



Tehran University of Medical
Sciences Publication
<http://tums.ac.ir>

Iran J Parasitol

Open access Journal at
<http://ijpa.tums.ac.ir>



Iranian Society of Parasitology
<http://isp.tums.ac.ir>

Original Article

Comparison of the Proteome Profiling of Iranian isolates of *Leishmania tropica*, *L. major* and *L. infantum* by Two- Dimensional Electrophoresis (2-DE) and Mass-spectrometry

*Homa HAJJARAN¹, Mitra MOHAMMADI BAZARGANI², Mehdi MOHEBALI¹,
Richard BURCHMORE³, Ghasem HOSSEINI SALEKDEH⁴, Elham KAZEMI-RAD^{4,5},
Mohammad Reza KHORAMIZADEH^{6,7}

1. Dept. of Medical Parasitology and Mycology, School of Public Health, Tehran University of Medical Sciences (TUMS), Tebran, Iran
2. Agriculture Institute, Iranian Research Organization for Science and Technology, Tebran, Iran
3. Institute of Infection, Immunity and Inflammation, College of Medical, Veterinary and Life Science, University of Glasgow, United Kingdom
4. Agricultural Biotechnology Research Institute of Iran, (ABRI), Karaj, Iran
5. Dept. of Parasitology, Pasteur Institute of Iran, Tebran, Iran
6. Biosensor Research Center, Endocrinology and Metabolism Molecular-Cellular Sciences Institute, TUMS, Tebran, Iran
7. Dept. of Medical Biotechnology, School of Advanced Technologies in Medicine, Tums, Tebran, Iran

Received 04 Feb 2015

Accepted 18 Sep 2015

Keywords:

Proteome,
2-Dimension electrophoresis,
Liquid chromatography,
Mass spectrometry,
L. tropica, *L. major*,
L. infantum

*Correspondence

Email:

hajjaranh@sina.tums.ac.ir

Abstract

Background: The mechanisms of virulence and species differences of *Leishmania* parasites are under the influence of gene expression regulations at posttranscriptional stages. In Iran, *L. major* and *L. tropica* are known as principal agents of cutaneous leishmaniasis, while *L. infantum* causes visceral leishmaniasis.

Methods: As a preliminary study, we compared the proteome mapping of the above three Iranian isolates of *Leishmania* species through the 2-dimension electrophoresis (2-DE), and identified the prominent proteins by Liquid Chromatography (LC) mass spectrometry.

Results: We reproducibly detected about 700 protein spots in each species by using the Melanie software. Totally, 264 proteins exhibited significant changes among 3 species. Forty nine protein spots identified in both *L. tropica* and *L. major* were similar in position in the gel, whereas only 35 of *L. major* proteins and 10 of *L. tropica* proteins were matched with those of *L. infantum*. Having identified 24 proteins in the three species, we sought to provide possible explanations for their differential expression patterns and discuss their relevance to cell biology.

Conclusion: The comparison of proteome profiling pattern of the 3 species identified limit up and limit down regulated or absent /present proteins. In addition, the LC-MS data analysis showed that most of the protein spots with differential abundance in the 3 species are involved in cell motility and cytoskeleton, cell signaling and vesicular trafficking, intracellular survival / proteolysis, oxidative stress defense, protein synthesis, protein ubiquitination / proteolysis, and stress related proteins. Differentially proteins distributed among the species maybe implicated in host pathogenicity interactions and parasite tropism to cutaneous or visceral tissue macrophages.

Introduction

Leishmaniasis refers to a variety of diseases caused by more than 20 species of intracellular protozoan parasites belonging to the genus *Leishmania*. The clinical spectrum of the disease ranges from simple self-limiting cutaneous ulcers to severe disfiguring mucocutaneous, and even to a fatal visceral disease known as Kala Azar. About 98 tropical and subtropical countries are known to be endemic for this disease, with 350 million people at risk and overall prevalence of 12 million worldwide (1). Iran is endemic to both cutaneous and visceral leishmaniasis. Cutaneous Leishmaniasis (CL) is commonly caused by *L. major* and *L. tropica* (2). Visceral leishmaniasis (VL), the most life threatening form, is caused by *L. infantum* and in very rare occasions by *L. tropica* (2, 3).

In VL fever and hepato-splenomegaly are the main clinical signs in which *Leishmania* parasite is dispersed to the internal viscera like spleen, liver and bone marrow (4). Based on the leishmaniasis clinical symptoms, it is evident that the host immunity factors, *Leishmania* species, and in some cases the *Leishmania* strain, determines the measure of pathogenicity (5). *Leishmania* spp. has about 8000 genes among only 78 genes are restricted to individual species (6). In spite of a few species parasite genes implicated in pathogenesis and clinical presentation, the parasite gene expression rates differ greatly among species (6).

In leishmaniasis, parasites are challenged by the host immune conditions throughout their life cycle such as temperature increase of visceral tissues (liver, spleen or bone marrow). Such challenges causes *Leishmania* experience biochemical changes in which post transcriptional modification are activated and may eventuate into the emergence of the leishmaniasis pathogenicity (7- 14). Proteomics is an invaluable tool for systematic analysis of the proteome. Analysis of proteome is most commonly performed by a combination of 2-

DE and mass spectrometry (MS). 2-DE method could separate proteins in first and second dimensions according to their isoelectric and molecular weight points. With the help of 2-DE and the MS, a variable mixture of proteins is separated, visualized and then identified (15-16).

