Original Investigation
Özgün Araştırma

Turkiye Parazitol Derg
DOI: 10.4274/tpd.galenos.2025.92400

Genetic Diversity Analysis of *Cysteine Proteinase B* Gene in *Leishmania major* Isolates from Southwest Iran

İran'ın Güneybatısındaki Leishmania major İzolatlarında Sistein Proteinaz B Geninin Genetik Çeşitlilik Analizi

Mahmoud Rahdar[1], Mohammad Reza Maghsoudi[2], Molouk Beiromvand[2], Tahereh Kardooni[2]

[1]Cellular and Molecular Research Center, Medical Basic Science Research Institute, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

[2]Ahvaz Jundishapur University of Medical Sciences Faculty of Medicine, Department of Parasitology, Ahvaz, Iran

Cite this article as: Rahdar M, Maghsoudi MR, Beiromvand M, Kardooni T. Genetic diversity analysis of *cysteine proteinase B* gene in *Leishmania major* isolates from southwest Iran. Turkiye Parazitol Derg. [Epub Ahead of Print]

ABSTRACT

Objective: Selecting an effective target for Leishmania vaccination requires identifying a protein antigen with low or no genetic diversity. The *cysteine proteinase B* (CPB) gene has emerged as a promising immunogenic target, though its diversity requires evaluation across different regions. Given Iran's status as a hyperendemic region for cutaneous leishmaniasis, this study aimed to collect and analyze 30 *Leishmania major* isolates from various areas of Khuzestan Province in Southwestern Iran.

Methods: The *CPB* gene was amplified via polymerase chain reaction, and its nucleotide sequence was determined. Sequence analysis was performed using MEGA5 software, with subsequent comparison to National Center for Biotechnology Information database entries. A phylogenetic tree was constructed to compare isolated strains with reference strains from other geographic regions and species.

Results: The phylogenetic analysis demonstrated that the *CPB* gene of the isolated strains in different regions of Khuzestan province formed a clade with certain *Leishmania major* strains from various parts of the world. Overall, the genotypic analysis of the *CPB* gene in Khuzestan province indicated genetic similarity among 29 isolates. In contrast, one sample from Dezful (north of Khuzestan Province) exhibited a significant difference from the reference strain, resulting in notable amino acid changes.

Conclusion: Based on these findings, the *CPB* gene holds promise as a potential candidate for vaccination development against cutaneous leishmaniasis in this region.

Keywords: *Leishmania major*, cysteine proteinase B, phylogeny

ÖZ

Amaç: Leishmania aşısı için etkili bir hedef seçimi, düşük veya sıfır genetik çeşitliliğe sahip bir protein antijenin belirlenmesini gerektirir. *Sistein proteinaz B* **(***CPB***)** geni, umut vaat eden bir immünojenik hedef olarak öne çıkmakla birlikte, çeşitliliğinin farklı bölgelerde değerlendirilmesi gerekmektedir. İran'ın kutanöz leishmaniasis için hiperendemik bir bölge olması nedeniyle, bu çalışma İran'ın Güneybatısındaki Huzistan Eyaleti'nin çeşitli bölgelerinden 30 *Leishmania major* izolatını toplamayı ve analiz etmeyi amaçlamıştır.

Yöntemler: *CPB* geni PCR yöntemiyle çoğaltıldı ve nükleotit dizisi belirlendi. Dizi analizi, MEGA5 yazılımı kullanılarak gerçekleştirildi ve sonrasında Ulusal Biyoteknoloji Bilgi Merkezi veritabanındaki dizilerle karşılaştırıldı. Coğrafi bölgeler ve türler arasındaki referans suşlarla izole edilen suşları karşılaştırmak üzere bir filogenetik ağaç oluşturuldu.

Bulgular: Filogenetik analiz, Huzistan Eyaleti'nin farklı bölgelerinden izole edilen suşların *CPB* geninin, dünyanın çeşitli bölgelerindeki *Leishmania major* suşlarıyla bir klad oluşturduğunu göstermiştir. Genotipik analizler, Huzistan Eyaleti'ndeki *CPB* geninin 29 izolat arasında genetik benzerlik sergilediğine işaret etmektedir. Buna karşılık, Dezful'dan (Huzistan Eyaleti'nin kuzeyi) elde edilen bir örnekte referans suşla belirgin farklılıklar gözlemlenmiş olup, bu durum amino asit düzeyinde kayda değer değişikliklerle sonuçlanmıştır.

