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ORIGINAL ARTICLE

Investigating the effect of *MAP2K1* gene (MEK1) in MAPK pathway in the induction of adult T-cell leukemia/lymphoma (ATLL)

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ABSTRACT

Background and Objectives: HTLV-1 is responsible for two important diseases, HAM/TSP and ATLL. Approximately 10 to 20 million people are infected with HTLV-1 worldwide. Identifying altered genes in different cancers is crucial for finding potential treatment strategies. One of the proteins of the RAS/MAPK signaling pathway is MEK1, which is made from the *MAP2K1* gene. The effects of the *MAP2K1* gene on the MAPK signaling pathway are not yet fully elucidated. The current study aims to determine the *MAP2K1* gene mutations and the level of *MAP2K1* gene expression in ATLL patients compared to healthy individuals.

Materials and Methods: Ten ATLL and 10 healthy control individuals were investigated in this study. We used ELISA test to screen anti-HTLV-I antibodies and PCR for confirmation of infection. Then, we extracted total RNA from fresh whole blood, and cDNA was synthesized. The expression levels of the *MAP2K1* gene were examined by qRT-PCR, and to check possible mutations in the *MAP2K1* gene; all samples were sequenced and analyzed by BioEdite Software.

Results: *MAP2K1* gene expression in the ATLL group was significantly higher than in the healthy control (P=0.001). The mutational sequencing analysis showed nucleotide 212 (S→R) change and identification mutations at different nucleotides that were entirely different from the nucleotide mutations defined in the UniProt database.

Conclusion: These results could be a perspective in the prevention, prognosis, and targeted treatment of diseases in which the *MAP2K1* gene plays a vital role.

Keywords: Human T-lymphotropic virus type 1; HTLV-I-associated myelopathy; Tropical spastic paraparesis; Adult T-cell leukemia/lymphoma; Mitogen-activated protein kinase kinase 1; MAP kinase/ERK kinase

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INTRODUCTION

The discovery of human T-cell lymphotropic virus type 1 (HTLV-1) in 1980 identified the first human oncoretroviral (1, 2). HTLV-1 is known as a member of the *Retroviridae* family and belongs to the *Orthoretrovirinae* subfamily and the *Deltaretrovirus* genus. The HTLV-1 infection rate is estimated at 10 to 20 million individuals globally. The two serious diseases are HTLV-1-associated myelopathy/ tropical spastic paraparesis (HAM/TSP) and adult T-cell leukemia (ATLL) caused by HTLV-1. Adult T-cell leukemia (ATLL) is a T-cell malignancy and an aggressive lymphoproliferative disorder of mostly CD4 T-cells caused by HTLV-1 (1, 3, 4). Approximately 4 to 5% of HTLV-1 carriers experience ATLL disorder (3). In HAM/TSP, inflammatory reactions play an important role in affecting the corticospinal tracts. Moreover, irreversible paraparesis and chronic neuromyopathy are the result of these inflammatory reactions and can affect the lower organs (5). In addition, HTLV-1 is involved in the development of a variety of autoimmune diseases and several other inflammatory diseases, such as bronchiectasis, arthritis, infective dermatitis, myopathies, and multiple different uveitides (6, 7). HTLV-1 is transmitted in various routes such as blood transfusion, intravenous injection, sexual contact, vertical transmission from mother to baby, breastfeeding, and organ transplant (8, 9).

In cancer, genetic changes occur in specific genes that lead to the ability of cells to grow and divide uncontrollably. In the last decade, the systematic study of these genetic alterations has been facilitated by the possibility of DNA sequencing. Now a better understanding of the processes of the signaling pathways has been developed (10). Given that specific medication targets genetic changes, DNA sequencing plays an important role in routine clinical surveillance (11-14). Furthermore, in different tumors, the altered genes and pathways are very diverse, and accurate identification of the altered genes and pathways in different cancers is critical to explore therapeutic options. In various cancers, different significant signaling pathways such as phosphatidylinositol 3 kinase (PI3K)/ protein kinase B(Akt) signaling, Receptor Tyrosine Kinase (RTK)/ Ras GTPase/ MAP kinase (*MAP-Kinase*), etc. have been recognized in the process of genetic change. The frequency of gene alteration in key pathways is not equal, some specific genes frequently changed that can be identified in cancer, while some other genes are rarely or never changed (10).

