*, 60(2):117-25.
14. Babakhani F, Bahavar A, Zare S, et al (2025). Phylogenetic and Mutational Analysis of the Tax Gene in the Human T-Lymphotropic Virus 1-Associated HAM/TSP in Comparison with Asymptomatic Carriers. *Iran J Public Health,* 54(2):435-42.

15. Azarpazhooh MR, Hasanpour K, Ghanbari M, et al (2012). Human T-lymphotropic virus type 1 prevalence in northeastern Iran, Sabzevar: an epidemiologic-based study and phylogenetic analysis. *AIDS Res Hum Retroviruses,* 28(9):1095-101.
16. Pourrezaei S, Shadabi S, Gheidishahran M, et al (2021). 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 *Iran J Microbiol*, 13(4):509-517.
17. Fujikawa D, Nakagawa S, Hori M, et al (2016). Polycomb-dependent epigenetic landscape in adult T-cell leukemia. *Blood*, 127(14):1790-802.
18. Pashabayg CR, Momenifar N, Malekpour SA, et al (2020). Phylogenetic and phylodynamic study of Human T-cell lymphotropic virus Type 1 (HTLV-1) in Iran. *Infect Genet Evol,* 85:104426.
19. Hall WW, Fujii M (2005). Deregulation of cell-signaling pathways in HTLV-1 infection. *Oncogene*, 24(39):5965-75.
20. Mueller N, Okayama A, Stuver S, et al (1996). Findings from the Miyazaki cohort study. *J Acquir Immune Defic Syndr Hum Retrovirol*, 13 Suppl 1:S2-7.
21. Voevodin A, Gessain A (1997). Common origin of human T-lymphotropic virus type-I from Iran, Kuwait, Israel, and La Réunion Island. *J Med Virol*, 52(1):77-82.
22. Karimi G, Zadsar M, Pourfathollah AA, et al (2017). Seroprevalence and geographical distribution of human T-lymphotropic virus type 1 among volunteer blood donors in endemic areas of Iran. *Virol J*, 14:14.
23. Ferdowsi S, Najafzadeh M, Ameli MR, et al (2019). Evaluating the frequency of human T-lymphotropic virus type 1 infection in Iranian population. *Iranian Journal of Blood and Cancer,* 11(2):47-50.
24. Yasunaga J, Matsuoka M (2011). Molecular mechanisms of HTLV-1 infection and pathogenesis. *Int J Hematol***,** 94(5):435-42.
25. Furukawa Y, Yamashita M, Usuku K, et al (2000). Phylogenetic subgroups of human t cell lymphotropic virus (HTLV) type in the tax gene and their association with different risks for HTLV-I—associated myelopathy/tropical spastic paraparesis. J Infect Dis, 182(5):1343-9.