BMC Infectious Diseases

RESEARCH Open Access

Transcriptional alterations of virulence factors in *Leishmania major* clinical isolates harboring *Leishmania* RNA virus 2 (LRV2)

Sara Sabeti[1], Mona Koosha[2], Elham Kazemirad[1], Zahra Mirabedini[1], Mehdi Mohebali[1], Reza Saberi[3], Mahdi Fakhar[4,5*] and Homa Hajjaran[1*]

Abstract

Background Leishmaniasis is a parasitic disease caused by an intracellular protozoan, *Leishmania*. Various factors, including host immunity and the *Leishmania* species, influence the manifestation and severity of the disease. Recent investigations have shed light on the potentially significant role of *Leishmania* RNA virus (LRV) in the clinical prognosis of leishmaniasis. This study aims to investigate the influence of LRV2 + on various pathogenic genes of *Leishmania*.

Materials and methods In this study, 35 *Leishmania* isolates were obtained from patients diagnosed with cutaneous leishmaniasis (CL). *Leishmania* species and the presence of LRV2 + were identified with the PCR-RFLP and semi-nested PCR methods, respectively. Additionally, the RNA expression levels of cysteine protease (CP), heat shock protein 70 (HSP70), heat shock protein 83 (HSP83), glycoprotein 63 (GP63), and mannose phosphate isomerase (MPI) were assessed in LRV2 + and LRV2- *Leishmania* clinical isolates using RT-qPCR.

Results Out of the 35 isolates, 20 were selected from CL patients, all confirmed as *Leishmania major*. These isolates were divided into two groups, LRV2 + and LRV2-, with 10 isolates in each group. RT-qPCR analysis revealed that HSP83, MPI, and GP63 gene expression levels were statistically upregulated in LRV2 + isolates compared to LRV2- isolates ($P < 0.05$). Although *HSP70* and *CP* genes showed slight up-regulation in LRV2 + isolates, it was not statistically significant compared to LRV2- isolates.

Conclusion The notable increase in gene expression levels, particularly for *GP63*, *HSP83*, and *MPI* genes, suggests that the presence of LRV2 + may significantly influence the expression of these factors in *L. major* clinical isolates.

Clinical trial number Not applicable.

Keywords *Leishmania major*, *Leishmania* RNA virus, HSP83, MPI, GP63, HSP70, CP, RT-qPCR

*Correspondence:
Mahdi Fakhar
mahdifakhar53@gmail.com
Homa Hajjaran
hhajjaran@yahoo.com; hajaranh@tums.ac.ir
[1]Department of Medical Parasitology and Mycology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
[2]Department of Vector Biology and Control of Diseases, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
[3]Toxoplasmosis Research Center, Communicable Disease Institute, Department of Parasitology, School of Medicine, Mazandaran University of Medical Science, Sari, Iran
[4]Iranian National Registry Center for Lophomoniasis and Toxoplasmosis, Imam Khomeini Hospital, Mazandaran University of Medical Sciences, Sari, Iran
[5]Department of Medical Microbiology and Immunology, School of Medicine, Qom University of Medical Sciences, Qom, Iran

Introduction

Leishmania species are crucial pathogens responsible for a broad spectrum of clinical manifestations, ranging from self-healing ulcers to potentially life-threatening forms [1, 2].

The three main forms of this disease include visceral leishmaniasis (VL), cutaneous leishmaniasis (CL), and mucocutaneous leishmaniasis (MCL) [3]. CL is estimated to have between 0.7 and 1 million new cases annually, predominantly endemic in tropical and subtropical countries [4, 5]. Also, CL is the predominant form of leishmaniasis in Iran, present in 25 out of 31 provinces, occurring in two forms: anthroponotic cutaneous leishmaniasis (ACL) and zoonotic cutaneous leishmaniasis (ZCL) [6, 7]. Recent evidence has highlighted the role of viruses as endosymbionts in protozoan pathogenesis [8–11].

The *Leishmania* RNA virus (LRV), belonging to the Totiviridae family, acts as an endosymbiont within the *Leishmania* parasite [12]. The presence of LRV provides these parasites with a survival advantage by suppressing immunity in the vertebrate host [8, 13, 14]. Based on the complete nucleotide sequence, LRVs are categorized into two types: LRV1 (New World) and LRV2 (Old World), displaying less than 40% similarity in their genomes [15]. The presence of LRV2 in Iran has primarily been confirmed in *L. major*, with occasional occurrences in *L. infantum* and *L. tropica* [16–18]. In recent decades, numerous studies have investigated the potential role of LRV in the pathogenesis of *Leishmania* infections, treatment failures, and immune responses. While there is a wealth of literature addressing these topics, particularly in the New World, there remains a significant gap in understanding these issues within Old World regions [8, 10, 14, 19, 20].