Mitogen-activated protein kinase (MAPK) pathways play an essential and critical role in cancer initiation and development. Specifically, mutations in RAF and RAS proteins cause improper activation of MAPK pathways, and as a result, constitutive activation occurs in the extracellular signal-regulated protein kinase 1/2 (ERK1/2) pathway (15). In various cancers, including thyroid, neuroblastoma, breast, and non-small cell lung cancer, the MAPK pathway has been identified as a primary cell signaling pathway (16). The MEK1 protein kinase is made by providing instructions from the *MAP2K1* gene. This protein is one of the components of the RAS/MAPK signaling pathway, which is involved in the transmission of extracellular chemical signals into the nucleus (17). Nevertheless, a perfect understanding of the functions of *MAP2K1* gene regulation of oncogenesis and host cellular proteins is yet to be determined. Our knowledge of these signaling pathways faces several challenges. The exact functions of the *MAP2K1* gene and its effects on the MAPK signaling pathway are still unknown. On the other hand, the insufficient knowledge of possible mutations that may have occurred in the *MAP2K1* gene in the MAPK signaling pathway is important and controversial. In addition, no study has been conducted to measure the levels of *MAP2K1* gene expression in individuals with ATLL. To the best of our knowledge, no comprehensive study has yet investigated the possible mutations of the *MAP2K1* gene in the MAPK signaling pathway. This is the first study to examine the challenges in the functions of the *MAP2K1* gene in the oncogenic signaling pathways in ATLL. The present study aimed to understand the possible mutations in the *MAP2K1* gene in the MAPK signaling pathway of ATLL carcinogenesis. Also, this research investigates the expression levels of the *MAP2K1* gene in healthy control compared to patients with ATLL.

MATERIALS AND METHODS

Samples. A cross-sectional study was done from 2021 to 2022 on 20 samples. In this study, there were two investigated groups. The first group included 10 ATLL patients collected from Shariati Hospital, Teh-

ran, Iran., The inclusion criteria for this group were no autoimmune infectious or immune deficiency diseases, and the second group comprising 10 healthy control was collected from the Alborz Blood Transfusion Organization, Alborz, Iran. The inclusion criteria for this group were HTLV-1 negative, no autoimmune, infectious, or immune deficiency diseases. We utilize serological test enzyme-linked immunosorbent assay (ELISA, Diapro, Italy) (18, 19) to conduct the seropositive test for HTLV-1. The positive specimens were confirmed by the Polymerase chain reaction (PCR) technique (20, 21). This study was approved by the Ethical Committee of Tehran University of Medical Sciences, Tehran, Iran (IR.TUMS.SPH.REC.1400.090).

Quantitative real-time PCR. Total RNA was extracted from fresh whole blood utilizing TriPure isolation reagent following the manufacturer's instructions (Roche, Germany). DNase treatment was used to remove genomic DNA contamination for all purified RNA samples before constructing complementary DNA (cDNA). Subsequently, cDNA was synthesized utilizing RT-ROSET Kit (ROJE Technologies, Iran). According to the manufacturer's protocol, H_2O 6 µl, Master Mix 10 µl, Primer Forward 1 µl, Primer Revers 1 µl, cDNA Template 2 µl. SYBR Green-based (TaKaRa, Otsu, Japan), and then RT-qPCR was used according to the manufacturer's instructions.

The following primers were designed and utilized to determine the expression levels of MEK1: (forward primer: 5′- GGGACCAGCTCTGCGGAGA-3′, reverse primer: 5′- GCCCCCAGCTCACTGATCTTCT-3′) which amplified 185 bp. The optimum annealing temperature was 30 seconds at 61°C and the extension time was 25 seconds at 72°C, repeated in 42 cycles. The 5 standard curves real-time PCR was conducted on the cDNA samples using the Q-6000 machine (Qiagen, Germany). We utilized the RPLP0 as a housekeeping gene (22) to normalize the mRNA expression levels and to control the sampling error (23). The normalized value of the expression for each gene was calculated as the ratio of relative copies number of the mRNA of interest/relative mRNA copies number of reference gene, indicated as the expression index.

Statistical analysis. Analysis was done with GraphPad Prism Software Version 8.0.2 (GraphPad Software, Inc). The output was analyzed using the statistical Mann-Whitney U test for statistical differences between gene expressions. We considered p-Value <0.05 as significant.