In this preliminary study, we compared the proteome mapping, in three Iranian isolates of *Leishmania* species including *L. tropica*, *L. major* and *L. infantum*, with immobilized pH gradient stripes with linear pH 4–7. Moreover, Liquid Chromatography (LC) - mass spectrometry was used for identification of a number of differentially expressed proteins among the three species.

Materials and Methods

Leishmania isolates and cell culture

The proteome of three *Leishmania* species including *L. tropica* (GenBank accession nos. **EF653267**, *L. major* (**JN860745**) and *L. infantum* (**JX289853**) compared and were analyzed. promastigote forms recovered from the “Iranian *Leishmania* parasite bank” located in Leishmaniasis lab, School of Public Health, Tehran University of Medical Sciences (TUMS). The identity of these strains was already obtained by other molecular DNA based methods (2, 17).

Cell culture – Promastigotes recovered from liquid nitrogen (-196 °C), were mass cultured in RPMI1640 medium (Gibco, Life technologies GmbH, Frankfurt, Germany) supplemented with 15% heat inactivated fetal bovine serum (Gibco, Germany) and 100U/ml penicillin and 100ug/ml streptomycin (Gibco, Germany) and incubated at 24°C. Promastigotes harvested in the stationary phase.

Parasites were harvested washed in sterile Phosphate Buffered Saline (PBS, pH: 7.2-7.4) and were used for protein extraction.

Protein Extraction

Proteomics analysis was performed on *L. tropica*, *L. major* and *L. infantum*, three species at the time of study. Promastigotes were harvested by centrifugation at 3000rpm, 4° C and 20 minutes and washed three times in sterile PBS (pH: 7.2-7.4) in the same condition for 10 minutes. The cells were resuspended in 5 mM Tris-HCl, pH 7.8, containing 1 mM phenylmethylsulfonyl fluoride (PMSF (Merck, Germany)). Proteins were precipitated by 10% (w/v) trichloroacetic acid (Merck, Germany) in acetone (Merck) with 0.07 % (w/v) dithiothreitol (DTT) (Merck) for 1 hour at -20°C. The samples were then centrifuged at 17500 g (Hettich, Germany) for 15 minutes at 4° C and the pellets were washed with ice-cold acetone containing 0.07% DTT, incubated at -20°C for 1 h and centrifuged at 4 °C. The samples were then solubilized in lysis buffer (9.5 M urea (Merck), 2% (w/v) CHAPS (Merck), 0.8% (w/v) Ampholyte (Bio-Rad, USA) pH 3-10, 1% (w/v) DTT) (18, 19). The concentration of protein was measured by the Bradford assay with bovine serum albumin BSA (Merck) as the standard (20).

Two-Dimensional electrophoresis (2-DE)

For analytical and preparative gels, 120 µg and 1.2 mg of extracted promastigotes proteins were loaded respectively. IEF was carried out on the 18 cm immobilized pH gradient (IPG) strips (pH 4-7) (Bio-Rad, USA). IPG strips were rehydrated overnight by loading the samples diluted with rehydration buffer containing 8 M urea, 4% CHAPS, 2% ampholyte, 50 mM DTT, and traces of bromophenol blue (Merck). Isoelectric focusing was conducted at 20 °C with Mutiphor II and a Dry-Strip kit (GE Healthcare, Germany). The running condition was as follows: 300 V for 90 minute, followed by 500 V for 90 min, 1000 V for 3 h and finally 3500 V for 16 h. The focused strips were equilibrated twice in equilibration solution. The first equilibration was performed in a solution containing 6 M urea, 20% (w/v) glycerol, 2% (w/v) SDS (Merck),

1% (w/v) DTT, and 50 mM Tris-HCl (Merck) buffer, pH 8.8. The second equilibration was performed in a solution with 2.5% (w/v) iodoacetamide (Merck). Separation in the second dimension was performed by SDS-PAGE in a vertical slab of acrylamide (Merck) (12% total monomer, with 2.6% cross-linker) using a PROTEAN II Multi Cell (BioRad). The protein spots in analytical and preparative gels were visualized by silver nitrate (Merck, Germany) and Coomassie brilliant blue CBB/G-250 (Sigma, Germany) respectively (19-21-22).

Gel image Analysis

GS-800 densitometer (Bio-Rad) was used for scanning of silver stain gels. Gels were analyzed using the Melanie 6 software (GeneBio, Geneva, Switzerland). The molecular masses of protein on gels were determined by co electrophoresis of standard protein markers (GE Heathcare) and *pI* of the proteins were determined by migration of the protein spots on 18 cm IPG (pH 4-7, linear) strips. 2-DE per sample (each species) was run for three biologically independent replicates, percent volume of each spot was estimated and analyzed by one-way analysis of variance (ANOVA) SAS software, and means were compared by the LSD test at $P \leq 0.01$. Spots were only considered to be significantly different in abundance at least between two *Leishmania* species when/at $P \leq 0.01$.

