Turkiye Parazitol Derg 2021;45(2):83-87
DOI: 10.4274/tpd.galenos.2020.6983

Expression of Hexokinase in the Proteome Profile of *Leishmania major* and *Crithidia*

Leishmania'nın Proteom Profilinde Hekzokinaz İfadesi

Mohammadreza Karimazar[1], Qasem Asgari[1], Sajad Rashidi[1], Mostafa Rezaei Tavirani[2], Mohammad Hossein Motazedian[1]

[1]Shiraz University of Medical Sciences Faculty of Medicine, Department of Parasitology and Mycology, Shiraz, Iran
[2]Shahid Beheshti University of Medical Sciences, Proteomics Research Center, Tehran, Iran

Cite this article as: Karimazar M, Asgari Q, Rashidi S, Tavirani MR, Motazedian MH. Expression of Hexokinase in the Proteome Profile of *Leishmania major* and Crithidia. Turkiye Parazitol Derg 2021;45(2):83-87.

ABSTRACT

Objective: The relationship between drug resistance and the expression of hexokinase (HK) has been indicated in leishmaniasis. According to the prolonged treatment period in cutaneous leishmaniasis (CL) patients co-infected with *Crithidia* in Iran, this study aims to investigate the expression of HK in the proteome of *Leishmania major* and *Crithidia* using a proteomic approach.

Methods: A total of 205 samples were removed from the lesions of patients in Fars province, Iran, for the characterization of *L. major* and *Crithidia* using polymerase chain reaction (PCR). After protein extraction, two-dimensional gel electrophoresis was employed for protein separation. Several spots were isolated for HK determination in the proteomes of *L. major* and *Crithidia* using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI TOF/TOF MS).

Results: The PCR results showed 5 positive cases for *Crithidia* and 96 positive cases for *L. major*. MALDI TOF/TOF MS indicated HK as a common protein in the proteome of *L. major* and *Crithidia*. HK was up-regulated in the *Crithidia* proteome in comparison with the *L. major* proteome.

Conclusion: Since a relationship between HK expression and drug resistance has been indicated in leishmaniasis, the overexpression of HK in *Crithidia* might be related to the increased duration of the treatment period in CL patients co-infected with *Crithidia*.

Keywords: *Leishmania major*, *Crithidia*, proteomics, hexokinase, drug resistance

ÖZ

Amaç: İlaç direnci ile hekzokinaz (HK) ekspresyonu arasındaki ilişki leishmaniasiste gösterilmiştir. Bu çalışmada, İran'da *Crithidia* ile enfekte olan kutanöz leishmaniasis (KL) ile takipli hastalarda uzun tedavi süresine göre, HK'nin *Leishmania major* proteomunda gösterilmesi ve *Crithidia*'nın proteomik bir yaklaşım kullanarak ekspresyonunun araştırılması amaçlanmıştır.

Yöntemler: Polimeraz zincir reaksiyonu (PZR) kullanılarak *L. major* ve *Crithidia*'nın karakterizasyonu için İran'ın Fars ilindeki 205 hastadan lezyon örnekleri toplanmıştır. Protein ekstraksiyonundan sonra protein ayrılması için iki boyutlu jel elektroforezi kullanıldı. MALDI TOF/TOF MS kullanılarak *L. major* ve *Crithidia* proteomunda HK'yi bulmak için birkaç nokta izole edildi.

Bulgular: PCR sonuçlarına göre *Crithidia* pozitif 5 olgu ve *L. major* pozitif olan 96 olgu mevcuttu. MALDI TOF/TOF MS, HK'yi *L. major* ve *Crithidia* proteomunda ortak bir protein olarak ortaya koymaktadır. HK, *Crithidia* proteomunda *L. major* proteom ile karşılaştırıldığında yukarı doğru düzenlenmiştir.