Sonuç: Bu bulgulara dayanarak, *CPB* geninin bu bölgede kutanöz leishmaniasis'e karşı aşı adayı olarak potansiyel taşıdığı sonucuna varılmıştır.

Anahtar Kelimeler: *Leishmania major*, sistein proteinaz B, filogeni

Received/Geliş Tarihi: 23.10.2023 **Accepted/Kabul Tarihi:** 20.06.2025 **Epub:** 07.07.2025

Address for Correspondence/Yazar Adresi: Prof. MD., Mahmoud Rahdar, Cellular and Molecular Research Center, Medical Basic Science Research Institute, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
E-mail/E-Posta: mrahdar2002@yahoo.com **ORCID ID:** orcid.org/0000-0001-6667-7020

Türkiye PARAZİTOLOJİ Dergisi

INTRODUCTION

Leishmania major, the causative agent of cutaneous leishmaniasis, places nearly 10% of the global population at risk of infection. Annually, approximately two million new leishmaniasis cases are reported worldwide (1), with Iran representing a key endemic region where the disease constitutes a significant public health burden. The World Health Organization has prioritized leishmaniasis as an emerging and uncontrolled disease, prompting extensive efforts to develop effective vaccines, including the exploration of numerous candidate proteins (2).

Among these, cysteine proteinase B (CPB) stands out due to its critical role in parasite immune evasion (3). This 36-kDa protein, encoded by a 12,939-*nucleotide* gene on chromosome 8, is expressed in both promastigote (flagellated) and amastigote (non-flagellated) life cycle stages of Leishmania. CPB facilitates disease pathogenesis and serves as a key antigenic target. Phylogenetically, three cysteine proteinase types A, B, and C have been identified in Leishmania, with CPA and CPB homologous to mammalian Cathepsin-B, and CPC resembling Cathepsin-L (4).

Notably, CPB exhibits marked interspecies variability. For instance, *Leishmania mexicana* harbors 19 tandemly repeated *CPB* gene copies, while Leishmania donovani and Leishmania infantum possess five and one copies, respectively (5). Functional studies reveal that CPB deficiency in *L. mexicana* disrupts glycoprotein 63 (GP63) expression and impairs phagolysosomal activity (6). Furthermore, Rafati et al. (6) demonstrated that natural CPB isoforms in *Leishmania major* (*L. major*) elicit immune responses in human lymphocytes, infected murine models, and cutaneous leishmaniasis patients. Genetic polymorphisms in CPB contribute to phenotypic and genotypic diversity across Leishmania strains, underscoring its evolutionary plasticity (7).

Despite these advances, no provincial-level analysis of CPB genetic diversity has been conducted in Khuzestan, a hyperendemic tropical region in Iran. This study addresses this gap by performing molecular and genotypic analyses of CPB in *L. major* isolates from diverse geographic locations within the province. Identifying structural homogeneity in CPB across isolates would support its candidacy for region-specific vaccine development, offering a targeted strategy to mitigate cutaneous leishmaniasis in this high-risk population.

METHODS

Ethical Approval and Sample Collection

This study received ethical clearance from the Medical Ethics Committee (IR.TUMS.MEDICINE.REC.1400.014). Thirty *L. major* isolates were obtained from cutaneous leishmaniasis patients across five geographic subdivisions of Khuzestan Province (north, south, east, west, and central; six samples per region). Promastigotes were cultured in biphasic NNN medium supplemented with RPMI 1640 until reaching stationary phase, followed by cryopreservation at -20 °C. Genomic DNA extraction was performed on 3×10^6 promastigotes using the Qiagen DNA Mini Kit (catalog no. 51304, South Korea), with PBS-washed parasites processed according to manufacturer protocols. DNA concentration was quantified spectrophotometrically at 260 nm.