Sequencing and mutational. The following primers were designed and used to PCR test for Full MEK1: (forward primer: 5′- GATCAGTGAGCTGGGGGCT-3′, reverse primer: 5′- CTGCTCTCTCTGCGGGGTTT-3′) which amplified 840 bp. The extension time was 40 seconds at 94°C, optimum annealing temperature was 35 seconds at 61°C and the extension time was 30 seconds at 72°C, repeated in 45 cycles.

All PCR products were sequenced bidirectionally using the specific MEK1 primer pairs. The raw sequence data were visualized and edited by the Clustal W program in BioEdit (24) software, we manually edited all acquired sequencing chromatograms to achieve contigs.

RESULTS

Demographic data. In this study, no significant differences were observed in the ages of the groups (25).

***MAP2K1* mRNA expression.** The mean *MAP2K1* mRNA expression level in the healthy control and ATLL was found as 0.006 ± 0.005 and 0.056 ± 0.042, respectively. The expression of the *MAP2K1* gene in ATLL was higher than in the healthy control (P=0.001).

Mutational analysis. All the samples were compared with the original sequence of the *MAP2K1* gene received from the NCBI database. We analyzed 280 amino acids. We used a uniport database of the *MAP2K1* gene to check mutation (Table 1), and all the mutations registered in this database were compared with the sequencing results of this study. In nucleotide 212, the S→R change was observed, while the mutation recorded in Uniport for this position was S→A, so the class of amino acids had changed (Fig. 1).

DISCUSSION

In this study, the *MAP2K1* gene expression level

Table 1. The registered mutations of the *MAP2K1* gene

Mutagenesis	Position	Description
K → A	97	Loss of catalytic function. Strongly reduce phosphorylation upon ultraviolet irradiation
K → R	97	Loss of catalytic activity. Exerts no effect on BRAF-KSR1 or BRAF-KSR2 dimerization
S → A	150	No loss of function.
S → A	212	No loss of function.
S → A	218	Loss of catalytic function. Exerts no effect on BRAF-KSR1 dimerization; when associated with A-222.
S → D	218	Exerts no effect on BRAF-KSR1 dimerization; when associated with D-222.
M → V	219	Can Increase interaction with BRAF and KSR1.
M → W	219	Can increase interaction with BRAF and KSR1; when associated with L-220.
A → L	220	Can increase interaction with KSR1 and BRAF; when associated with w-219.
N → Y	221	Can increase interaction with KSR1 and BRAF.
S → A	222	Loss of catalytic function. Exerts no effect on BRAF-KSR1 dimerization; when associated with A-218.
S → D	222	Exerts no effect on BRAF-KSR1 dimerization; when associated with D-218.
F → S	311	Loss of interaction with KSR1 and BRAF. Loss of BRAF-KSR1 dimerization.

was investigated. In addition, by sequencing samples (both ATLL patients and healthy controls), possible mutations in the *MAP2K1* gene were evaluated in both groups. The most important result obtained from this research was the increase in *MAP2K1* gene expression level in ATLL patients compared to healthy control subjects. In addition, an amino acid change at nucleotide position 212 (S→R) was detected.

According to the results of the present study and previous studies, although infrequently, *MAP2K1* gene mutations are involved in various forms of cancers (26). Identification of one mutation in *MEK1*, mainly, a heterozygous missense D67N substitution, was reported in 1 / 15 ovarian cancer cell lines (27). The Q56P alteration was detected in 1 / 85 NSCLC cell lines. In addition, between 207 primary tumor samples, one somatic K57N mutation of *MAP2K1* in two cases of NSCLC were identified (28). One somatic amino acid alteration (R201H or E203K) of the *MAP2K1* gene in two cases of 55 colorectal cancer cases was detected. Moreover, screening of 38 breast cancer cases revealed no *MAP2K1* mutations (29).

Interestingly, sequencing of our samples revealed the identification of mutations at different nucleotides that were completely different from the nucleotide mutations defined in the UniProt database. For instance, in nucleotides, position 85 (V→A), 94 (M→V), 107 (I→V), 130 (Y→C), and 136 (D→E) were detected. Thus, according to the findings of this study, the existence of novel *MAP2K1* mutations is also possible.