Sonuç: Leishmaniaziste HK ekspresyonu ve ilaç direnci ilişkisi gösterildiğinden, *Crithidia*'da HK'nin aşırı ekspresyonu, *Crithidia* ile birlikte enfekte olan KL hastalarında tedavi süresinin artmasıyla ilişkili olabilir.

Anahtar Kelimeler: *Leishmania major*, *Crithidia*, proteomiks, heksokinaz, ilaç direnci

INTRODUCTION

Leishmaniasis is one of the endemic diseases in the tropical and subtropical regions of the world which is caused by protozoan parasites of the genus *Leishmania*. According to the World Health Organization data, endemic leishmaniasis has been reported in 98 countries on five continents. *Leishmania major* is considered one of the main agents of cutaneous leishmaniasis (CL) in the world. Iran is an endemic region for CL and it is estimated that the annual incidence of CL is 20,000-60,000 in this country (1-4). *Crithidia* species (flagellate protozoa) belongs to the order of lower *Trypanosomatida*. Different orders of insects including *Diptera*, *Hemiptera*, and *Hymenoptera* are considered as possible vectors for these flagellated protozoa (5). In comparison to the *Leishmania* genus

Received/Geliş Tarihi: 31.10.2020 **Accepted/Kabul Tarihi:** 11.11.2020

Address for Correspondence/Yazar Adresi: Mohammad Hossein Motazedian, Shiraz University of Medical Sciences Faculty of Medicine, Department of Parasitology and Mycology, Shiraz, Iran
Phone/Tel: +00987132305291 **E-mail/E-Posta:** motazedm@sums.ac.ir **ORCID ID:** orcid.org/0000-0003-0117-9666

Türkiye PARAZİTOLOJİ Dergisi

(heterogeneous parasites), *Crithidia* spp. are considered single-host parasites (5).

Recently, the co-infection of *L. major* and *Crithidia* has been reported in CL patients in Iran (4). Clinical investigations have indicated the increased duration of the treatment period in CL patients co-infected with *Crithidia* (4). According to the obtained gene sequencing data, the sequence of the *internal transcribed spacer 1* (*ITS1*) gene in *L. major* has shown a 10% similarity to *Crithidia fasciculata* (6-8). The presence of similar genes in *L. major* and *Crithidia* genomes suggests the expression of common functional proteins involved in the pathogenicity and treatment of these parasites (6).

In comparison with the genomic data, proteomic data reveal more details regarding the structure and the function of the expressed proteins in the pathogens and diseases. The different aspects of *Leishmania* parasites have been investigated using proteomic studies in recent years; however, there is no information regarding the proteome of the *Crithidia* spp. (9-11). The use of proteomics concerning the involved proteins in drug resistance in leishmaniasis provides further information in the treatment of leishmaniasis in the future.

The relationship between drug resistance and the expression of the hexokinase (HK) has been indicated in leishmaniasis. According to the prolonged the treatment period in CL patients co-infected with *Crithidia* in Iran, this study was conducted to investigate the possible expression of the HK in the proteome of *Leishmania major* and *Crithidia* using a proteomic approach and two-dimensional gel electrophoresis (2-DE).

METHODS

Ethical Approval

All human specimens were obtained from the CL patients with the approval of the ethical committee of Shiraz University of Medical Sciences, Shiraz, Iran (IR.SUMS.REC.1395.S1).

Samples Collecting

Totally, in this study, 205 patients were selected. In the first step, the important criterion for patient selection was the observation of cutaneous lesion in individuals. All patients had one or more lesions on their hand, leg, and face. Two hundred five samples were taken from the lesions of the patients from Marvdasht and Kharameh cities, Fas province, Iran. Other significant clinical manifestations were not seen. After sample collecting, all samples were checked for *L. major* and *Crithidia* using microscopic, cultivation, and polymerase chain reaction (PCR) methods. The ages of the patients ranged from 6 months to 70 years old.