Peptide extraction and mass analysis

The protein spots of interest were excised from coomassie brilliant blue (CBB) stained gels and analyzed using an Amazon ion trap MS/MS (Bruker Daltonics) Mass spectrometer. Briefly, peptides were solubilized in 0.5 % formic acid and fractionated on a nano flow uHPLC system (Thermo RSLCnano) before online analysis by electrospray ionisation (ESI) mass spectrometry on an Amazon ion trap MS/MS (Bruker Daltonics). Peptide separation was performed on a Pepmap C18 reversed phase column (LC Packings), using a 5

- 85% v/v acetonitrile gradient (in 0.5% v/v formic acid) run over 45 min. at a flow rate of 0.2 μ l / min. Mass spectrometric (MS) analysis was performed using a continuous duty cycle of survey MS scan followed by up to ten MS/MS analyses of the most abundant peptides, choosing the most intense multiply charged ions with dynamic exclusion for 120s. MS data was processed using Data Analysis software (Bruker) and the automated Matrix Science Mascot Daemon server (v2.1.06) (23). Protein identifications were assigned using the Mascot search engine to interrogate in house databases of protein sequences for *L. major*.

Results

Comparison of proteome patterns

Protein extracts from the three *Leishmania* species including *L. tropica*, *L. major* and *L. in-*

fantum were separated by 2-DE gel electrophoresis. Multiple gels from the three independent replications were run to ensure reproducibility of the protein homogenates on the 2-DE gels. The analytical gels were visualized by silver staining. Fig. 1 show a representative example of the proteins separated/detected on a 2- DE gel in *L. tropica*, *L. major* and *L. infantum*, where a total weight of 120 μ g of proteins had been applied. The gel images were analyzed by Melanie software, and the percent volume of the spots was estimated and compared across the gels. We succeeded in detecting 600 ± 100 spots on the 2-DE gels, from which 638, 590, and 546 spots were statistically analyzed across the replicates in *L. tropica*, *L. major*, and *L. infantum* respectively. A number of 478 spots could be paired in all of the three species (Supplementary Table 1).

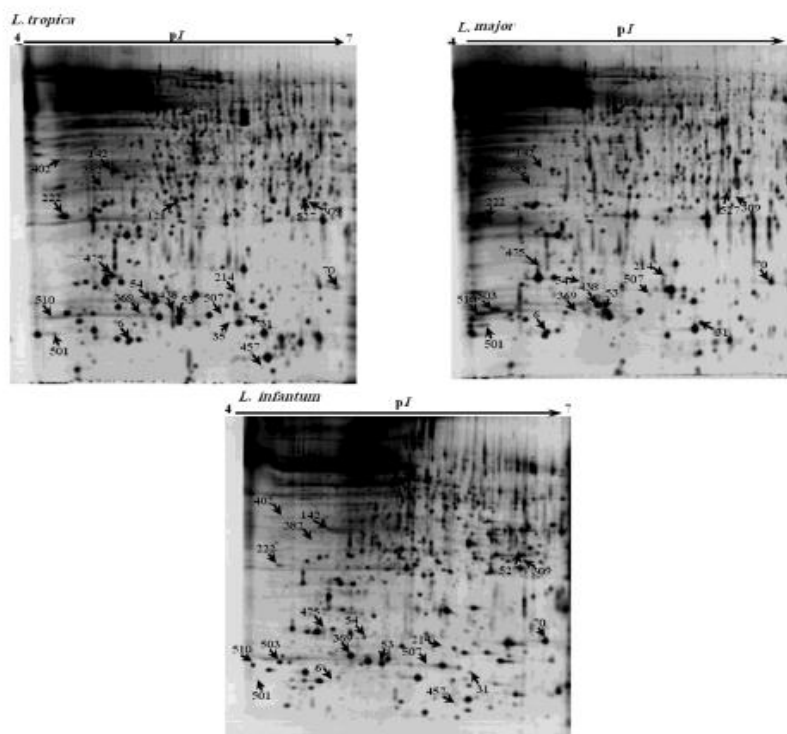


Fig. 1: 2-DE gels analysis of proteins extracted from different *Leishmania* species: *L. tropica*, *L. major*, and *L. infantum*. In first dimension (IEF), protein was loaded on a 18-cm IPG strip with a linear gradient of pH 4–7. In the second dimension, 12% SDS-PAGE gels were used, with a well for molecular weight standards. Proteins were visualized by silver staining. Arrows represent spots identified by LC Mass spectrometry. All spot numbers correspond to the spot numbers of Table 1

However 34, 33, and 4 protein spots of *L. tropica*, *L. major*, and *L. infantum* were identified as *Leishmania* species-specific spots (Fig. 2). Altogether, 265 protein spots exhibited reproducible quantitative ($P \leq 0.01$) changes across the three samples in *Leishmania* species (Supplementary Table 2). Among them, 35, 22 and 11 protein spots were different between the *L. tropica* and *L. major* (presented by **a**), *L. tropica* and *L. infantum* (presented by **b**), *L. major* and *L. infantum* (presented by **c**) respectively. Seven protein spots were different between all of the three species (presented by **abc**).