The findings of the present study are in line with the results of former studies that showed the key role of *MAP2K1* gene mutations in the progression of different types of cancers. For instance, between 172 samples or cell lines, mutations in the *MAP2K1* gene were observed in 3.5% of human epithelial cancers. One mutation (D67N) and four new mutations (P306H, R47Q, R49L, and I204T) were recognized in the *MAP2K1* gene (26). Among 7119 melanoma cases, small in-frame deletions were identified in 37 cases (0.5%), most likely caused by known or putative activation of the *MAP2K1* gene. E102_I103del, P105_A106del, Q58_E62del, I103_K104del, I99_K104del, L98_I103del, and E41_F53del were identified in 11, 8, 6, 5, 3, 3 and 1 cases respectively (30). Fifteen patients with melorheostosis were investigated. The obtained results showed *MAP2K1* gene mutation in 5/15 cases using whole exome sequencing (31). Eight cases of Langerhans cell histiocytosis were investigated by next-generation sequencing. It reported the identification of an E102_I103del mutation in the *MAP2K1* gene in a BRAF wild-type subject, which was subsequently confirmed definitively by the Sanger sequencing method (32).

Despite the obvious strengths of this study, it is suggested that in the following studies, protein quantitative measurement was used for confirmation of the results.

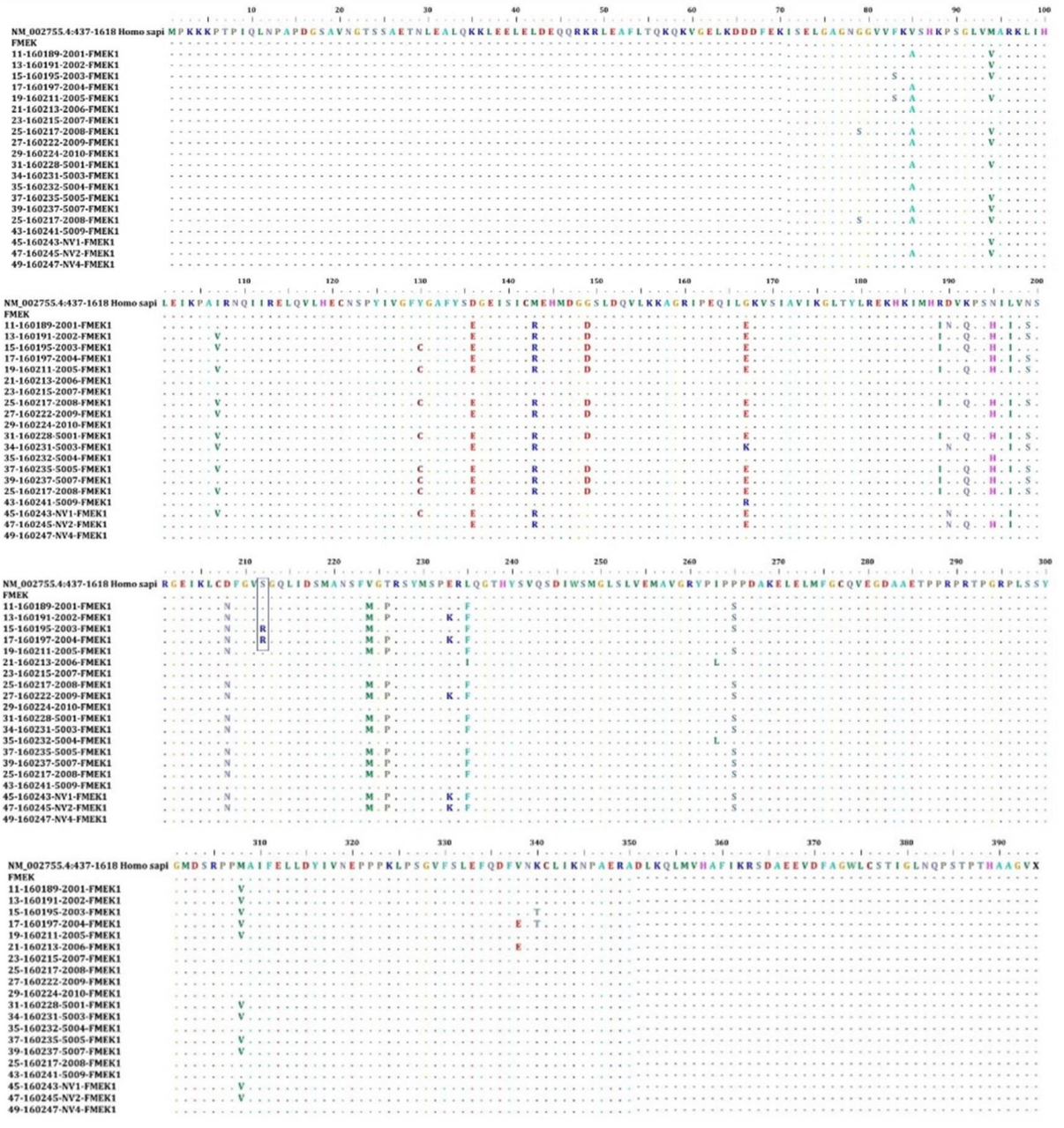

Fig. 1. Multiple sequence alignments (MSAs) of *MAP2K1* gene in the healthy and ATLL groups to show possible mutations. In nucleotide 212, the S→R change was observed, while the mutation recorded in Uniport for this position is S→A. Therefore, the class change of amino acid was observed.