Primary Polymerase Chain Reaction (PCR)

Primary PCR amplification targeting kinetoplast DNA minicircles (kDNA) was conducted to *differentiate L. major* from *L. tropica*, utilizing a Sinaclone PCR Kit (Shiraz, Iran). The species-specific 620-bp amplicon was generated using primers:

Forward: 5'-GGGGTTGGTGTAAAATAGGG-3'

Reverse: 5'-TTTGAACGGGATTTCTG-3'

Reactions (25 µL total volume) contained 12.5 µL master mix, 2.5 µL primer pair (10 pmol/µL), 5 µL template DNA, and 5 µL DNase-free water. Thermocycling parameters included: Initial denaturation: 95 °C for 3 min

35 cycles of:

Denaturation: 93 °C for 40 s

Annealing: 63 °C for 40 s

Extension: 72 °C for 1 min

Final extension: 72 °C for 5 min

Amplification products were resolved on 2% agarose gels stained with ethidium bromide (0.5 µg/mL) and visualized under ultraviole illumination.

CPB Proliferation

CPB gene was used to design primers amplification 1100 bp nucleotide band. Primers included:

Forward: 5'-ACGGTCTTAGCGTGCGAGTTGTG-3'

Reverse: 5'-TCGTGCAGCACATGTCGCTTG-3'

PCR reactions (25 µL) comprised 12.5 µL master mix, 1 µL each primer (10 pmol/µL), 5 µL DNA template, and 5.5 µL DNase-free water. Thermal cycling conditions were:

5 minutes at 95 °C for initial denaturation and 35 cycles including 94 °C for 1 min, 57 °C 30 seconds for annealing, 72 °C 1 minute for extension. The PCR process was ended at 72 °C 10 min for final extension. The electrophoresis of PCR products was performed using gel agarose 2% and ethidium bromide, and the bands appeared by gel documentation apparatus.

Phylogeny Analysis

Sequence alignments and phylogenetic trees were constructed using MEGA5 software. Evolutionary relationships were inferred via the Neighbor-joining algorithm with Kimura 2-parameter distance modeling. Topological robustness was evaluated through 1,000 bootstrap replicates, with clade stability thresholds set at ≥70%. Nucleotide substitution patterns were analyzed under a gamma-distributed rate variation model (shape parameter α=0.5).

Statistical Analysis

Statistical analysis was performed using SPSS software, version 22 (SPCC Inc., Chicago, IL, USA). Descriptive analysis was used to calculate the mean and standard deviation and parametric tests (ANOVA) and non-parametric tests (Mann-Whitney and Wilcoxon) were used. Significant differences were considered at a p-value of less than 0.05.

Demographic data

The study cohort comprised 30 cutaneous leishmaniasis patients, with a male-to-female ratio of 12:18 (40% vs. 60%). Age distribution revealed a predominance of cases in the 21-30-year demographic, though infections spanned ages 2 to 80 years. Cutaneous lesions localized predominantly to the hands (21/30 cases, 70%), followed by facial involvement (9/30, 30%). Four

patients exhibited lesions at multiple anatomical sites, and 50% of cases presented with more than one lesion.

Primary PCR

Primary PCR amplification targeting kDNA minicircles confirmed *L. major* as the sole causative agent across all isolates, evidenced by a species-specific 620-bp amplicon (Figure 1). Electrophoretic differentiation from *L. tropica*, which produces an 800-bp fragment under identical conditions, excluded coinfection or misclassification. Subsequent amplification of the *CPB* gene yielded a conserved ~1,170 bp product in all isolates (Figure 2), confirming target gene integrity across geographically distinct *L. major* strains from Khuzestan Province.

Sequencing Analysis

Sequencing of 10 representative isolates (GenBank accessions OM037016-OM037025) revealed minimal nucleotide divergence from the *L. major* Friedlin reference strain, with one notable exception: Isolate OM037023 exhibited 14 nonsynonymous substitutions, resulting in substantial amino acid alterations within the CPB catalytic domain (Figures 3, 4). Clinically, infections involving OM037023 correlated with atypical presentations, including larger lesion sizes (>5 cm diameter), multidermatomal spread, and reduced responsiveness to antimonial therapy. The clinical changes in this study may be due to the presence of *Leishmanis* RNA virus 2 (LRV2). Çulha et al. (8) in Hatay-Türkiye failed to show LRV2 in 20 isolates of Leishmania included 17 *L. tropica*, 2 *L. major* and 1 *L. infantum*. Mirabedini et al. (9) in Iran, out of four isolates of *L. major*, three samples were positive for LRV2. The changes mentioned are described in Table 1.

