Iran J Public Health, Vol. 54, No.2, Feb 2025, pp.435-442

Original Article

Phylogenetic and Mutational Analysis of the Tax Gene in the Human T-Lymphotropic Virus 1-Associated HAM/TSP in Comparison with Asymptomatic Careers

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(Received 12 Jun 2024; accepted 15 Sep 2024)

Abstract

Background: Human T-lymphotropic virus 1 (HTLV-1) is a member of the Retroviridae family that can cause two groups of diseases: HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP) and adult T-cell leukemia/lymphoma (ATLL). Despite HTLV having seven different subtypes, cosmopolitan subtype A is responsible for most HTLV-1-related disorders and is the most widely dispersed type globally, including in the Middle East, known to be an endemic area for the virus. Therefore, due to the importance of determining the subtypes of this virus, we aimed to explore the subtypes of HTLV-1 in Iran.

Methods: In this cross-sectional study, we screened 140 Blood samples for HAM/TSP infection and approximately 4,500 samples for asymptomatic carriers (ACs) from Sina Hospital, Iran between 2020 and 2021. Positive samples were used for phylogenetic and mutational analysis to compare ACs and HAM/TSP cases via the Tax segment of HTLV. To identify the genotype of positive samples, the Maximum Likelihood method was used to construct the phylogenetic tree based on the positive samples.

Results: All Iranian isolates were clustered as HTLV-1a subgroups. Moreover, all of our samples have undergone positive selection pressure. Furthermore, we detected unique mutations in Iranian HAM/TSP and ACs sequences.

Conclusion: All of the Iranian Tax proteins are under positive selection pressure with respect to Japanese isolates. Interestingly, we detect specific mutation patterns in the sequences. Positions 51, 82, 109, 172, 232, and 339 in the aa sequence have undergone mutations specific to Iranian HTLV-1; and in positions 22 and 146 aa we detected mutations unique to ACs and HAM/TSP, respectively.

Keywords: Human T-lymphotropic virus 1 (HTLV-1); Phylogenetic analysis; Mutational analysis

DOI: 10.18502/ijph.v54i2.17914

Introduction

Human T-lymphotropic virus 1 (HTLV-1), which belongs to the Retroviridae family, can cause two groups of diseases: HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP) and adult T-cell leukemia/lymphoma (ATLL) (1-3). This virus can be transmitted in different ways: from mother to infant, mostly by prolonged breastfeeding, transfusion of blood, and via sexual contact (since the virus transmits from male to female, women are more endangered) (4-6).

HTLV-1 has a diploid RNA genome and based on the long terminal repeat (LTR) sequence and Tax segment, seven different subtypes are recognized from HTLV-1. The Cosmopolitan subtype is known as HTLV-1A, African subtypes are known as B, D, E, F, and G, and Melanesian clustered in the C subtype (7-10). Previous studies have confirmed the variety of HTLV-1 prevalence among different populations. Moreover, the prevalence of HTLV-1 increases proportionally to age and also, is more prevalent in females (11, 12).

Among the seven characterized subtypes, cosmopolitan subtype A is responsible for most of the HTLV-1-related disorders. In addition, this type is the most transferred type dispersed worldwide, however, southern western Japan, the Caribbean islands, South America, West Africa, the Melanesian Islands, and the Middle East are known to be the endemic areas for the virus. This virus has infected more than five million people globally (13-16).

HTLV-1 creates an important protein known as Tax proteins associated with controlling the cellular signaling pathways in order to development of chronic inflammation and the induction of related diseases (17, 18).

Since the most abundant HTLV-1 sequence is LTR which is available in databases and covers a wide range of countries, most of the studies targeted the LTR region for their phylogenetic analysis. There are few studies in Iran that analyze the phylogeny of the Tax segment in the infected HAM/TSP patients versus ACs ones, while the Tax sequence is informative for characterizing different subtypes of the virus. Furthermore, this segment can be considered for mutational analysis in order to detect positive and negative selection.

Although Iran is reported to be one of the endemic regions and has a prevalence of infection of approximately 4% in some northeast cities [PMID: 26899860], there are few comprehensive studies to conduct phylogenetic insights into the Tax segment. In this study, we aim to compare this segment in HAM/TSP and ACs patients via phylogenetic and mutational analysis. Previous phylogenetic studies in Iran revealed that the HTLV-1 genotype belongs to the subtype A (19). We aimed to perform a phylogenetic analysis of the HTLV-1 based on the Tax sequence to determine the subtypes of the virus in Iran. Moreover, mutational analysis of the HTLV-1 based on the Tax segment is covered in this study.