CONCLUSION

The present study found a higher level of *MAP2K1* gene expression in patients with ATLL compared to healthy individuals. In addition, mutations that differed from *MAP2K1* gene data were discovered in the UniProt database. Therefore, it seems that more studies are needed to confirm these mutations. Considering the importance of ATLL in society, the obtained results can be a turning point in the prevention, prognosis, and efficient treatment of this disease and disorders in which the *MAP2K1* gene plays a key role.

REFERENCES

1. 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.
2. Mohanty S, Harhaj EW. Mechanisms of oncogenesis by HTLV-1 Tax. *Pathogens* 2020; 9: 543.
3. Miura M, Naito T, Saito M. Current perspectives in human T-cell leukemia virus type 1 infection and its associated diseases. *Front Med (Lausanne)* 2022; 9: 867478.
4. Mozhgani S-H, Piran M, Zarei-Ghobadi M, Jafari M, Jazayeri S-M, Mokhtari-Azad T, et al. An insight to HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP) pathogenesis; evidence from high-throughput data integration and meta-analysis. *Retrovirology* 2019; 16: 46.
5. Sato T, Yagishita N, Tamaki K, Inoue E, Hasegawa D, Nagasaka M, et al. Proposal of classification criteria for HTLV-1-associated myelopathy/tropical spastic paraparesis disease activity. *Front Microbiol* 2018; 9: 1651.
6. Martin F, Taylor GP, Jacobson S. Inflammatory manifestations of HTLV-1 and their therapeutic options. *Expert Rev Clin Immunol* 2014; 10: 1531-1546.
7. Hermine O, Ramos JC, Tobinai K. A review of new findings in adult T-cell leukemia–lymphoma: A focus on current and emerging treatment strategies. *Adv Ther* 2018; 35: 135-152.
8. Higuchi Y, Yasunaga J-i, Matsuoka M. HTLV-1's Foxy strategy for survival and transmission. *Front Virol* 2022; 1: 792659.
9. Kalinichenko S, Komkov D, Mazurov D. HIV-1 and HTLV-1 transmission modes: Mechanisms and importance for virus spread. *Viruses* 2022; 14: 152.
10. Sanchez-Vega F, Mina M, Armenia J, Chatila W, Luna A, La K, et al. Oncogenic signaling pathways in The Cancer Genome Atlas. *Cell* 2018; 173: 321-337. e10.
11. Hartmaier RJ, Albacker LA, Chmielecki J, Bailey M, He J, Goldberg ME, et al. High-throughput Genomic profiling of adult solid tumors reveals novel insights into cancer pathogenesis. *Cancer Res* 2017;77:2464-2475.
12. Schram AM, Berger MF, Hyman DM. Precision oncology: Charting a path forward to broader deployment of genomic profiling. *PLoS Med* 2017; 14(2): e1002242.
13. Sholl LM, Do K, Shivdasani P, Cerami E, Dubuc AM, Kuo FC, et al. Institutional implementation of clinical tumor profiling on an unselected cancer population. *JCI Insight* 2016; 1(19): e87062.
14. Zehir A, Benayed R, Shah RH, Syed A, Middha S, Kim HR, et al. Mutational landscape of metastatic cancer revealed from prospective clinical sequencing of 10,000 patients. *Nat Med* 2017; 23: 703-713.
15. Rovida E, Tusa I. Targeting MAPK in cancer 2.0. *Int J Mol Sci* 2022; 23: 5702.
16. Chilamakuri R, Agarwal S. Direct targeting of the raf-MEK-ERK signaling cascade inhibits Neuroblastoma growth. *Curr Oncol* 2022; 29: 6508-6522.
17. Wang W, Xu C, Wang D, Zhu Y, Zhuang W, Fang M, et al. P70. 05 The Association between *MAP2K1* mutation class and clinical features in *MAP2K1*-mutant East Asian non-small cell lung cancer patients. *J Thorac Oncol* 2021; 16: S564.
18. Ghobadi MZ, Emamzadeh R, Mozhgani S-H. Deciphering microRNA-mRNA regulatory network in adult T-cell leukemia/lymphoma; the battle between oncogenes and anti-oncogenes. *PLoS One* 2021; 16(2): e0247713.
19. Pourrezaei S, Shadabi S, Gheidishahran M, Rahimi-foroushani A, Akhbari M, Tavakoli M, et al. 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* 2021; 13: 509-517.
20. Rafatpanah H, Torkamani M, Valizadeh N, Vakili R, Meshkani B, Khademi H, et al. Prevalence and phylogenetic analysis of HTLV-1 in a segregated population in Iran. *J Med Virol* 2016; 88: 1247-1253.
21. Mozhgani S-H, Jahantigh HR, Rafatpanah H, Valizadeh N, Mohammadi A, Basharkhah S, et al. Interferon lambda family along with HTLV-1 proviral load, tax, and HBZ implicated in the pathogenesis of myelopathy/tropical spastic paraparesis. *Neurodegener Dis* 2018;18:150-5.
22. Aghamahdi F, Shafiee A, Rostami S, Mokhames Z, Safavi M, Yaslianifard S, et al. Comparative study of CNR1 and CNR2 cannabinoid receptors expression levels in COVID-19 patients with and without diabetes mellitus: Recommendations for future research targets. *Diabetes Metab Syndr* 2022; 16: 102499.
23. Ramezani S, Shirdel A, Rafatpanah H, Akbarin MM, Tarokhian H, Rahimi H, et al. Assessment of HTLV-1 proviral load, LAT, BIM, c-FOS and RAD51 gene expression in adult T cell leukemia/lymphoma. *Med Microbiol Immunol* 2017; 206: 327-335.
24. Meysami P, Alavian SM, Monavari SH, Mozhgani SH, Taghizadeh M, Farahani M, et al. Molecular characterization of hepatitis D virus genotypes circulating in Iran. *Future Virol* 2018;13: 265-273.
25. Soltani S, Mozhgani SH, Siri G, Emadi MS, Foroushani AR, Jazayeri SM, et al. High Expression of Inflammatory Cytokines and Chemokines in Human T-lymphotropic Virus 1-Associated Adult T-cell Leukemia/Lymphoma. *Jundishapur J Microbiol* 2022; 15(10): e132348.
26. Choi YL, Soda M, Ueno T, Hamada T, Haruta H, Yamato A, et al. Oncogenic *MAP2K1* mutations in human epithelial tumors. *Carcinogenesis* 2012; 33: 956-961.
27. Estep AL, Palmer C, McCormick F, Rauen KA. Mutation analysis of BRAF, MEK1 and MEK2 in 15 ovarian cancer cell lines: implications for therapy. *PLoS One* 2007; 2(12): e1279.