Using the neighbour-joining method, the phylogenetic analysis of the gene encoding CPB DNA sequence was performed in *L. major*, along with the rest strains available in Genebank. The sequence patterns of the *CPB* gene in *L. major* isolated from patients in this study were compared to those of species in GenBank, demonstrating that all isolates, except for isolate number 8, exhibit a high degree of similarity with all isolates and standard Friedlin strain (Figure 5).

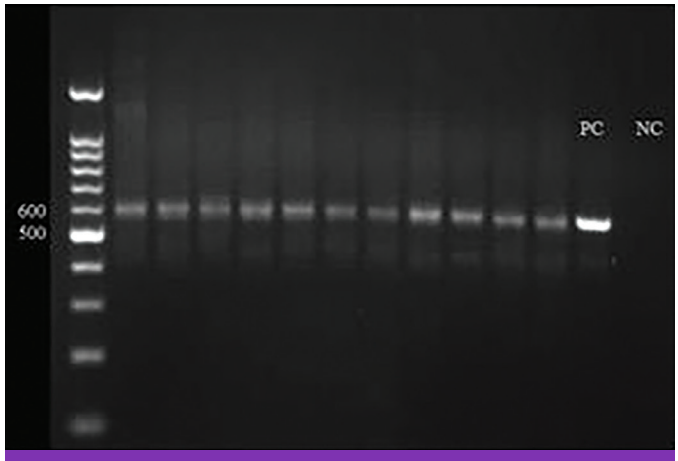

Figure 1. The electrophoresis of isolated *Leishmania major* show 620 bp nucleotides in PCR

PC: Positive control, NC: Negative control, PCR: Polymerase chain reaction

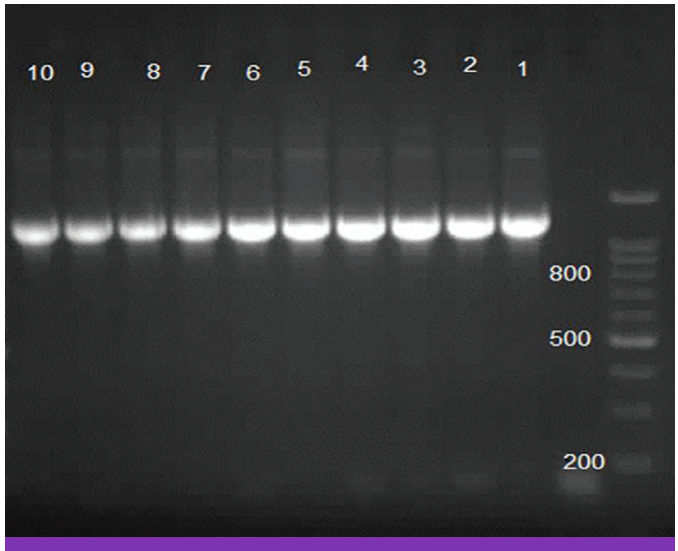

Figure 2. The electrophoresis of *cysteine proteinase B* gene PCR show a 1170 bp nucleotide in all isolated samples

PCR: Polymerase chain reaction

DISCUSSION

CPB constitutes a critical virulence factor in *Leishmania* spp., mediating parasite survival through differentiation roles in host cell invasion, immune evasion, and nutrient acquisition (10-12). Experimental validation of CPB's pathogenicity was demonstrated by Denise et al. (13), who observed attenuated cutaneous lesion

Figure 3. Nucleotide alignment of OM037023 isolate with Friedlin standard strain

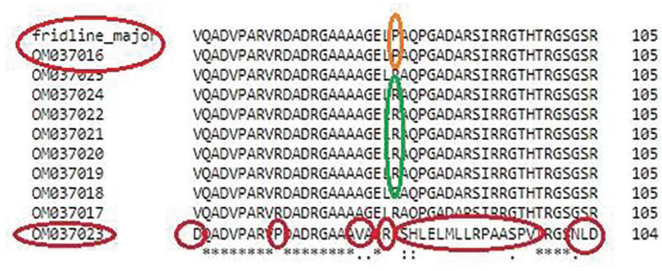

Figure 4. Amino acid alignment of OM037023 and other isolates with Friedlin standard strain

progression in BALB/c mice infected with CPB-deficient *L. mexicana* mutants. This phenotype correlated with enhanced Th1-polarized immunity (e.g., elevated IFN-γ), whereas *CPB* gene complementation restored lesion expansion and parasite proliferation via Th2 axis activation, evidenced by upregulated interleukin (IL)-4, immunoglobulin (Ig)G1, and IgE titers (13,14). Such immunological modulation underscores CPB's dual role as both a virulence effector and immunomodulator, motivating ongoing efforts to develop CPB-targeted chemotherapeutics and subunit vaccines (15,16).