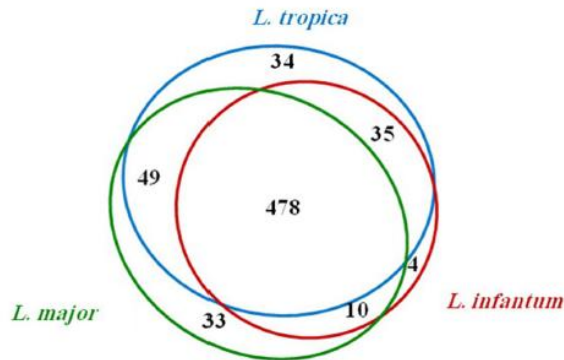


Fig. 2: Venn diagram showing shared and unique protein expression between different *Leishmania* species: *L. tropica*, *L. major*, and *L. infantum*. Regions of overlap between circles indicate gene expression which paired in all the three species/two species. Regions that do not overlap between circles indicate unique gene expression of particular/each species as species-specific spots

Protein identification

Within differentially expressed proteins, which were detected on the analytical gels, we could reliably detect and excise a total number of 28 protein spots on CBB-stained preparative gels. Due to the lack of the protein amount, the remaining proteins could not be detected. The excised protein spots were then analyzed by LC/MS leading to the identification of 24 proteins (Table 1). The identified protein spots by mass spectrometry have been labeled in Fig. 1. These proteins were classified in multiple categories according to their

species, functions, and biological processes: Cell motility and cytoskeleton, Cell signaling and vesicular trafficking, Intracellular survival / Nucleotid metabolism, Protein synthesis, oxidative stress defense, microtubule motor movement proteolysis, Lipid metabolism, Amino-acid biosynthesis, Protein ubiquitination / proteolysis, Transport, Stress related proteins/Protein folding and hypothetical proteins (Unknown) (Table 1). It is worth noting that some of these proteins are hypothetical and their functions in *Leishmania* still remain to be elucidated.



Fig. 3: Clustering pattern of differential proteins expressed in *L. tropica*, *L. major* and *L. infantum*. All quantitative information is showed using a color scale in which the color ranges from green for the highest down- regulation to red for the highest up-regulation. Black color indicates no changes in expression pattern of 3 *Leishmania* species

Table 1: Proteins identified in all three Iranian isolates of *Leishmania* species using LC/MS analysis

Spot No. ^a	Protein name & biological process	Access. No	(PI/MW) Exp. ^c	(PI/MW) Theo. ^d	% Volume			Score. ^e
					<i>L. tropica</i>	<i>L. major</i>	<i>L. infantum</i>	
Cell motility and cytoskeleton								
382	<i>beta tubulin</i>	A4HTR1	4.79/45	4.74/49	0.05	0.02	0.02	407
457	<i>hypothetical protein, conserved (Contains Myosin_tail_1 domain) 1 HECT (E6AP-type E3 ubiquitin-protein ligase) domain.)</i>	Q4QCC9	6.08/14	4.95/347	0.02	ND	ND	43
510	<i>ADF/Cofilin</i>	A4I4A3	6.35/17	6.61/15	0.17	0.14	0.10	540
Cell signaling and vesicular trafficking								
309	<i>hypothetical protein, conserved (Contains Ras_like_GTPase superfamily)</i>	A4I8E6	6.46/42	5.92/24	0.02	0.07	0.05	820
501	<i>calmodulin, putative</i>	A4HU13	4.46/15	4.10/16	0.05	0.03	0.15	773
Intracellular survival / proteolysis								
31	<i>calpain-like cysteine peptidase, putative,cysteine peptidase, Clan CA, family C2, putative</i>	E9AD27	6.14/15	5.14/700	0.06	0.12	0.07	83
35	<i>calpain-like cysteine peptidase, putative,cysteine peptidase, Clan CA, family C2, putative</i>	E9AD27	5.96/14	5.14/700	0.04	ND	ND	57
507	<i>calpain-like cysteine peptidase, putative,cysteine peptidase, Clan CA, family C2, putative</i>	E9AD27	6.54/14	5.14/700	ND	0.10	ND	42
774	<i>calpain-like cysteine peptidase, putative,cysteine peptidase, Clan CA, family C2, putative</i>	E9AD27	5.68/66	5.14/700	ND	0.09	ND	45
121	<i>hs1vu complex proteolytic subunit-like,hs1vu complex proteolytic subunit-like, threonine peptidase, Clan T(1), family T1B</i>	Q8I496	5.38/42	6.05/24	0.07	ND	ND	501
Lipid metabolism								
70	<i>hypothetical protein, conserved (Contains Acyl transferase domain ;Acyl transferase domain. S-malonyltransferase)</i>	E9AFH1	6.68/19	6.3/59	0.12	0.34	0.25	41
microtubule motor movement								
53	<i>hypothetical protein, conserved (Contains Ankarin repeat domain)</i>	A4IC52	5.42/16	5.63/38	0.52	0.48	0.28	587
Nucleotid metabolism								
369	<i>hypothetical protein, conserved (Contains Nucleoside 2-deoxyribosyltransferase domain)</i>	E9AH20	5.1/17	4.95/16	0.03	0.04	0.30	351
oxidative stress defense								
54	<i>tryparedoxin</i>	Q6RYT3	5.22/18	5.20/16	0.35	0.06	0.01	322
Protein synthesis								
142	<i>translation elongation factor 1-beta, putative</i>	A4IDB2	4.88/51	4.61/23	0.16	0.01	0.06	742
503	<i>60S acidic ribosomal protein P2</i>	E9AGM1	4.61/16	4.18/10	ND	0.15	0.18	408
Amino-acid biosynthesis								
527	<i>pyrroline-5-carboxylate reductase</i>	E9AGK4	6.32/43	6.21/28	0.12	0.03	0.14	529
Protein ubiquitination / proteolysis								
214	<i>hypothetical protein, conserved (Contains 1 HECT (E6AP-type E3 ubiquitin-protein ligase) domain.)</i>	Q4Q4M9	5.88/19	6.12/454	0.03	0.51	ND	45
Transport								
438	<i>calcium channel protein, putative,ion transporter, putative</i>	Q4Q3G0	5.36/16	8.6/291	0.06	0.34	0.05	50
Stress related proteins/Protein folding								
6	<i>fk506-binding protein 1-like protein</i>	Q4QBK4	5.02/14		0.23	0.35	ND	204
222	<i>hypothetical protein, conserved (Contains alpha-crystallin-Hsps_p23-like Superfamily)</i>	A4IBY7	4.48/39	4.15/21	0.52	0.07	0.17	441
Unknown								
402	<i>hypothetical protein, conserved</i>	Q4Q8Y7	4.52/54	6.27/95	0.02	0.06	0.02	45
419	<i>hypothetical protein, conserved</i>	Q4Q2Z8	5.42/72	9.93/129	0.02	0.10	0.01	32
475	<i>hypothetical protein</i>	Q4QE59	4.87/26	10.88/58	0.12	0.01	ND	35