Samples Cultivation

The obtained samples were transferred to the Novy-MacNeal-Nicolle (NNN) media in sterile conditions. After the growth of the promastigote in NNN, the promastigotes were transferred to RPMI-1640 (Shelmax Company, China) supplemented with 10% (v/v) heat-inactivated fetal calf serum, 100 µg/mL streptomycin and 100 U/mL penicillin at 25 °C and mass-cultivated for obtaining the logarithmic growth phase of the parasites.

Characterization of the *L. major* and *Crithidia*

Genus identification

The cultivated promastigotes were characterized by PCR for finding and confirming the presence of *Leishmania* and *Crithidia* spp. The positive controls of *L. major* (MHOM/TM/1973/5ASKH) and *Crithidia* spp. were provided by the Department of Parasitology and Mycology, Shiraz University of Medical Sciences, Shiraz, Iran (4).

DNA extraction

In the first step, the DNA of the cultivated promastigotes (*Leishmania* and *Crithidia*) was extracted using a commercial kit (QIAGEN 28106, USA).

PCR

Specific primers were designed using Kinetoplastid Genomics Resource (TriTrypDB) (http://tritrypdp.org/tritrypdp) and based on the genomic sequence of the Glyceraldehyde-3-phosphate dehydrogenase in *L. major* and *Crithidia*. Used primers (Forward: 5'ATGGTCAAAGTGGGCATTAACGG3' and Reverse: 5'TCCATGTGCGAGGACAACGTGCT3') were able to characterize the *Leishmania* and *Crithidia* spp. (4,12). The amplification program was set to start denaturation at 94 °C for 3 min; followed by 10 cycles each at 95 °C for 30 sec, 62 °C for 30 sec, and 72 °C for 45 sec and a final extension stage at 72 °C for 5 min (4,12).

Agarose-gel electrophoresis

5 µL of loading buffer (3X) (Ampliqon Company, Cat. No. A608304) was added to 10 µL of the final PCR products. Then, PCR products were run to electrophoresis in 1.5% agarose gel. After running, the gels were visualized under ultraviole light with ethidium bromide.

Protein extraction

The cultivated promastigotes of the *L. major* and *Crithidia* were centrifuged at 3.000×g at 4 °C for 20 min. The obtained pellets were washed 3 times with PBS (pH: 7.2-7.4), each time at 3.000×g in 4 °C for 10 min. For protein extraction, 10 cc of the extraction buffer (acetone solution containing 10% TCA) was added to the obtained pellets and were kept for 1 h at-20 °C for protein precipitation. In the next step, the tubes were centrifuged at 17.500×g in 4 °C for 15 min. Then, the obtained pellets were dissolved in an acetone solution containing dithiothreitol (DTT) for 1 h at-20 °C for more protein precipitation (13). After centrifugation at 12.000×g at 4 °C, the precipitated proteins were dissolved in a lysis buffer (9.5 M urea, 2 M thiourea, 2% CHAPS, 8% immobilized pH gradient (IPG) buffer (pH 3-10) (GE Healthcare, Uppsala, Sweden) and stored at -70 °C. The protein concentration was measured by using the Bradford method.

2-DE

For each experiment, 250 µgr of extracted protein was developed in the first dimension on the IPG strips (18 cm, pH=3-10, GE healthcare) and the rehydration was done overnight at 50 V. Rehydration solution contained 8 M urea, 2% CHAPS, 50 mM DTT, and 0.5% IPG buffer. After rehydration, isoelectric focusing (IEF) (first dimension) was performed at 50-55.000 Vh using the protean IEF cell (Bio-Rad). Then the strips were equilibrated for 15 min in equilibration buffer (50 mM Tris-HCl, pH 8.8, 6 M urea, 30% glycerol, and 2% SDS) containing 65 mM DTT which was followed by a 15 min incubation in equilibration buffer

containing 135 mM iodoacetamide. In the second dimension, the equilibrated strips were sealed to the top of the 12% SDS-PAGE using a twin gel apparatus (Sci Plus/15 mA/gel) until the tracking dye reached the bottom of the gel (14). All experiments were performed in triplicate for confirming the results.