Leishmania virulence factors (VFs) play a significant role in the pathogenesis of the parasite by stimulating the immune response [9, 10, 21].

Heat shock proteins (HSPs) play an essential role for various critical processes, including differentiation, proliferation, and survival throughout different life cycle stages [22]. The MPI protein (mannose phosphate isomerase) is a vital virulence factor in *Leishmania*. The absence or functional impairment of MPI in *Leishmania* species has been associated with slow growth rates [23].

The gene region associated with the metalloprotease GP63 is integral to the survival and virulence of *Leishmania* parasites, with GP63 recognized as one of the most significant virulence factors. This protein contributes to the parasite's ability to evade the host immune system by modulating macrophage signaling, which is critical for establishing and propagating the parasite within these immune cells. Studies indicate that mutants lacking GP63 exhibit increased susceptibility to host defenses, underscoring its protective role against immune attacks. Furthermore, GP63 has been shown to degrade host antimicrobial peptides, enabling *Leishmania* to survive in hostile environments. Overall, GP63 is not only involved in immune evasion but also impacts cellular processes at a molecular level. It can translocate to host cell nuclei, influencing protein transport and modulating gene expression related to immune responses [24].

Cysteine proteases (CPs) are critical VFs and have been considered drug targets and candidates for preparing vaccines [25, 26]. In Iran, numerous studies have explored the expression levels of VF genes and observed changes in expression under in vitro conditions. These studies have suggested that these expression changes may be attributed to the presence of LRV2 [11, 14].

Given the significant roles of VFs, this study aims to explore the differential expression of *HSP83*, *MPI*, *GP63*, *HSP70*, and *CP* genes in LRV2 + and LRV2- *L. major* isolates using the qRT-PCR.

Methods

Sample collection

In this study, 20 *Leishmania* isolates (10 LRV2 positive and 10 LRV2 negative) were obtained from patients diagnosed with CL in the ZCL regions of Golestan (northern Iran) and Isfahan (central Iran) provinces in Iran. The dermal lesions were sterilized with 70% ethanol, and sample collection was done by scraping the swollen edge of the lesions. The exudate materials were initially cultured on a Novy-MacNeal-Nicolle (NNN) medium. After 3–5 days, promastigotes were then transferred to RPMI 1640 medium (Gibco, Germany) with 10% heat-inactivated fetal bovine serum (Gibco, Germany), 100 U/mL penicillin, and 100 µg/ml streptomycin (Gibco, Germany), and incubated at 26 ℃ [27].

Leishmania species identification by PCR-RFLP

DNA extraction was conducted using a commercial kit from Bioneer. PCR amplification targeted a 1450 bp fragment of the N-acetyl glucosamine-1-phosphate transferase (NAGT) gene in *Leishmania* isolates, utilizing specific forward and reverse primers. Species identification was performed through restriction fragment length polymorphism (RFLP) analysis, employing the Acc1 enzyme for digestion [27].

RNA extraction and cDNA synthesis

Total RNA from 1×10^6 promastigotes in the stationary phase was extracted according to the manufacturer's protocol (YTZ, Favorgen, Taiwan). Complementary DNA (cDNA) was synthesized from 400 ng of total RNA using a cDNA synthesis kit (YTZ, Favorgen, Taiwan) following the manufacturer's instructions [14].

Leishmania LRV2 detection by semi-nested PCR

A previous study described a method for detecting LRV2. Briefly, the initial PCR employed outer primers LRV F1 and LRV R to amplify a 526 bp fragment. This was followed by a semi-nested PCR using inner primers LRV F2 and LRV R to target a 315 bp product of the RdRp gene of LRV2. The PCR protocol included 35 cycles at 94 °C, 60 °C, and 72 °C, with a final extension at 72 °C for 5 min [28].

Real-time PCR

Quantitative Real-time PCR (RT-qPCR) was used to investigate the expression of the *HSP83*, *MPI*, *Gp63*, *HSP70*, and *CP* genes compared to *L. major* standard isolate (MRHO/IR/75/ER). Primers for targeted genes were designed using primer 3 software (bioinfo.ut.ee/primer3-0.4.0/). Alpha-tubulin (ALT) genes were included for normalization purposes. The sequences of the primers are shown in Table 1.