LC/MS analysis./ ^aThe numbering corresponds to the 2-DE gel in Figure 1./ ^bAccession number in Swiss-Prot./ ^cExperimental pI and molecular weight./ ^dTheoretical pI and molecular weight./ ^eMascot score./ **ND**: Not Detected (Spot)

The clustering of protein expression pattern of differentially expressed proteins in *L. tropica*, *L. major* and *L. infantum* is presented in Fig. 3. All quantitative information is showed using a color scale in which the color ranges from green for the highest down- regulation to red for the highest up-regulation. Black color indicates no changes in expression pattern of the 3 *Leishmania* species. A comparison among the 3 species revealed that the changes in expression pattern were more pronounced in *L. infantum* compared with *L. tropica* and *L. major*. In addition, the number of up regulated proteins was higher than that of down- regulated proteins. The functional annotation of the 3 species identified proteins in *L. tropica*, *L. major* and *L. infantum* classified by biological function and processes described in Table 1. The biological function pie charts of the cutaneous

species (*L. tropica* and *L. major*) are highly similar whereas, the pie chart for the biological function of the visceral species (*L. infantum*) is different from those of cutaneous species (Fig. 4). Among the cutaneous species, most of the proteins functionalities are observed in the following categories: intracellular survival / nucleotid metabolism and cell motility/ cytoskeleton (20-22%). Moreover, the least functionalities are observed among cell signaling/ vesicular trafficking and lipid metabolism (5%). Moreover, in the *L. infantum* species, most functionality are observed among protein synthesis and cell motility/ cytoskeleton (15%) and least of them are observed among amino-acid biosynthesis and oxidative stress defense (7%).

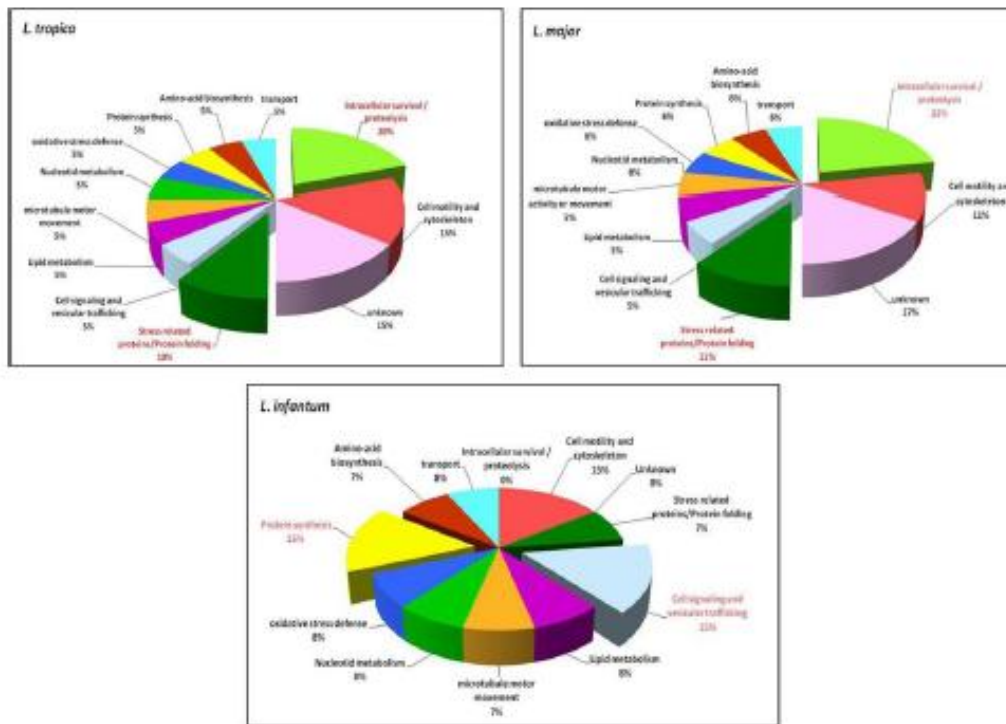


Fig. 4: Functional annotation of the identified proteins of 3 species *L. tropica*, *L. major* and *L. infantum* classified by biological function and processes in described in Table 1