Gel staining, imaging, and image analysis

After fixation of the 2-DE gels with fixation buffer (5% acetic acid, 30% ethanol), the gels were stained with silver staining method (silver stain solution: 12.5 mL of 1 N silver nitrate solution per liter, development solution: 30 g anhydrous potassium carbonate, 250 µL of 37% formaldehyde and 125 µL of 10% thiosulfate solution per liter) (15). After washing, the gels were scanned using GS-800 calibrated densitometer (Bio-Rad) and were analyzed using the progenesis same spot software (version 4.1). Matching and analysis were performed with both automatic and manual methods.

In-gel digestion of protein samples, MS, and database search

In-gel digestion of protein spots and MALDI TOF/TOF MS analysis were done by the metabolomics and proteomics lab technology facility, Department of Biology, University of York. MALDI TOF/TOF MS was performed on a Bruker Autoflex Mass Spectrometer. The punched gels were placed into the wells of a ZipPlate (Montage In-Gel-DigestZP Kit, Millipore). The proteins in the punched gels were destained, digested with trypsin (Promega), extracted, purified on a C18 reverse phase matrix, and eluted in 8 µL of 60% acetonitrile, 0.1% trifluoroacetic acid (TFA). MALDI TOF/TOF MS was done on a BrukerAutoflex Mass Spectrometer (BrukerDaltonics, Bremen). Measurements were done in the reflection mode, using Ion source 1 voltage: 19 kV; ion source 2 voltages: 16.5 kV; reflector voltage 20 kV; lens voltage 8 kV; 40 ns pulse time; 120 ns pulse extraction time; and matrix suppression <500 Da. Using the Xtof analysis software package, version 5.1.5 (BrukerDaltonics), all spectra were analyzed. Using a mix of peptides (Sigma-Aldrich), the mass spectra we calibrated (9,16). Obtained masses generated by MALDI TOF/TOF MS were searched in the MASCOT program. MASCOT scores more than 62 were significant ($p<0.05$) for obtained proteins.

Statistical Analysis

Statistical analysis was not applicable in this study.

RESULTS

PCR

For identification of the parasites' genus, PCR was performed on the cultivated samples (promastigotes). PCR amplification and electrophoresis results for *L. major* and *Crithidia* have been shown in Figure 1, 2, respectively. In Figure 1, positive control and protein ladder have been indicated as Figure 1 a, b, respectively. Samples that produced a 760 bp band were positive for *L. major* (Figure 1 c, d) (e: negative control, f: positive sample with a weak band). In Figure 2, samples that illustrated a band in 858 bp were positive for *Crithidia* spp. Protein ladder (Figure 2 a), positive control (Figure 2 b), positive samples for *Crithidia* spp. (Figure 2 c-e), and negative control (Figure 2 f) have been represented in Figure 2. Generally, the PCR results showed 5 positive cases of *Crithidia* spp. and 96 positive cases of CL (*L. major*).