Real-time PCR for these genes was performed using StepOnePlus Real-Time PCR System (Applied Biosystems, Thermo Fisher Scientific) in an equal amount of cDNA were run in duplicate and amplified in 15 µL reaction containing 1.2 µL target cDNA, 2.2 µL forward and reverse primers, 3.8 µl sterile deionized water, 7.5 µL SYBR green (YTZ, Favorgen, Taiwan) and 0.3 µL Rox (YTZ, Favorgen, Taiwan). The reaction was programmed with the following details: Holding Stage: 3 min at 95 °C, Cycling Stage: 45 Cycles of 15 s at 95°c and 38 s at 60 °C, Melt Curve Stage: 15 s at 95 °C, 1 min at 60°c and then 15s at 95 °C. Results were analyzed using Step One Plus™ software (Applied Biosystems).

The expression level of the studied genes, using the CT obtained for the reference gene and the target genes, after putting the corresponding values in the $2^{-\Delta\Delta CT}$ formula in a relative manner using REST software (Relative Expression Software Tool) obtained, which will be presented in the results section. The acceptable significance level was 95%, and the p-value was < 0.05.

Results

Leishmania specimen characterization

In the present study, isolates were examined using NAGT PCR-RFLP to identify *Leishmania* species. NAGT PCR produced a 1450 bp band in all *Leishmania* isolates. Digestion of the PCR product with ACC1 generated 500 bp and 950 bp fragments, indicating *L. major* species. All isolates from CL patients with complete demographic data were characterized as *L. major* infections (Suppl Fig. 1) (Table 2).

LRV2 detection

The first and second rounds of RdRp semi-nested PCR on LRV2 + samples revealed approximately 526 bp and 315 bp products, respectively. In the present study, 35 isolates were examined, of which 10 were identified as LRV2 + and 25 as LRV2- (Suppl Fig. 2) (Table 2). The isolates were then divided into LRV2 + and LRV2- groups. Finally, 10 LRV2 + isolates and 10 LRV2- isolates were selected.

Virulence factors expression

The relative RNA expression levels of the virulence factor genes in LRV2- and LRV2 + isolates were compared to the standard strain (Fig. 1; Table 3).

HSP83

The average RNA expression level of the *HSP83* gene in LRV2+ (S12-S21) and LRV2- (S2-S11) groups were 3.47 and 2.17 fold, respectively. The relative expression level of the *HSP83* gene was significantly upregulated by 1.816-fold in LRV2 + group compared to LRV2- ones ($p < 0.001$) (Fig. 1). In all the isolates, the expression was increased compared to standard isolate except one LRV2- isolate. However, the expression in LRV2 + isolates was higher,

Table 1 Designed primer sequences for parasite VF genes

Genes	Primer name	Sequence (5′-3′)	Length (mer)	Melt temperature	GC (%)	Amplicon size (bp)	Ref
HSP83	HSP83 F	ACGAAGCACTTCTCTGTGGAG	21	59.66	53.38	108	[14]
	HSP83 R	GATGTTGTTGCGCTTCTTGTT	21	60.31	42.86		
MPI	MPI F	AGTGCCCTACCTGCTGAAGA	20	60.01	55	138	
	MPI R	ATGAGCTCTGGCTTGTGGTT	20	59.87	50		
Gp63	GP63 F	ATCTGTGGCGACTTCAAGGT	20	59.31	50	136	
	GP63 R	CAGAGAACGTCTGGCAGGTC	20	60.39	60		
CP	CP F	ACAGCTCCTCTTTCATGGAC	20	57.22	50	92	[11]
	CP R	AATGTGTGAGGACAGGTACG	20	57.26	50		
HSP70	HSP70 F	GTTTGATGTGACGCTGCTGA	20	59.13	50	153	
	HSP70 R	GCCAGGTTCTTACCCTTGTTC	21	58.84	52.38		
Alpha Tubulin	ALT F	CAGGTGGTTGTCGTCTCTGAC	20	60.04	60	119	
	ALT R	TAGCTCGTCAGCACGAAGTG	20	60.11	55		