Based on this, much attention has been paid to this enzyme for therapeutic purposes as well as vaccine production. Denise et al. (13) demonstrated that in Balb/C mice infected with Mexican *L. mexicana* strains with *CPB* gene disruption, disease severity was reduced, and induction of protective TH1 response led to weak expansion of cutaneous lesions. Reintroducing the *CPB* gene into the mutant strains increased lesion growth and parasite proliferation and led to TH2 axis activity, as evidenced by increased levels of IL4, IgG1, and IgE (13).

Prophylactic strategies leveraging CPB have shown promise in preclinical models. Doroud et al. (17) reported sustained protective immunity in BALB/c mice immunized with a CPB DNA vaccine adjuvanted with nanoparticles, manifesting as reduced lesion sizes and robust Th1 responses (IFN-γ/IL-5 ratio >3.2) compared to controls.

The existence of homogeneity with low diversity in the *CPB* gene in *Leishmania* species gives hope that the production of an immunogenic vaccine against the *CPB* gene can be used against all *Leishmania* species. Still, due to the molecular diversity of the CPB enzyme in different *Leishmania* species, it is hindered for this purpose. Gomes et al. (18) showed that the supply of *CPB* gene is different in various developmental stages of the parasite and identified stage-specific CPB expression dynamics, with promastigotes producing 1.5-fold higher protein levels than amastigotes, modulated by environmental pH and temperature fluctuation.

Saadati et al. (19) were able to isolate *L. donovani* from 3477 mosquitoes. Examining the *CPB* gene in this parasite species showed the difference between the genotypes of this gene in *L. donovani* and other species of *Leishmania*; therefore, they concluded that one type of vaccine could not be used to protect this parasite from other species. Mohammadpour et al. (20) in Fars province examined the *CPB* gene in 100 clinical samples of *Leishmania* cutaneous and showed many differences in the *CPB* gene sequence in clinical samples. Their results are different from the present study, which showed that only one of the 10 sequenced samples had genotypic and clinical differences with other isolates, proving the importance of making vaccines based on local species. The Chaouch et al. (5) study showed that the sequences of *L. tropica* and *L. major* have the least diversity, and intraspecies nucleotide polymorphisms largely lead to silent mutations, especially in the case of *L. major*. However, in *L. donovani,* there are many differences as a result of the deletion of a 39 nucleotide segment in the cysteine proteinase genotype (5).

In the present study, out of the ten samples examined in the nucleotide sequence, nine isolates were similar to the Friedline standard strain, except in a few areas that led to changes in the nucleotide sequence, and no differences were observed, and these changes did not lead to amino acid changes. Therefore, there was no change in the parasite's behaviour. Only in sample no. OM037023, significant nucleotide sequence alterations were

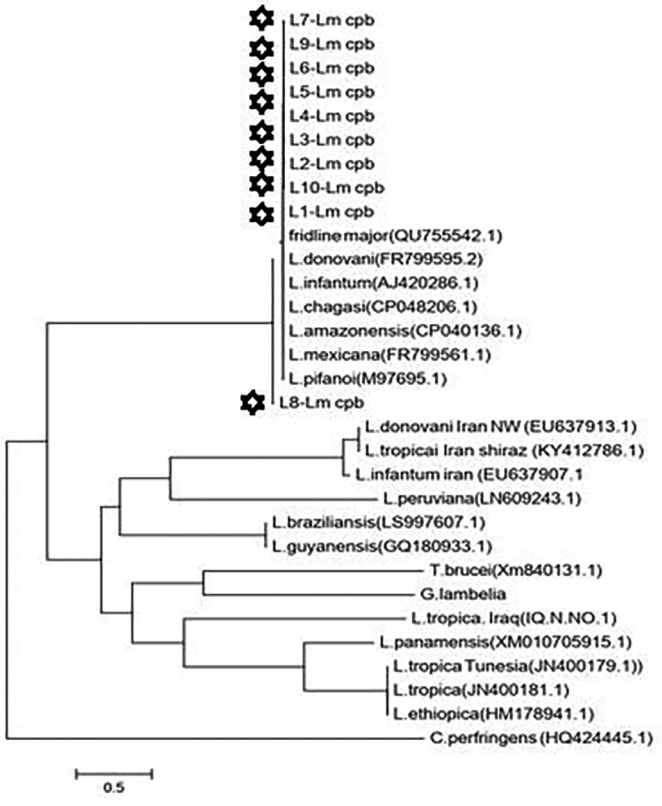

Figure 5. The phylogeny tree of studied isolates in comparison to other species of *Leishmania* in gene bank.*. Studied isolates