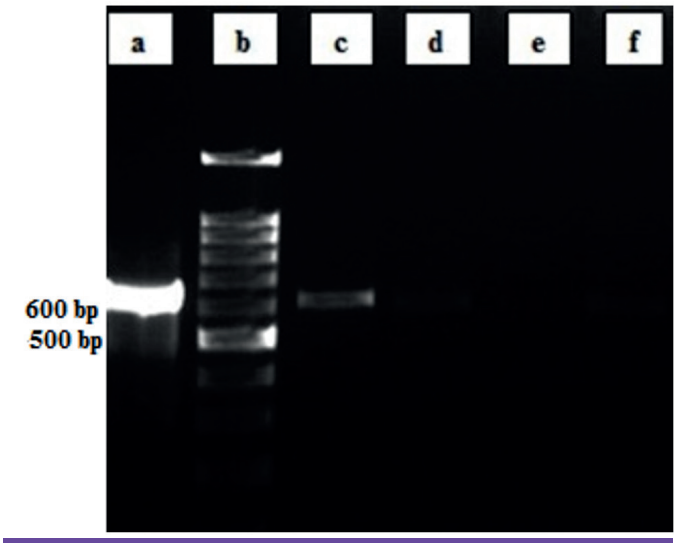

Figure 1. Characterization of *L. major* by PCR: positive control (a), ladder 1.000 bp (b), positive patients for *L. major* (c-f), negative control (e)
PCR: Polymerase chain reaction

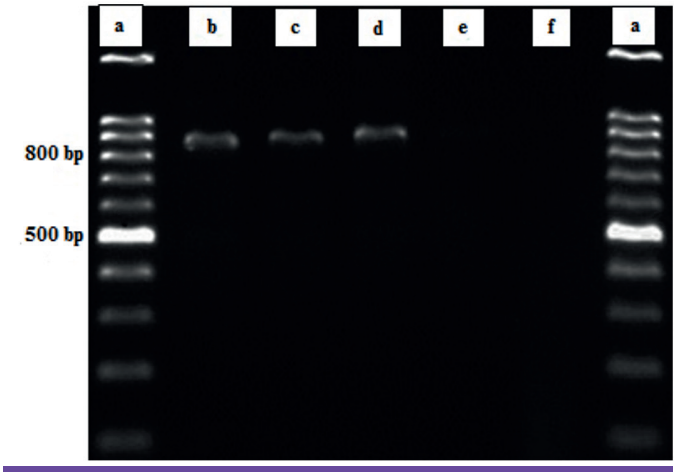

Figure 2. Characterization of *Crithidia* spp. by PCR: ladder 1.000 bp (a), positive control (b), positive patients for *Crithidia* spp. (c-e), negative control (f)
PCR: Polymerase chain reaction

2-DE and MS

As shown in Figure 3, different spots were matched in this study. The sharp and repeatable spots in different 2-DE experiments were selected for MS analysis (Figure 3). In peptide mass fingerprinting, unknown proteins (peptide profile obtaining from MS data) are compared with theoretical peptide libraries provided from sequences in the different protein databases (17). Accordingly, the results of the MALDI TOF/TOF MS indicated the HK (spot 48) as a common and repeatable protein in the proteome of *L. major* and *Crithidia* (Table 1). The score of the obtained protein was significant (>62). The up-regulation of the HK in *Crithidia* proteome (Figure 4 a) in comparison with *L. major* proteome (Figure 4 b) has been shown in obtained graph from progenesis same spot software (Figure 4 c). The putative role of the identified protein (HK) was investigated using *L. major* and *Crithidia* spp.

genome project database (www.genedb.org).

DISCUSSION

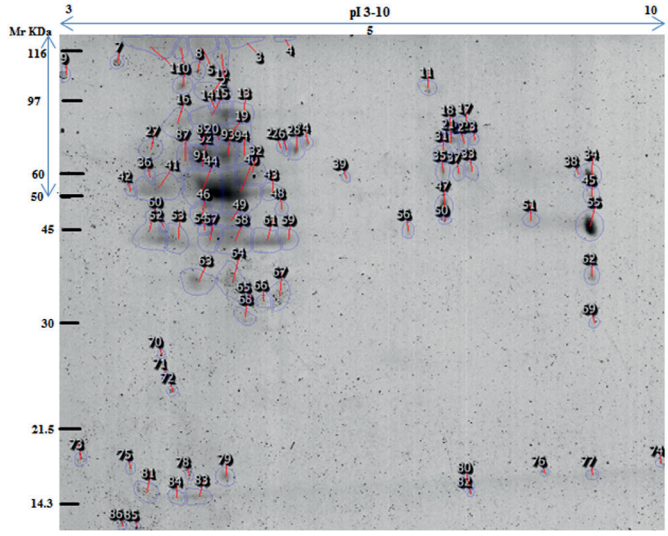

Figure 3. *L. major* 2-DE gel (silver nitrate staining): Common proteins matched in the proteome of the *Crithidia* spp. and *L. major*