Materials and Methods

Population study

In this cross-sectional study, blood samples of 140 suspected HAM/TSP patients were collected from people referred to Sina Hospital in Tehran, Iran. All collected blood samples were analyzed for anti-HTLV1 antibodies. Sera-positive samples were checked by PCR to confirm HTLV-1 infection using specific primers based on PLTR and PHBZ genes. Subjects' agreements were obtained for participating in the study.

Each month around 1500 individuals referring to the Blood Transfusion Organization of Alborz (BTOA) for blood donation were screened to be asymptomatic careers. All the samples from suspected HAM/TSP patients and ACs were first screened for the presence of HTLV-1 antibodies by an Enzyme-linked immunosorbent assay

(ELISA, Dia. Pro-Italy). Then, the PCR test was implemented to confirm the positive sera samples. The HAM/TSP-positive samples were collected between 2020 and 2021, while the ACs samples were collected in 3 months starting from Apr 2020 to Jun 2020.

DNA extraction and PCR amplification

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. Two pairs of primers were designed, Partial LTR (PLTR) and Partial HBZ (PHBZ) for infection confirmation, and Full Tax (FTax) for sequencing. PLTR forward primer (5-GGCTCGCATCTCCCCTTCAC-3) and PLTR reverse primer (5-GAGCAAGCAGGGTCAGGCAA-3), PHBZ forward primer (5-ACGTCGCCCGGAGAAAACA-3) and PHBZ reverse primer (5-CTCCACCTCGCCTTCCAACT-3). A T100 thermocycler (Biorad ,USA) was used to amplify the PLTR and PHBZ regions with the following amplification program for PLTR: 94 °C for 4 min, 45 cycles of 95 °C for 40 sec, 62 °C for 40 sec, 72 °C for 40 sec and for final extension step 72 °C for 5 min and hold in 4 °C for 2 min. Moreover, the program used for amplifying the PHBZ region is as follows: 94 °C for 4 min, 45 cycles of 95 °C for 40 sec, 60 °C for 40 sec, 72 °C for 30 sec and for final extension step 72 °C for 5 min and hold in 4 °C for 2 min.

DNA sequencing and phylogenetic analysis

The Tax region 1062 bp was amplified with the following primers: forward strand (5-GGATAGCAAACCGTCAAGCAC-3) and reverse primer (5-GGTGAGGGGTTGTCGTCAA-3). For the amplification section, each PCR run consisted of 94 °C for 4 min, 45 cycles of 95 °C for 40 sec, 58 °C for 35 sec, 72 °C for 80 sec and for the final extension step 72 °C for 5 min and hold in 4 °C for 2 min. Then, the amplified segments of the Tax genes were sent to the Codon institute for sequencing. The Tax gene segments were determined, using the sanger method. Then, the sequences were converted into the blast and the accuracy was assessed in NCBI, the confirmed sequences were documented in Gene bank. To analyze the sequences BioEdit was used.

Afterward, Phylogenetic tree reconstructions were conducted using MEGA software (version X) with the maximum likelihood method in the Kimura two-parameter substitution model with 1000 bootstrap sampling.

Mutational analysis

Iranian isolates were considered for mutational analysis to explore the probable changes in the amino acid sequence of the Tax region. For this purpose, we used the Japanese Tax segment, which was the most similar Tax isolate to the Iranian sequences, as the reference sequence. Then, we obtained amino acid changes in the Iranian sequences.

Ethics approval and consent to participate

Informed consent for study participation was obtained from all subjects. The study was approved by the Ethics Committee of Tehran University of Medical Science. (IR.400.12.1417).

Results

opulation study

We obtained 9 HAM/TSP-positive samples out of 140 and 4 Asymptomatic HTLV-1 carriers out of almost 4500 samples. Of the 9 HAM/TSP samples, 3 were male and 6 were female. Additionally, 2 of them were in the 20-40 age group, and 7 were between 41 and 60. No positive sample was detected to be more than 60 yr old. Demographic information on the samples indicated that the mean age of HAM/TSP patients was 44.5, while for ACs samples it was 47.5. Furthermore, all HAM/TSP samples had the same marital status and were married, while only one out of four ACs samples was single. Most pa-

tients had no hospitalization history, and 5 out of 9 HAM/TSP and 50% of the ACs samples had undergone surgery (Table 1).