Discussion

The aim of this preliminary study was to apply 2-DE, which is a valuable method in the proteomics arena to analyze the protein profile patterns of the three Iranian cutaneous and visceral *Leishmania* species including *L. tropica*, *L. major* and *L. infantum* to search for species-specific *Leishmania* proteins. In recent years by completion of genome sequencing *Leishmania* parasites coupled with protein separation techniques analysis has given us an insight in better understanding the mechanisms of pathogenesis of *Leishmania* species. Proteomics approaches at the protein expression level provide additional information for further analysis with a biological function. Moreover, comparative proteomics has been successful in determining the virulence biomarkers (24).

Overall, the proteome 2-DE maps of *L. tropica*, *L. major* and *L. infantum* were strikingly similar in terms of protein distribution and positioning. By using 18-cm IPG strips at the pI4-7 range in 2-DE comparative analysis, we successfully identified more than 700 protein spots for all the three species. These numbers represent about 9% of total proteins in *Leishmania* genome projects (25). The analysis and discussion about the detected proteins in this study could be categorized into three parts. First, we discuss comprehensively about the biological function of proteins whose expression abundances were common among each of the three species. In the second and third part, we represent the biological function of the proteins which are different and absent/present in the three different species. As it is described in Table 1 and Venn diagram (Fig.2), in the first part we have studied a total number of 478 proteins among which we will discuss the common ones. Among the common proteins of three *Leishmania* species, which were surveyed, we would like to mention significant proteins such as beta tubulin and ADF/Cofilin. These proteins could be

categorized in the cell mobility and cytoskeleton group. ADF/cofilin is existent in all eukaryotic organisms and has been involved in cell motility and cytokinesis. *Leishmania* parasites express only one isoform of ADF/cofilin, which is essential for flagellar assembly and motility (26). Beta tubulin is known as one of the members of distinct microtubule networks in *Leishmania* and is implicated in locomotion, cell shape and division (27).

Within other common proteins we could name trypanothione, calpain-like cysteine peptidase, and calmodulin putative from the groups of oxidative stress defense, Inter-cellular survival/proteolysis, cell signalling, and vesicular trafficking, respectively. Trypanothione is special thiol disulfide oxidoreductases related to thioredoxins, which play a crucial role in hydroperoxide detoxification cascades of Kinetoplastida (28).

One of the cell signaling proteins was calmodulin-like protein. Calmodulin is a kind of calcium binding protein. It participates in calcium signaling pathways that regulate multiple critical processes such as growth and proliferation (29, 30). The biological mechanism of elongation factor puts it in the protein synthesis group. Elongation factor1alpha plays a role in protein synthesis and assembly. It is a highly conserved GTP-binding protein involved in protein translation. In addition, it is an actin/microtubule-binding protein which interacts with the cytoskeleton (31- 33). Another common protein could be calcium channel protein that is a member of the transporter group. A huge number of proteins exist under the group of hypothetical proteins whose biological mechanisms are still not well discovered.

The second group of proteins, which were studied, was the ones, which were different in all three *Leishmania* species. The differences in proteins mainly occur between *L. tropica* and *L. major* and the least differences occur between *L. major* and *L. infantum* (Fig. 2). It is an interesting point that the proteins, which are different between *L. tropica* and *L. infantum* are less than those of *L. major* and *L. tropica*. *L.*

tropica is the main cause of dry cutaneous leishmaniasis lesions; whereas, *L. major* is the main cause of wet CL lesion and *L. infantum* causes visceral leishmaniasis. Recently there have been reports of viscerotropic forms caused by *L. tropica* in either humans or dogs infected to visceral leishmaniasis in Iran and different parts of the world (34, 2). Among significant proteins which are different in *L. tropica* and *L. major* we could mention calcium channel proteins, tryparedoxin and Elongation factor from the groups of oxidative stress defense and protein synthesis respectively whose mechanism have been described previously. Recently, it was demonstrated that *Leishmania* EF-1alpha acts as a virulence factor (35). This protein could diffuse into the cytosol of infected macrophages, where it is able to activate tyrosine phosphatase-1 leading to macrophage deactivation (31). Among the proteins different in *L. tropica* and *L. infantum* we could mention proteins such as calmadolin and tryparedoxin in which the former role is to transfer the material into the cells and the latter role is defending the host cell against oxidative factors and preventing its death. Other proteins are hypothetical and have an unknown mechanism. Nevertheless, some of these proteins have a specific domain and have a different mechanism such as hypothetical protein, conserved contains nucleoside 2-deoxyribosyltransferase domain, that have a role in nucleotide metabolism.

Among the most significant proteins between *L. major* and *L. infantum* we could mention Calmadolin. The remaining proteins are partially hypothetical. Another group of proteins, found in this study, was too rare and introduced as absent/present.