Table 2 Characteristics of isolates from CL patients

Isolate code	Duration of infection (Weeks)	Number of wounds	Lesion site	Type of wound	LRV2+/LRV2-
S1 (Standard)	-	-	-	-	LRV2-
S2	3	6	Hand and Leg	Wet	LRV2 -
S3	4	2	Hand	Wet	
S4	8	3	Hand	Wet	
S5	3	2	Hand	Wet	
6 S	8	5	Hand and Leg	Wet	
S7	8	6	Hand	Wet	
S8	4	1	Leg	Wet	
S9	3	1	lower limb	Wet	
S10	4	2	Upper limb	Wet	
S11	4	2	Hand	Wet	
S12	8	2	Hand	Wet	LRV2+
S13	8	1	Hand	Wet	
S14	3	1	waist	Wet	
S15	4	8	Upper limb	Wet	
S16	3	1	Leg	Wet	
S17	4	15	Hand and Face	Wet	
S18	1	1	Hand	Wet	
S19	4	1	waist	Wet	
S20	2	1	Leg	Wet	
S21	8	2	Leg and Face	Wet	

ranging from 1.73 to 5.26-fold, compared to LRV2- isolates, ranging between 1.54 to 3.16-fold (Figs. 1A and 2).

MPI

The average RNA expression level of the *MPI* gene in LRV2+ (S12-S21) and LRV2- (S2-S11) groups were 4.12 and 2.12, respectively, displaying a 2.043-fold increase in LRV2 + group. The *MPI* was significantly overexpressed in LRV1 + isolates ranging from 3.123 to 5.95, except for one isolate, which was insignificant compared to the standard isolate. In six LRV2- isolates, *MPI* slightly increased (2.09 to 3.97-fold), while in the remaining isolates, it was insignificant (Figs. 1B and 2).

GP63

The average RNA expression level of the *GP63* gene in LRV2 + was 2.96. The remaining LRV2 + isolates were upregulated from 2.16 to 4.87-fold, except for one isolate ($p < 0.05$). Also, in LRV2- group, the average RNA expression level was 1.76; it was increased in LRV2- isolates from 2.15 to 3.158 and in one isolate deceased by 0.1675-fold (Figs. 1C and 2).

HSP70

The average RNA expression level of the *HSP70* gene was 1.95 and 2.34 in LRV2- and LRV2 + isolates, respectively. The average gene expression was 1.256 times higher in the LRV2 + group compared to the LRV2- one, which was insignificant ($P > 0.05$) (Figs. 1D and 2).

CP

The average RNA expression level of the *CP* gene was significantly increased by 1.078-fold in LRV2 + isolates compared to LRV2- isolates; however, it was not statistically significant. Significant upregulation was detected in three LRV2 + and four LRV2- isolates compared to the standard isolate. Moreover, *CP* was downregulated in the three LRV2 + and four LRV2- isolates (Figs. 1E and 2).

Discussion

A precise interaction between innate and acquired immune cells is required to treat leishmaniasis infection effectively. However, various factors arising from the host, the vector, and the species of the *Leishmania* parasite can influence the host's immunity and alter the clinical outcome; one such influential factor is the presence of LRV [29]. Numerous studies have explored the impact of LRV on the *Leishmania* parasite, and some have suggested LRV as a potentially effective agent on the pathogenicity of the parasite [8, 9, 11, 13, 14]. While the role of LRV1 in the severity of CL in the New World is well recognized, there is limited understanding of the involvement of LRV2 in the pathogenicity of Old World CL [11, 14]. In this context, our evaluation of the role of LRV2 in CL has focused on targeting five fundamental VFs of *L. major*, including HSP83, MPI, GP63, HSP70, and CP.

The HSP90 family members are one of the most abundant heat shock proteins in eukaryotic cells [22]. HSP90 is an ATP-dependent chaperone predominantly expressed in the cytosol and assembles a large protein complex with co-chaperone molecules [30]. In the *Leishmania* parasite, the HSP90 homolog is referred to as HSP83. There are 18 gene copies of *HSP83* in *Leishmania*, accounting for approximately 2.8-3% of the total protein content in the promastigote stage.

In this study, we observed a significant increase in the expression of the *HSP83* gene in LRV2 + isolates compared to LRV2- isolates. Previous studies have established that HSP83 is involved in protein folding and stress response, both of which are critical for parasite survival under host immune pressure [14]. In 2018, a study conducted by Bifeld and colleagues highlighted