28. Marks JL, Gong Y, Chitale D, Golas B, McLellan MD, Kasai Y, et al. Novel MEK1 mutation identified by mutational analysis of epidermal growth factor receptor signaling pathway genes in lung adenocarcinoma. *Cancer Res* 2008; 68: 5524-5528.
29. Bentivegna S, Zheng J, Namsaraev E, Carlton VE, Pavlicek A, Moorhead M, et al. Rapid identification of somatic mutations in colorectal and breast cancer tissues using mismatch repair detection (MRD). *Hum Mutat* 2008; 29: 441-450.
30. Williams EA, Montesion M, Shah N, Sharaf R, Pavlick DC, Sokol ES, et al. Melanoma with in-frame deletion of MAP2K1: A distinct molecular subtype of cutaneous melanoma mutually exclusive from BRAF, NRAS, and NF1 mutations. *Mod Pathol* 2020; 33: 2397-2406.
31. Mohammadpour M, Najd Mazhar F, Rajei M, Razi S, Rashidi H, Hassanzdeh M. Melorheostosis in proximal phalanx and metacarpal bone: a short-mid-term follow up. *Mod Med Lab J* 2022; 5: 56-60.
32. Brown NA, Furtado LV, Betz BL, Kiel MJ, Weigelin HC, Lim MS, et al. High prevalence of somatic *MAP2K1* mutations in BRAF V600E–negative Langerhans cell histiocytosis. *Blood* 2014; 124: 1655-1658.