Table 1: Demographic information of the positive samples

Isolate	Sex	Age	Marital statues	Collected date
3001-FTax (ACs)	M	35	M	4-2020
3002-FTax (ACs)	M	55	M	4-2020
3003-FTax (ACs)	M	49	M	5-2020
3004-FTax (ACs)	M	51	M	6-2020
4001-FTax (HAM/TSP)	F	45	M	10-2020
4002-FTax (HAM/TSP)	M	51	M	11-2020
4003-FTax (HAM/TSP)	F	42	M	10-2020
4004-FTax (HAM/TSP)	F	38	M	10-2020
4005-FTax (HAM/TSP)	F	46	M	10-2020
4006-FTax (HAM/TSP)	F	54	M	11-2020
4007-FTax (HAM/TSP)	M	37	M	12-2020
4008-FTax (HAM/TSP)	M	47	M	1-2021
4009-FTax (HAM/TSP)	F	41	M	12-2020

Phylogenetic analysis

The phylogenetic analysis, conducted using 18 other sequences from different genotypes, indicated that all Iranian isolates belong to the transcontinental subgroup (A) of the Cosmopolitan subtype (Fig. 1). This outcome showed that HTLV-3 from the USA was the outgroup and the most distant variant, while Japanese HTLV-1 Tax was the closest sequence to Iranian isolates.

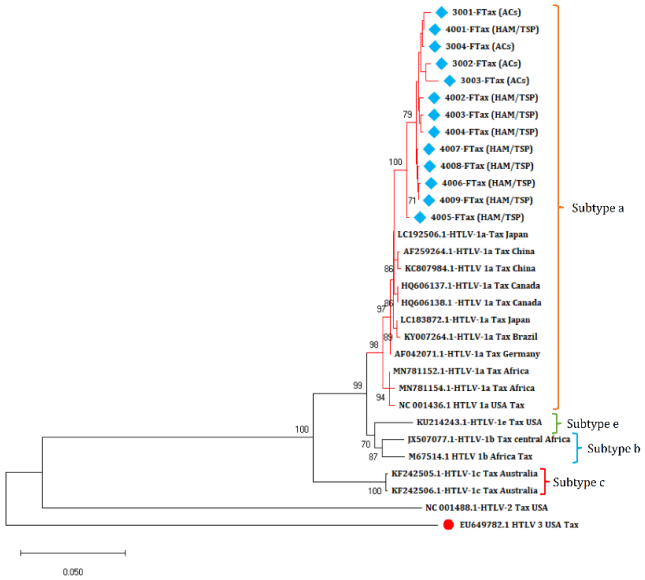

Fig. 1: Phylogenetic tree constructed with ML method in Kimora-2-parameter model. Different subtypes are noted in the tree. Iranian isolates are clustered in the subtype A clade. Out group of tree is HTLV-3 of USA

Mutational analysis

From the results of the mutational analysis, all collected samples showed evidence of positive selection pressure. Detailed information on the number of observed mutations is shown in Table 2. We found signatures in the mutation patterns that were specific to Iranian HTLV-1 samples, highlighted with gray boxes. Furthermore, we detected mutations that were specific to ACs groups, noted with red boxes. Moreover, HAM/TSP isolates showed unique mutations specific to them, shown with blue boxes (Fig. 2).