Table 1. Nucleotide and amino acid change in OM037023 isolate in comparison with Friedlin standard strain

Position in nucleotide sequence	Nucleotide		Amino acid	
	From	To	From	To
	G	C	Alanine	Proline
58	T	C		Stop codon
59	A	T		Stop codon
182	T	A	Valine	Asparagine
209	G	C	Arginine	Proline
236	C	T	Alanine	Valine
237	T	G	Alanine	Valine
	G	C	Glycine	Alanine
243	A	G	Without change	Without change
245	T	G	Leucine	Arginine
250	T	G	Alanine	Serine
257	C	T	Proline	Leucine
258	T	G	Proline	Leucine
260-272			Amino acids changed	
280-289			Amino acids changed	
307-318			Amino acids changed	

identified when compared to the Friedline reference strain and other samples isolates. These genetic modifications induced substantial amino acid substitutions, resulting in altered parasite pathogenicity manifested through increased lesion severity, prolonged disease duration, and reduced therapeutic efficacy. This divergence parallels reports of LRV2-associated hyperpathogenicity, though LRV2 detection protocols were not explicitly performed here. The observed genetic homologous in the *CPB* gene of *L. major* highlights its potential utility as a molecular target for region-specific vaccine development.

However, the outlier OM037023 underscores the necessity for pan-regional genomic surveillance to capture emergent virulent strains. Future studies should integrate proteomic profiling to quantify CPB expression variability across parasite life stages and assess cross-reactivity of CPB-directed antibodies against heterologous *Leishmania* species.

CONCLUSION

Vaccination in Leishmaniasis is the important and safty method for controlling the disease in endemic areas. To select of appropriated target for candidate vaccine need to identification and characterization of immunogenic and homologous gene or proteines. This study presented that *CPB* gene and protein are suitable target for designing an effective vaccine in this region. Further study is needed for evaluation LRV2 detection gene in isolated samples and increasing sample size for reaching to confidental results.

*Ethics

Ethics Committee Approval: This study received ethical clearance from the Medical Ethics Committee (IR.TUMS.MEDICINE.REC.1400.014).

Informed Consent: Informed consent was obtained from all patients to participate in this study, and this study received approval from the Research Ethics Committee.

Acknowledgments

This paper has been extracted from the M.Sc thesis of Mohammad Reza Maghsoudi and financially supported by Cellular and Molecular Research Center, Medical Basic Science Research Institute, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran as grant number CMRC-0002.

Footnotes

*Authorship Contributions

Surgical and Medical Practices: M.R.M., M.B., Concept: M.R., Design: M.R., Data Collection or Processing: M.R.M., Analysis or Interpretation: T.K., Literature Search: M.R., Writing: M.R.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: This manuscript is financially supported by Cellular and Molecular Research Center, Medical Basic Science Research Institute, as grant number CMRC-0002.