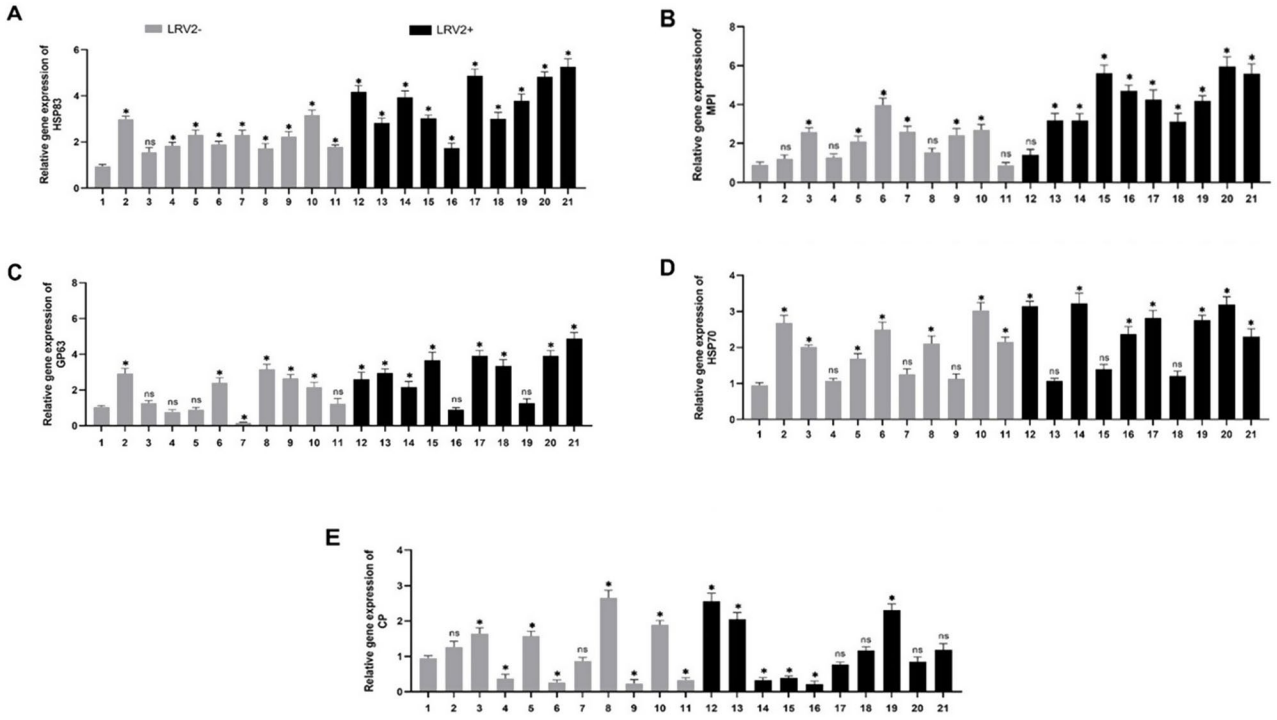

Fig. 1 Relative gene expression patterns of target genes in LRV2 + and LRV2- isolates. (**A**) *HSP83*, (**B**) *MPI*, (**C**) *GP63*, (**D**) *HSP70*, and *CP*. The expression of alpha-tubulin was used to normalize the data. Data analysis was done using two-way ANOVA for repeated measurements, followed by the Tukey test. The values are the mean ± SD of two independent experiments. ns: not significant; *, $p < 0.05$

Table 3 The relative expression levels of the target genes in LRV2- and LRV2 + isolates were compared to the standard strain

	Isolates	HSP83	MPI	GP63	HSP70	CP
LRV2+	*1 (Standard)	1	1	1	1	1
	2	2.975	1.205	2.929	2.679	1.27
	3	1.544	2.585	1.264	2.009	1.641
	4	1.84	1.279	0.7724	1.066	0.3727
	5	2.302	2.093	0.8823	1.685	1.571
	6	1.897	3.976	2.4	2.489	0.2645
	7	2.297	2.603	0.1675	1.26	0.8653
	8	1.718	1.536	3.158	2.103	2.66
	9	2.231	2.41	2.649	1.124	0.232
	10	3.169	2.694	2.154	3.029	1.896
	11	1.777	0.8782	1.239	2.148	0.3307
LRV2-	12	4.179	1.418	2.614	3.143	2.554
	13	2.816	3.19	2.951	1.073	2.049
	14	3.94	3.179	2.169	3.221	0.3291
	15	3.026	5.611	3.671	1.39	0.3912
	16	1.731	4.711	0.9004	2.365	0.2184
	17	4.882	4.256	3.933	2.814	0.7688
	18	3.005	3.123	3.348	1.203	1.164
	19	3.792	4.176	1.254	2.753	2.301
	20	4.831	5.953	3.908	3.194	0.8538
	21	5.264	5.593	4.872	2.302	1.19