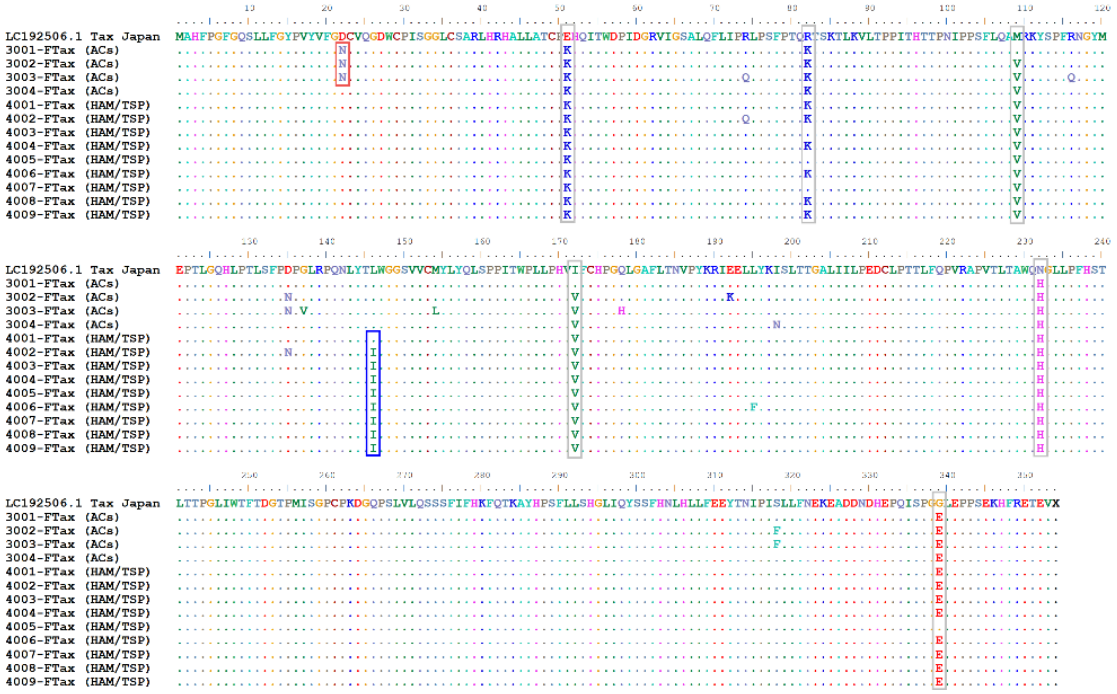

Fig. 2: Mutational analysis of the amino acid sequence of the Iranian HTLV-1 isolates with respect to the Japanese isolate

Table 2: Mutational signatures found in the ACs and HAM/TSP sequences

Isolate	Total	S.M	NS.M	Ratio	Type
3001-FTax (ACs)	10	5	5	1	Positive
3002-FTax (ACs)	13	4	9	2.25	Positive
3003-FTax (ACs)	18	7	11	1.57	Positive
3004-FTax (ACs)	11	4	7	1.75	Positive
4001-FTax (HAM/TSP)	11	5	6	1.2	Positive
4002-FTax (HAM/TSP)	13	5	8	1.6	Positive
4003-FTax (HAM/TSP)	11	5	6	1.2	Positive
4004-FTax (HAM/TSP)	12	5	7	1.4	Positive
4005-FTax (HAM/TSP)	6	1	5	5	Positive
4006-FTax (HAM/TSP)	12	4	8	2	Positive
4007-FTax (HAM/TSP)	10	4	6	1.5	Positive
4008-FTax (HAM/TSP)	11	4	7	1.75	Positive
4009-FTax (HAM/TSP)	11	4	7	1.75	Positive
Total	**149**	**57**	**92**	**1.61**	**Positive**

Discussion

In this study, the Tax segment was used to screen the phylogenetic tree, since there were reports that the Tax segment has undergone mutations that can affect the function of the coded protein. Moreover, Tax proteins can alter the functions of regulatory proteins via direct interactions and play and important role in replicating infected T cells continuously and uncontrollably, preventing DNA repair by affecting topoisomerase-I function, and regulating different cellular genes for overexpression. Therefore, Tax has an important impact on the process of cancer (20).

Phylogenetic analysis of this study revealed that all Iranian isolates were clustered as the Transcontinental (A) subgroup of the Cosmopolitan (a) subtype. Similarly, all the Iranian HTLV isolates were in genotype -1a (19, 21). Additionally, Japanese HTLV-1a was observed as the most similar sequence to Iranian isolates, suggesting that Iranian HTLV-1a may have originated from this endemic area (22). There are hypotheses that suggest how this virus was transmitted to Iran via pilgrims and travelers to the holy Muslim shrine, wars with attacking groups like Mongols, trade and Silk Road and African slaves (8, 23-25). The fact that Japanese sequences are more similar to Iranian may support the idea that the Silk Road and wars from the East are more probable to transmit the virus to Iran. Iran. Moreover, the Silk Road improved economic relations between countries, but it also caused the spread of diseases such as plague, smallpox, and HTLV-1 (20). The age of HLTV dates back to 700 years ago (15), suggesting that pilgrimages to the holy Muslim shrine can have a lower impact on transmission.