However, further studies are needed to define precise biological function for the mentioned proteins in the process of pathogenesis. Moreover, the examination of these species must be repeated with other strains in order to sanction the results. In addition, such differences must be evaluated and approved by oth-

er methods such as real time PCR and western blotting with monoclonal antibodies.

Conclusion

The analysis of proteome mapping of 3 *Leishmania* species including, *L. tropica*, *L. major* and *L. infantum* by 2-DE and mass spectrometry demonstrated that the vast majority of *Leishmania* proteins are commonly expressed among 3 species. Therefore, differentiation, virulence and pathogenesis may be related not only to the immunity situation of the host but also to the differentiation expression of a number of proteins like Stress related proteins/Protein folding and protein ubiquitination/proteolysis. It must be pointed out that further studies must be undertaken using western blotting or real time PCR in order to support the results of the current study.

Acknowledgments

The authors would like to express their gratitude to Prof. Ghasem Hosseini Salekdeh from Agricultural Biotechnology Research Institute of Iran, (ABRI), for their kind cooperation in proteomics laboratory. We are grateful to Elham Sarhadi from ABRI for valuable technical assistant. Also special thanks from Prof. Burchmore and Ms Suzzane Eideman from Institute of Infection, Immunity and Inflammation, College of Medical, Veterinary and Life Science, University of Glasgow, United Kingdom for Mass spectrometry analysis. In addition, we are grateful to Dr S.R. Naddaf from Pasteur Institute of Iran for valuable assistant. The authors would like to express their gratitude to Dr. B. Akhoundi and Mrs. S. Charedar for their kind cooperation. This study was financially supported by the Vice Chancellor for Research, School of Public Health, and Tehran University of Medical Sciences, Iran (NO.10695). The authors declare that there is no conflict of interests.

References

- Alvar J, Velez ID, Bern C, Herrero M, Desjeux P, Cano J, et al. Leishmaniasis worldwide and global estimates of its incidence. *PLOS One*. 2012; 7: e35671.
- Hajjaran H, Mohebbali M, Mamishi S, Vasigheh F, Oshaghi MA, Naddaf SR, et al. Molecular identification and polymorphism determination of cutaneous and visceral leishmaniasis agents isolated from human and animal hosts in Iran. *BioMed Res Int*. 2013; 789326.
- Jafari S, Hajiabdolbaghi M, Mohebbali M, Hajjaran H, Hashemian H. Disseminated leishmaniasis caused by *Leishmania tropica* in HIV-positive patients in the Islamic Republic of Iran. *East Mediterr Health J*. 2010; 16 (3): 340-343.
- Mohebbali M. Visceral leishmaniasis in Iran: Review of the epidemiological and clinical features. *Iran J Parasitol*. 2013; 8(3):348-358.
- Cohen-Freue G, Holzer TR, Forney JD, McMaster WR. Global gene expression in *Leishmania*. *Int J Parasitol*. 2007; 37:1077-86.
- Peacock CS, Seeger K, Harris D, Murphy L, Ruiz JC, Quail MA, et al. Comparative genomic analysis of three *Leishmania* species that cause diverse human disease. *Nat Genet*. 2007; 39 (7): 839-47.
- Devault A, Bañuls A-L. The promastigote surface antigen gene family of the *Leishmania* parasite: differential evolution by positive selection and recombination. *BMC Evol Biol*. 2008; 8: 292.
- Doyle MA, MacRae JI, De Souza DP, Saunders EC, McConville MJ, Likić VA. LeishCyc: a biochemical pathways database for *Leishmania major*. *BMC Syst Biol*. 2009; 3: 57.
- Haile S, Papadopoulou B. Developmental regulation of gene expression in trypanosomatid parasitic protozoa. *Curr Opin Microbiol*. 2007; 10 (6): 569-77.
- Kazemi-Rad E, Mohebbali M, Khadem-Erfan MB, Hajjaran H, Hadighi R, Khamesipour A, et al. Overexpression of Ubiquitin and Amino Acid Permease Genes in Association with Antimony Resistance in *Leishmania tropica* Field Isolates. *Korean J Parasitol*. 2013; 51(4):413-19.
- Quijada L, Soto M, Alonso C, Requena JM. Analysis of post-transcriptional regulation operating on transcription products of the tandemly linked *Leishmania infantum* hsp70 genes. *J Biol Chem*. 1997; 272: 4493-99.
- Wallach M, Fong D, Chang K-P. Post-transcriptional control of tubulin biosynthesis during Leishmanial differentiation. *Nature*. 1982; 299 (5884): 650-52.
- Duncan R, Gannavaram S, Dey R, Debrabant A, Lakhali-Naouar I, Nakhasi HL. Identification and characterization of genes involved in *Leishmania* pathogenesis: The potential for drug target selection. *Mol Biol Int*. 2011; p. 428486.
- Rochette A, Raymond F, Ubeda JM, Smith M, Messier N, Boisvert S, Rigault P, Corbeil J, Ouellette M, Papadopoulou B. Genome-wide gene expression profiling analysis of *Leishmania major* and *Leishmania infantum* developmental stages reveals substantial differences between the two species. *BMC Genomics*. 2008; 9:255.
- Gahlaut A, Dahiya M, Gothwal A, Kulharia M, Chhillar AK, Hooda V, Dabur R. Proteomics & metabolomics: Mapping biochemical regulations. *Drug Invention Today*. 2013; 5:321-326.
- McNicoll F, Drummelsmith J, Müller M, Madore É, Boilard N, Ouellette M, Papadopoulou B. A combined proteomic and transcriptomic approach to the study of stage differentiation in *Leishmania infantum*. *Proteomics*. 2006; 6 (12):3567-3581.
- Hajjaran H, Mohebbali M, Teimouri A, Oshaghi MA, Mirjalali H, Kazemi-Rad E, Shiee MR, Naddaf SR. Identification and Phylogenetic Relationship of Iranian Strains of Various *Leishmania* Species Isolated from Cutaneous and Visceral Cases of Leishmaniasis Based on N-Acetyl Glucosamine-1-phosphate Transferase Gene. *Infect Genet Evol*. 2014; 26: 203–12.
- Bazargani MM, Sarhadi E, Bushehri A-AS, Matros A, Mock H-P, Naghavi M-R, Hajihoseini V, Mardi M, Hajirezaei MR, Moradi F, Ehdai B, Salekdeh GH. A proteomics view on the role of drought-induced senescence and oxidative stress defense in enhanced stem reserves remobilization in wheat. *J Proteomics*. 2011; 74 (10): 1959–73.
- Sarhadi E, Mahfoozi S, Hosseini SA, Salekdeh GH. Cold acclimation proteome analysis reveals close link between the up-regulation of low-temperature associated proteins and