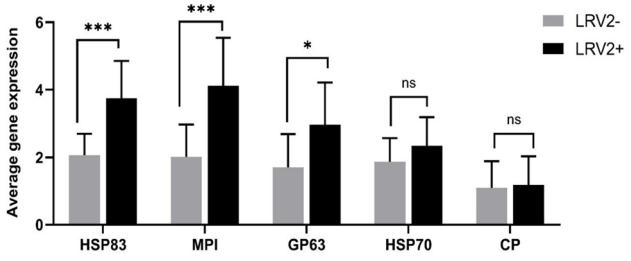

Fig. 2 Comparison of the average RNA expression of target genes in LRV2 + and LRV2- isolates. The values are the mean ± SD of two independent experiments. ns: not significant; *, $p < 0.05$; ***, $p < 0.01$

the importance of HSP90 activity on gene expression in *Leishmania donovani*. The findings confirmed the inhibitory effect of HSP90 on the synthesis of Leishmania proteins associated with the intracellular pathogenicity of the parasite [31]. In this line, Mirabedini et al. reported an expression of the *HSP83* gene in LRV2 + isolates compared to LRV2- in *L. major* [14]. In contrast to our result, Kariyawasam et al. reported higher expression of the *HSP90* gene in LRV1- isolates than in LRV1 + isolates [10]. Therefore, it appears that the role of species of *Leishmania* and the presence of LRV1/2 may influence the expression of HSPs during the infection.

Leishmania species synthesize large quantities of glycolipids and glycoproteins that contain mannose. These molecules are crucial for the virulence of this parasitic protozoan and enable *Leishmania* to resist the host [23]. It was demonstrated that the slow growth of various parasite species is associated with a lack of *MPI* [9]. Furthermore, MPI has been linked to enhanced virulence due to its role in glycoprotein synthesis, which is vital for Leishmania's ability to resist host defenses. Our finding revealed a significant increase in *MPI* gene expression in LRV2 + isolates compared to LRV2- isolates. Kariyawasam et al. reported higher expression of *MPI* gene in LRV1- compared to LRV1 + isolates [10]. Meanwhile, Mirabedini et al. demonstrated that MPI gene expression was higher in LRV2 + isolates than in LRV2 isolates [14]. Therefore, it can be inferred that LRV2 plays a significant role in the up-regulation of VF genes.

GP63 is a surface glycoprotein found on the *Leishmania* parasite. This 63-kilo Dalton glycoprotein, also known as MSP (Major Surface Protease) and Leishmanolysin, is primarily present in the promastigote form of the parasite [32]. GP63 is a zinc-containing metalloprotease encoded by seven genes, comprising about 1% of the total protein in Leishmania promastigotes [33].

In this study, the expression of the *GP63* gene showed an increase in LRV2 + isolates compared to LRV2- isolates. This is consistent with the findings of Kariyawasam et al., which reported no significant difference in the expression of the *GP63* gene for LRV1 + isolates compared to LRV1- isolates [10]. Furthermore, Rahmanipour et al. and Mirabedini et al. demonstrated a similar trend toward higher *GP63* levels in LRV2 + samples compared to LRV2- isolates [11, 14].

Members of the HSP70 family are ATP-dependent chaperones that carry out their function with the assistance of several co-chaperone molecules. When ATP is absent, members of this family strongly bind to misfolded protein substrates. The subsequent binding of ATP to the N-terminal region of the chaperon leads to the dissociation of the Hsp70/substrate complex. This sequential binding and release of the misfolded protein is repeated until complete refolding occurs [34–36].

Our findings suggest that LRV2 + isolates may induce elevated levels of HSP70, thus promoting the parasite's survival. These results align with the findings of Rahmanipour et al. but differ from those of Kariyawasam et al. [10, 11].

In protozoa, proteases play a crucial role in life cycle transitions, host invasion, tissue barrier traversal, haemoglobin and blood protein breakdown, immune system invasion, and the induction of inflammatory responses in mammalian hosts [25, 37, 38]. One of the functions of proteases is to cleave host complement factor C3b to C3bi, which enables the *Leishmania* parasite to attach to the macrophage surface through C3bi [25].

Our findings indicate increased *CP* gene expression in LRV2 + isolates compared to LRV2- isolates. It suggests that the presence of LRV2 is associated with higher levels of *CP* gene expression. However, Kariyawasam et al. reported no difference in *CP* gene expression between LRV1 + and LRV1- strains [10]. This indicates that LRV1 does not significantly impact *CP* gene expression. These findings suggest that different LRV strains may have varying effects on *CP* gene expression in this context [21]. Notably, the results from Rahmanipour et al. coincide with our findings. They reported increased *CP* gene expression in LRV2 + isolates compared to LRV2- [11]. This consistency across different studies strengthens the evidence supporting the association between LRV2 + strains and increased *CP* gene expression. It also reinforces the idea that LRV1 may not significantly impact CP *gene* expression, as observed in Kariyawasam et al.'s study. This kind of convergence in research findings helps to build a more comprehensive understanding of the relationship between LRV strains and *CP* gene expression [11].