This study conducted mutational analysis on collected samples that revealed all the samples were under the pressure of positive selection with respect to the Japanese isolates. From 149 nucleotide acid mutations, 92 were nonsynonymous and 57 were observed to be synonymous, resulting in a $\frac{NS.M}{S.M}$ ratio of 1.61, indicating positive selection. The mutational analysis also provided information about mutations specific to the Iranian subtype. Iranian-specific mutations were observed at positions 51, 82, 109, 172, 232, and 339 in the amino acid sequence. Additionally, an ACs-specific mutation was detected at position 22 with a change from D to N. Furthermore, a mutation unique to the HAM/TSP sequence was found at position 146, with a change from L to I. These differences in mutation patterns between ACs and HAM/TSP may warrant further com-

prehensive analysis to investigate differences between active Tax proteins in these two groups.

Conclusion

We used 9 HAM/TSP and 4 ACs samples of the Tax segment of HTLV-1 for our comparative analysis. Both phylogenetic and mutational analyses were implemented to investigate the difference of these two groups based on the Tax protein. The results of the phylogenetic analysis illustrated that all of the Iranian HAM/TSP and ACs isolates belonged to the HTLV-1 subtype A. Furthermore, we found Japanese isolates as the most similar isolates to Iranian HTLV-1. Based on the amino acid sequence, all of the Iranian Tax proteins are under positive selection pressure with respect to Japanese isolates. Interestingly, we detect specific mutation patterns in the sequences. Positions 51, 82, 109, 172, 232, and 339 in the aa sequence have undergone mutations specific to Iranian HTLV-1; and in positions 22 and 146 aa we detected mutations unique to ACs and HAM/TSP, respectively.

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.

Acknowledgements

No financial support was received for this study.

Conflict of interest

The authors declare that there is no conflict of interests.

References

1. Gessain A, Barin F, Vernant JC, Gout O, Maurs L, Calender A, de Thé G (1985). Antibodies to human T-lymphotropic virus type-I in patients with tropical spastic paraparesis. *Lancet,* 2 (8452):407-10.
2. Kannian P, Green PL (2010). Human T Lymphotropic Virus Type 1 (HTLV-1): Molecular Biology and Oncogenesis. *Viruses,* 2 (9):2037-2077.
3. Nejmeddine M, Bangham CRM (2010). The HTLV-1 Virological Synapse. *Viruses,* 2 (7):1427-1447.
4. Boxus M, Willems L (2009). Mechanisms of HTLV-1 persistence and transformation. *Br J Cancer,* 101 (9):1497-501.
5. Li HC, Biggar RJ, Miley WJ, Maloney EM, Cranston B, Hanchard B, Hisada M (2004). Provirus load in breast milk and risk of mother-to-child transmission of human T lymphotropic virus type I. *J Infect Dis,* 190 (7):1275-8.
6. Okochi K, Sato H, Hinuma Y (1984). A retrospective study on transmission of adult T cell leukemia virus by blood transfusion: seroconversion in recipients. *Vox Sang,* 46 (5):245-53.
7. Grassmann R, Aboud M, Jeang KT (2005). Molecular mechanisms of cellular transformation by HTLV-1 Tax. *Oncogene,* 24 (39):5976-85.
8. Romanelli MG, Diani E, Bergamo E, Casoli C, Ciminale V, Bex F, Bertazzoni U (2013). Highlights on distinctive structural and functional properties of HTLV Tax proteins. *Front Microbiol,* 4:271.
9. Van Dooren S, Gotuzzo E, Salemi M, Watts D, Audenaert E, Duwe S, Ellerbrok H, Grassmann R, Hagelberg E, Desmyter J, Vandamme AM (1998). Evidence for a post-Columbian introduction of human T-cell lymphotropic virus [type I] [corrected] in Latin America. *J Gen Virol,* 79 (Pt 11):2695-708.
10. Vidal AU, Gessain A, Yoshida M, Tekaia F, Garin B, Guillemain B, Schulz T, Farid R, De Thé G (1994). Phylogenetic classification of human T cell leukaemia/lymphoma virus type I genotypes in five major molecular and geographical subtypes. *J Gen Virol,* 75 (Pt 12):3655-66.
11. Guimarães de Souza V, Lobato Martins M, Carneiro-Proietti ABdF, Januário JN, Ladeira RVP, Silva CMS, Pires C, Gomes SC, Martins

CdS, Mochel EG (2012). High prevalence of HTLV-1 and 2 viruses in pregnant women in São Luis, state of Maranhão, Brazil. *Rev Soc Bras Med Trop*, 45(2):159-62.