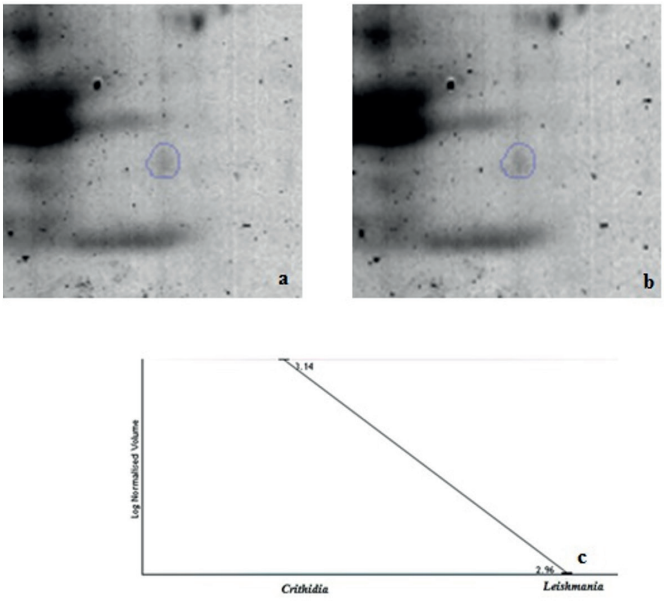

Figure 4. Progenesis same spot software graph: HK enzyme in *Crithidia* (a), HK enzyme in *L. major* (b), progenesis same spot software graph (c). According to the obtained graph (descending graph), the expression of HK enzyme has been up-regulated in *Crithidia* proteome in comparison with *L. major* proteome
HK: Hexokinase

Co-infection of the *Crithidia* spp. and *Leishmania* parasites in CL patients has received attention in recent years in Iran (4). It seems that molecular techniques should be used more frequently in the diagnosis of co-infection of *Leishmania* and *Crithidia* spp. in research and clinical fields in the future.

Protein profiling of the microorganisms can be changed due to the routine subculture *in vitro* conditions. Since sample preparation is an important stage in proteomic studies, for increasing the accuracy of the obtained data, *L. major* and *Crithidia* were taken from the CL patients and it was a remarkable issue in our study.

Although the information regarding HK is rare in *Crithidia* spp., genomic data have revealed the presence of two types of HK (HK1, HK2) on chromosome 21 in *Leishmania* parasites. The obtained results of our proteomic study confirmed the expression of HK in both proteomes of *L. major* and *Crithidia*. The report of the expression of the HK in the proteome of the *Crithidia* by Alcolea et al. (11) in 2014 confirms our results.

The proteomic studies have elucidated the critical functions of portions involving mitochondrion and metabolic pathways in the pathogenicity, diagnosis, treatment, and vaccination of protozoan parasites (18). HK, as an identified protein in the proteome profile of the *Crithidia* spp. and *L. major* belongs to the glycolysis pathway. Glycolysis is one of the important pathways in the metabolism of *Leishmania* parasites and the associated-glycolysis enzymes are located in glycosome organelle (19).

A study in 1999 indicated the relationship of drug resistance in *Leishmania* parasite and the expression of the *HK* gene. Their results showed that the up-regulation of the *HK* gene increases the drug resistance of the *Leishmania* parasites (20). The inhibitory effect of anti-leishmanial drugs against HK highlights the important role of HK as a therapeutic target and a possible protein in drug resistance in *Leishmania* parasites (21,22). According to our results, the up-regulation of the HK in *Crithidia* proteome in comparison to the *L. major* proteome might be related to the increased duration of the treatment period with an unknown mechanism in CL patients co-infected with *Crithidia*.