Here, we used a higher number of LRV + and LRV- *L. major* isolates compared to previous surveys; nevertheless, some divergences were also detected in the gene expression of some LRV- isolates, suggesting gene expression of virulence factor can vary between isolates of the same species, which also reported in other species [39]. The limitation of the present study was the investigation of RNA expression in the promastigote stage. Since gene

regulation relies mainly on post-transcriptional modification [40], further studies are needed to evaluate both RNA and protein levels of target genes at promastigote and amastigote stages to provide better insight into the impact of LRV on the pathogenicity of *Leishmania*.

Conclusion

Our result revealed the upregulation of pathogenesis factors, particularly GP63, HSP83, and MPI genes, in *L. major* clinical isolates infected with LRV2. The results on the RNA level of the promastigote stage suggested the potential involvement of LRV2 in the pathogenicity of *L. major* clinical isolates. Further studies are necessary to fully confirm the role of LRV2 in the pathogenesis of *Leishmania*, which can provide new insights into managing and treating *Leishmania* infections.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12879-025-10717-9.

Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Supplementary Material 4

Supplementary Material 5

Supplementary Material 6

Supplementary Material 7

Supplementary Material 8

Acknowledgements
The authors express their appreciation to the Tehran University of Medical Science and Mazandaran University of Medical Science for their cooperation.

Author contributions
All the authors had access to the full dataset (including the statistical reports and tables) and took responsibility for the data's integrity and data analysis. H.H, M.M and M.F conceived and designed the study. R.S was involved in the case and sample collection. E.K, M.K, S.S and Z.M designed the analysis, and interpreted the data. S.S wrote the frst draft of the paper. H.H and E.K reviewed and approved the final report. All authors have read and approved the final manuscript.

Funding
This work was supported by Tehran University of Medical Sciences, Tehran, Iran.

Data availability
The data sets generated during and/or analyzed during the current study are available from the corresponding authors on reasonable request.

Declarations

Ethics approval and consent to participate
Approvals for this study were obtained from the Ethics of Tehran University of Medical Science (code number IR.TUMS.SPH.REC.1400.047) and Mazandaran University of Medical Sciences (IR.MAZUMS.REC.1400.016). Samples were collected upon informing patients about the scope of this study and signing the informed consent form. Informed written consent was obtained from adult participants and the parents of children, adhering to guidelines, and their responses were documented using a structured questionnaire.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Received: 5 October 2024 / Accepted: 25 February 2025
Published online: 06 March 2025