12. van Tienen C, van der Loeff MF, Peterson I, Cotten M, Holmgren B, Andersson S, Vincent T, Sarge-Njie R, Rowland-Jones S, Jaye A, Aaby P, Whittle H (2010). HTLV-1 in rural Guinea-Bissau: prevalence, incidence and a continued association with HIV between 1990 and 2007. *Retrovirology,* 7:50.
13. Gessain A, Cassar O (2012). Epidemiological Aspects and World Distribution of HTLV-1 Infection. *Front Microbiol,* 3:388.
14. Naderi M, Paryan M, Azadmanesh K, Rafatpanah H, Rezvan H, Mirab Samiee S (2012). Design and development of a quantitative real time PCR assay for monitoring of HTLV-1 provirus in whole blood. *J Clin Virol,* 53 (4):302-7.
15. Rahman S, Quann K, Pandya D, Singh S, Khan Z, Jain P (2012). HTLV-1 Tax Mediated Downregulation of miRNAs Associated with Chromatin Remodeling Factors in T Cells with Stably Integrated Viral Promoter. *PloS one,* 7:e34490.
16. Yamashiro T, Kamiya H, Miyara T, Gibo S, Ogawa K, Akamine T, Moromizato H, Yara S, Murayama S (2012). CT scans of the chest in carriers of human T-cell lymphotropic virus type 1: presence of interstitial pneumonia. *Acad Radiol,* 19 (8):952-7.
17. Macaire H, Riquet A, Moncollin V, Biémont-Trescol MC, Duc Dodon M, Hermine O, Debaud AL, Mahieux R, Mesnard JM, Pierre M, Gazzolo L, Bonnefoy N, Valentin H (2012). Tax protein-induced expression of antiapoptotic Bfl-1 protein contributes to survival of human T-cell leukemia virus type 1 (HTLV-1)-infected T-cells. *J Biol Chem,* 287 (25):21357-70.
18. Stoppa G, Rumiato E, Saggioro D (2012). Ras signaling contributes to survival of human T-cell leukemia/lymphoma virus type 1 (HTLV-1) Tax-positive T-cells. *Apoptosis,* 17 (3):219-28.
19. Rafatpanah H, Torkamani M, Valizadeh N, Vakili R, Meshkani B, Khademi H, Gerayli S, Mozhgani SH, Rezaee SA (2016). Prevalence and phylogenetic analysis of HTLV-1 in a segregated population in Iran. *J Med Virol,* 88 (7):1247-53.
20. Fujikawa D, Nakagawa S, Hori M, Kurokawa N, Soejima A, Nakano K, Yamochi T, Nakashima M, Kobayashi S, Tanaka Y, Iwanaga M, Utsunomiya A, Uchimaru K, Yamagishi M, Watanabe T (2016). Polycomb-dependent epigenetic landscape in adult T-cell leukemia. *Blood,* 127 (14):1790-802.
21. Safavi M, Habibian-Sezavar F, Letafati A, Solouki S, Yaslianifard S, Kaboli P, Mohammadzadeh M, Kabir K, Haj MS, Mozhgani SH (2024). Determination of molecular epidemiologic pattern of human T-lymphotropic virus type 1 (HTLV-1) in Alborz province, Iran. *Virus Genes*.
22. Pourrezaei S, Shadabi S, Gheidishahran M, Rahimiforoushani A, Akhbari M, Tavakoli M, Safavi M, Madihi M, Norouzi M (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.
23. Azran I, Schavinsky-Khrapunsky Y, Aboud M (2004). Role of Tax protein in human T-cell leukemia virus type-I leukemogenicity. *Retrovirology,* 1:20.
24. Hall WW, Fujii M (2005). Deregulation of cell-signaling pathways in HTLV-1 infection. *Oncogene,* 24 (39):5965-75.
25. Matsuoka M, Yasunaga J (2013). Human T-cell leukemia virus type 1: replication, proliferation and propagation by Tax and HTLV-1 bZIP factor. *Curr Opin Virol,* 3 (6):684-91.