- vernalization fulfillment. J Proteome Res. 2010; 9 (11): 5658–67.
20. Kruger NJ. The Bradford Method for Protein Quantitation, in: The Protein Protocols Handbook 2002. pp. 15–21.
 21. Blum H, Beier H, Gross HJ. Improved silver staining of plant proteins, RNA and DNA in polyacrylamide gels. Electrophoresis. 1987; 8(2): 93-9.
 22. Neuhoff V, Arold N, Taube D, Ehrhardt W. Improved staining of proteins in polyacrylamide gels including isoelectric focusing gels with clear background at nanogram sensitivity using Coomassie Brilliant Blue G-250 and R-250. Electrophoresis. 1988; 9 (6): 255-62.
 23. Daneshvar H, Wyllie S, Phillips S, Hagan P, Burchmore R. Comparative proteomics profiling of a gentamicin-attenuated *Leishmania infantum* cell line identifies key changes in parasite thiol-redox metabolism. J Proteomics. 2012; 75 (5): 1463–71.
 24. Tambor V, Fucikova A, Lenco J, Kacerovsky M, Rehacek V, Stulik J, Pudil R. Application of proteomics in biomarker discovery: a primer for the clinician. Physiol Res. 2010; 59 (4): 471-97.
 25. Ivens AC, Peacock CS, Worthey EA, Murphy L, Aggarwal G, Berriman M, et al. The genome of the kinetoplastid parasite, *Leishmania major*. Science. 2005; 309 (5733): 436-42.
 26. Tammana TS, Sahasrabudde AA, Bajpai VK, Gupta CM. ADF/cofilin-driven actin dynamics in early events of *Leishmania* cell division. J Cell Sci. 2010; 123 (11): 1894–1901.
 27. Hiam A, Sebastien D, George B, Arlette F, Kalil J, Le Pape P. Microtubule target for new antileishmanial drugs based on ethyl 3-haloacetamidobenzoates. J Enzyme Inhib Med Chem. 2006; 21(3): 305–12.
 28. Nogoceke E, Gommel DU, Kieß M, Kalisz HM, Flohé L. A unique cascade of oxidoreductases catalyses trypanothione-mediated peroxide metabolism in *Crithidia fasciculata*. Biol Chem. 1997; 378 (8): 827–36.
 29. Chin D, Means AR. Calmodulin: a prototypical calcium sensor. Trends Cell Biol. 2000; 10(8): 322-8.
 30. Zhang M, Abrams C, Wang L, Gizzi A, He L, Lin R, Chen Y, Loll PJ, Pascal JM, Zhang JF. Structural basis for calmodulin as a dynamic calcium sensor. Structure. 2012; 20(5): 911-23.
 31. Gross SR, Kinzy TG. Translation elongation factor 1A is essential for regulation of the actin cytoskeleton and cell morphology. Nat Struct Mol Biol. 2005; 12: 772-78.
 32. Ladeira de Campos CB, Lopes UG. *Leishmania braziliensis*, molecular characterization of an elongation factor 1alpha gene. Gene. 1997;198: 281-88.
 33. Nandan D, Cherkasov A, Sabouti R, Yi T, Reiner NE. Molecular cloning biochemical and structural analysis of elongation factor-1 from *Leishmania donovani*. Comparison with the mammalian homologue. Biochem Biophys Res Commun. 2003; 302(4): 646–52.
 34. Dillon DC, Day CH, Whittle JA, Magill AJ, Reed SG. Characterization of a *Leishmania tropica* antigen that detects immune responses in Desert Storm viscerotropic leishmaniasis patients. Proc Natl Acad Sci. 1995; 92(17): 7981–85.
 35. Torrecilhas AC, Schumacher RI, Alves MJM, Colli W. Vesicles as carriers of virulence factors in parasitic protozoan diseases. Microbes and Infection. 2012;14:1465-74.