REFERENCES

1. Ashwin H, Sadlova J, Vojtkova B, Becvar T, Lypaczewski P, Schwartz E, et al. Characterization of a new Leishmania major strain for use in a controlled human infection model. vvvNat Commun. 2021; 12: 215.
2. Farash BRH, Shamsian SAA, Mohajery M, Fata A, Sadabadi F, Berenji F, et al. Changes in the epidemiology of cutaneous leishmaniasis in Northeastern Iran. Turkiye Parazitol Derg. 2020; 44: 52-7.
3. Mahmoudzadeh-Niknam H, McKerrow JH. Leishmania tropica: cysteine proteases are essential for growth and pathogenicity. Exp Parasitol. 2004; 106: 158-63.
4. Judice WAS, Ferraz LS, Lopes RM, Vianna LDS, Siqueira FDS, Di Iorio JF, et al. Cysteine proteases as potential targets for anti-trypanosomatid drug discovery. Bioorg Med Chem. 2021; 46: 116365.
5. Chaouch M, Fathallah-Mili A, Driss M, Lahmadi R, Ayari C, Guizani I, et al. Identification of Tunisian *Leishmania* spp. by PCR amplification of cysteine proteinase B (cpb) genes and phylogenetic analysis. Acta Trop. 2013; 125: 357-65.
6. Rafati S, Nakhaee A, Taheri T, Taslimi Y, Darabi H, Eravani D, et al. Protective vaccination against experimental canine visceral leishmaniasis using a combination of DNA and protein immunization with cysteine proteinases type I and II of L. infantum. Vaccine. 2005; 23: 3716-25.
7. Zadeh-Vakili A, Taheri T, Taslimi Y, Doustdari F, Salmanian AH, Rafati S. Immunization with the hybrid protein vaccine, consisting of leishmania major cysteine proteinases type I (CPB) and type II (CPA), partially protects against leishmaniasis. Vaccine. 2004; 22: 1930-40.
8. Çulha G, Kaya T, Özpınar N. Investigation of leishmania RNA virus 2 (LRV2) in cutaneous leishmaniasis strains isolated from Hatay, Turkey. Iran J Parasitol. 2022; 17: 436-9.
9. Mirabedini Z, Mirjalali H, Kazemirad E, Khamesipour A, Samimirad K, Koosha M, et al. The effects of leishmania RNA virus 2 (LRV2) on the virulence factors of L. major and pro-inflammatory biomarkers: an *in vitro* study on human monocyte cell line (THP-1). BMC Microbiol. 2023; 23: 398.
10. Klemba M, Goldberg DE. Biological roles of proteases in parasitic protozoa. Annu Rev Biochem. 2002; 71: 275-305.
11. Rosenthal PJ. Proteases of protozoan parasites. Adv Parasitol. 1999; 43: 105-59.
12. Sajid M, McKerrow JH. Cysteine proteases of parasitic organisms. Mol Biochem Parasitol. 2002; 120: 1-21.
13. Denise H, McNeil K, Brooks DR, Alexander J, Coombs GH, Mottram JC. Expression of multiple CPB genes encoding cysteine proteases is required for leishmania mexicana virulence *in vivo*. Infect Immun. 2003; 71: 3190-5.
14. Buxbaum LU, Denise H, Coombs GH, Alexander J, Mottram JC, Scott P. Cysteine protease B of leishmania mexicana inhibits host Th1 responses and protective immunity. J Immunol. 2003; 171: 3711-7.
15. McKerrow JH, Engel JC, Caffrey CR. Cysteine protease inhibitors as chemotherapy for parasitic infections. Bioorga Med Chem. 1999; 7: 639-44.
16. Freitas PG, Castilho TE, Almeida LB, MacielRezende CM, Caffarena ER. An *in silico* study of benzophenone derivatives as potential non-competitive inhibitors of *Trypanosoma cruzi* and leishmania amazonensis cysteine proteinases. Braz Chem Soc. 2018; 29: 515-27.
17. Doroud D, Zahedifard F, Vatanara A, Taslimi Y, Vahabpour R, Torkashvand F, et al. C-terminal domain deletion enhances the protective activity of cpa/cpb loaded solid lipid nanoparticles against leishmania major in BALB/c mice. PLoS Negl Trop Dis. 2011; 5: e1236.
18. Gomes CB, Silva FS, Charret KD, Pereira BA, Finkelstein LC, Santos-de-Souza R, et al. Increasing in cysteine proteinase B expression and enzymatic activity during *in vitro* differentiation of Leishmania (Viannia) braziliensis: first evidence of modulation during morphological transition. Biochimie. 2017; 133: 28-36.
19. Saadati M, Nazarian S, Barati B, Mehdizadeh H, Shirzad HS, Sadeghizadeh M. Detection and identification of Leishmania parasites within sand flies using kDNA, rDNA and CPB loci. Pathobiology Research. 2008; 11: 87.
20. Mohammadpour I, Hatam GR, Handjani F, Bozorg-Ghalati F, PourKamal D, Motazedian MH. Leishmania cytochrome b gene sequence polymorphisms in southern Iran: relationships with different cutaneous clinical manifestations. BMC Infect Dis. 2019; 19: 98.