Evidence shows that the identification of diverse HK-zymodemes leads to distinguishing the different strains of *L. major* (23). Indication of the HK as a common protein in the proteome of *L. major* and *Crithidia* might be suggested for the detection of *Leishmania* and *Crithidia* spp. according to the HK-zymodemes profiles. Therefore, the results of the isoenzyme and zymography studies regarding HK might be used in the detection of the co-infection of *L. major* and *Crithidia* spp. in CL patients.

CONCLUSION

The report of *Crithidia* spp. in CL patients in Iran suggests more attention be given to the presence of this co-infection in other parts of the world. This study suggested the expression of the HK in *Crithidia* spp. as a possible factor in the increased duration of the

Table 1. The obtained MS data for HK in *L. major* and *Crithidia**

Protein name	Spot number on the 2-DE gel	Matched peptides	Mr	pI	Score	Protein sequence coverage	Accession no.
Putative hexokinase	48	2	52.2	8.84	172	6%	930561242

Mr: Molecular weight, pI: Isoelectric point
*Since score more than 62 (according to the company procedures) was significant, the obtained MS informant was adapted to the HK enzyme, HK: Hexokinase

treatment period in CL patients; however, further investigation such as using HK-inhibitors can reveal more information in this regard.

ACKNOWLEDGEMENTS

This article was extracted from Mohammadreza Karimazar's Ph.D. thesis which was supported by Shiraz University of Medical Sciences (grant no: 95-7660).

* Ethics

Ethics Committee Approval: All human specimens were obtained from the CL patients with the approval of the Ethical Committee of Shiraz University of Medical Sciences, Shiraz, Iran (IR.SUMS.REC.1395.S1).

Informed Consent: Informed consent was taken from all present patients in this study.

Peer-review: Internally peer-reviewed.

* Authorship Contributions

Concept: M.R.T., M.H.M., Design: M.R.T., M.H.M., Data Collection or Processing: M.K., Q.A., S.R., Analysis or Interpretation: M.K., Q.A., S.R., Literature Search: M.K., S.R., Writing: M.K., Q.A., S.R., M.R.T., M.H.M.

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

Financial Disclosure: This study was supported by Shiraz University of Medical Sciences (grant no: 95-7660).

REFERENCES

1. Georgiadou SP, Makaritsis KP, Dalekos GN. Leishmaniasis revisited: Current aspects on epidemiology, diagnosis and treatment. J Transl Int Med 2015; 3: 43-50.
2. Kermanjani A, Akhlaghi L, Oormazdi H, Hadighi R. Isolation and identification of cutaneous leishmaniasis species by PCR-RFLP in Ilam province, the west of Iran. J Parasit Dis 2017; 41: 175-9.
3. Alvar J, Vélez ID, Bern C, Herrero M, Desjeux P, Cano J, et al. Leishmaniasis worldwide and global estimates of its incidence. PLoS One 2012; 7: 35671.
4. Ghobakhloo N, Motazedian MH, Naderi S, Ebrahimi S. Isolation of Crithidia spp. from lesions of immunocompetent patients with suspected cutaneous leishmaniasis in Iran. Trop Med Int Health 2019; 24: 116-6.
5. Wallace FG. The trypanosomatid parasites of insects and arachnids. Exp Parasitol 1966; 18: 124-93.
6. Doudi M, Karami M, Eslami G, Setorki M. A study of genetic polymorphism of *Crithidia* in Isfahan, Iran. Zahedan J Res Med Sci 2015; 17: 971.
7. Doudi M, Setorki M, Bidabadi LS. Genotyping of isolated cutaneous leishmaniasis species by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis in endemic foci of Isfahan, Iran. AJMR 2011; 5; 4607-14.
8. Eslami G, Salehi R, Khosravi S, Doudi M. Genetic analysis of clinical isolates of Leishmania major from Isfahan, Iran. J Vector Borne Dis 2012; 49: 168-74.
9. Rashidi S, Mojtahedi Z, Shahriari B, Kalantar K, Ghalamfarsa G, Mohebali M, et al. An immunoproteomic approach to identifying immunoreactive proteins in *Leishmania infantum* amastigotes using sera of dogs infected with canine visceral leishmaniasis. Pathog Glob Health 2019; 113: 124-32.
10. Singh S, Dubey VK. Quantitative Proteome Analysis of Leishmania *donovani* under Spermidine Starvation. PLoS One 2016; 11: 0154262.
11. Alcolea PJ, Alonso A, García-Tabares F, Toraño A, Larraga V. An Insight into the proteome of *Crithidia fasciculata* choanomastigotes as a comparative approach to axenic growth, peanut lectin agglutination and differentiation of *Leishmania spp.* promastigotes. PLoS One 2014; 9: 113837.
12. de Cássia-Pires R, de Melo MF, Barbosa RD, Roque AL. Multiplex PCR as a tool for the diagnosis of *Leishmania spp*. kDNA and the gapdh housekeeping gene of mammal hosts. PLoS One 2017; 12: 0173922.
13. Bresson A, Jorge V, Dowkiw A, Guerin V, Bourgait I, Tuskan GA, et al. Qualitative and quantitative resistances to leaf rust finely mapped within two nucleotide-binding site leucine-rich repeat (NBS-LRR)-rich genomic regions of chromosome 19 in poplar. New Phytol 2011; 192: 151-63.
14. Brotherton MC, Racine G, Foucher AL, Drummelsmith J, Papadopoulou B, Ouellette M. Analysis of stage-specific expression of basic proteins in *Leishmania infantum*. J Proteome Res 2010; 9: 3842-53.
15. Rabilloud T. Silver staining of 2-D electrophoresis gels. Methods Mol Biol 1999; 112: 297-305.
16. Mojtahedi Z, Clos J, Kamali-Sarvestani E. Leishmania major: identification of developmentally regulated proteins in procyclic and metacyclic promastigotes. Exp Parasitol 2008; 119: 422-9.
17. Barrett J, Brophy PM, Hamilton JV. Analysing proteomic data. Int J Parasitol 2005; 35: 543-53.
18. Rashidi S, Kalantar K, Hatam G. Using proteomics as a powerful tool to develop a vaccine against Mediterranean visceral leishmaniasis. J Parasit Dis 2018; 42: 162-70.
19. Harris MT, Mitchell WG, Morris JC. Targeting protozoan parasite metabolism: glycolytic enzymes in the therapeutic crosshairs. Curr Med Chem 2014; 21: 1668-78.
20. Foulquié MR, Louassini M, Castanys S, Gamarro F, Benítez R, Adroher FJ. Different catalytic activities of hexokinase and phosphofructokinase in wild type and glucantime-resistant *Leishmania* promastigotes appears not causatively related to resistance. Eur J Protistol 1999; 35: 338-41.
21. Mottram JC, Coombs GH. *Leishmania mexicana*: enzyme activities of amastigotes and promastigotes and their inhibition by antimonials and arsenicals. Exp Parasitol 1985; 59: 151-60.
22. Pabón MA, Cáceres AJ, Gualdrón M, Quiñones W, Avilán L, Concepción JL. Purification and characterization of hexokinase from *Leishmania mexicana*. Parasitol Res 2007; 100: 803-10.
23. Azmi K, Schonian G, Schnur LF, Nasereddin A, Ereqat S, Abdeen Z. Development of assays using hexokinase and phosphoglucomutase gene sequences that distinguish strains of *Leishmania tropica* from different zymodemes and microsatellite clusters and their application to Palestinian foci of cutaneous leishmaniasis. PLoS Negl Trop Dis 2013; 7: 2464.