References

1. Bray R, Leishmania. Annual reviews microbiol. 1974;28(1):189–217.
2. Bruschi F, Gradoni L. The leishmaniases: old neglected tropical diseases. Springer;2018.
3. Akilov OE, Khachemoune A, Hasan T. Clinical manifestations and classification of old world cutaneous leishmaniasis. Int J Dermatol. 2007;46(2):132–42.
4. 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(5):e35671.
5. Ruiz-Postigo JA, Jain S, Madjou S, Agua JFV, Maia-Elkhoury AN, Valadas S, et al. Global leishmaniasis surveillance, 2022: assessing trends over the past 10 years/surveillance mondiale de La leishmaniose, 2022: evaluation des tendances des 10 dernieres annees. Wkly Epidemiol Rec. 2023;98(40):471–88.
6. Karami M, Gorgani-Firouzjaee T, Chehrazi M. Prevalence of cutaneous leishmaniasis in the middle East: a systematic review and meta-analysis. Pathogens Global Health. 2023;117(4):356–65.
7. Sharifi I, Khosravi A, Aflatoonian MR, Salarkia E, Bamorovat M, Moghadam MN, et al. Cutaneous leishmaniasis situation analysis in the Islamic Republic of Iran in Preparation for an elimination plan. Front Public Health. 2023;11:1091709.
8. Kariyawasam R, Grewal J, Lau R, Purssell A, Valencia BM, Llanos-Cuentas A, et al. Influence of leishmania RNA virus 1 on Proinflammatory biomarker expression in a human macrophage model of American tegumentary leishmaniasis. J Infect Dis. 2017;216(7):877–86.
9. Kariyawasam R, Lau R, Valencia BM, Llanos-Cuentas A, Boggild AK. Leishmania RNA virus 1 (LRV-1) in leishmania (Viannia) Braziliensis isolates from Peru: a description of demographic and clinical correlates. Am J Trop Med Hyg. 2020;102(2):280.
10. Kariyawasam R, Mukkala AN, Lau R, Valencia BM, Llanos-Cuentas A, Boggild AK. Virulence factor RNA transcript expression in the leishmania Viannia subgenus: influence of species, isolate source, and leishmania RNA virus-1. Trop Med Health. 2019;47(1):1–9.
11. Rahmanipour M, Mohebali M, Koosha M, Kazemirad E, Yasami-Khiabani S, Mirjalali H et al. Effect of leishmania RNA virus 2 on virulence factors and cytokines gene expression in a human macrophage infected with leishmania major: A preliminary study. 2023;246:108459.
12. Tarr PI, Aline RF Jr, Smiley BL, Scholler J, Keithly J, Stuart K. LR1: a candidate RNA virus of Leishmania. Proceedings of the National Academy of Sciences. 1988;85(24):9572-5.
13. de Carvalho RV, Lima-Junior DS, da Silva MVG, Dilucca M, Rodrigues TS, Horta CV, et al. Leishmania RNA virus exacerbates leishmaniasis by subverting innate immunity via TLR3-mediated NLRP3 inflammasome Inhibition. Nat Commun. 2019;10(1):5273.
14. 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(1):398.
15. Saberi R, Fakhar M, Mohebali M, Anvari D, Gholami S. Global status of synchronizing leishmania RNA virus in leishmania parasites: A systematic review with meta-analysis. Transbound Emerg Dis. 2019;66(6):2244–51.
16. Hajjaran H, Mahdi M, Mohebali M, Samimi-Rad K, Ataei-Pirkooh A, Kazemi-Rad E, et al. Detection and molecular identification of leishmania RNA virus (LRV) in Iranian leishmania species. Arch Virol. 2016;161:3385–90.
17. Moin-Vaziri V, Zare F, Seyyed Tabaei SJ, Saberi R, Hajjaran H. Successful isolation of leishmania RNA virus (LRV) from leishmania major in a cutaneous leishmaniasis focus in central Iran: an update on cases. Acta Parasitol. 2022;67(3):1290–8.
18. Saberi R, Fakhar M, Hajjaran H, Ataei-Pirkooh A, Mohebali M, Taghipour N, et al. Presence and diversity of leishmania RNA virus in an old zoonotic

cutaneous leishmaniasis focus, Northeastern Iran: haplotype and phylogenetic based approach. Int J Infect Dis. 2020;101:6–13.
19. Rahmanipour M, Mohebali M, Koosha M, Kazemirad E, Yasami-Khiabani S, Mirjalali H, et al. Effect of leishmania RNA virus 2 on virulence factors and cytokines gene expression in a human macrophage infected with leishmania major: a preliminary study. Exp Parasitol. 2023;246:108459.
20. Valencia BM, Lau R, Kariyawasam R, Jara M, Ramos AP, Chantry M, et al. Leishmania RNA virus-1 is similarly detected among metastatic and non-metastatic phenotypes in a prospective cohort of American tegumentary leishmaniasis. PLoS Negl Trop Dis. 2022;16(1):e0010162.
21. Omoruna O, Mukkala AN, Kariyawasam R, Shao E, Challa P, Klowak MA. A systematic review of virulence factors in the leishmania genus. Am J Trop Med Hyg. 2019;101:566.
22. Rico AI, Angel SO, Alonso C, Requena JM. Immunostimulatory properties of the leishmania infantum heat shock proteins HSP70 and HSP83. Mol Immunol. 1999;36(17):1131–9.
23. Atayde VD, Hassani K, da Silva Lira Filho A, Borges AR, Adhikari A, Martel C, et al. Leishmania exosomes and other virulence factors: impact on innate immune response and macrophage functions. Cell Immunol. 2016;309:7–18.
24. McGwire BS, Chang K-P, Mosser DM. Role of the leishmania surface protease gp63 in complement fixation, cell adhesion, and resistance to complement-mediated lysis'. 1995.
25. Kazemirad E, Reisi Nafchi H, Latifi A, Raoofian R, Mohebali M, Hajjaran HJJMM et al. Comparison of cysteine protease B gene expression between clinical isolates of leishmania tropica, leishmania major and leishmania infantum. 2019;7(3):72–8.
26. Grimm F, Jenni L, Bouvier J, Etges R, Bordier, CJAt. The promastigote surface protease of leishmania donovani infantum in the midgut of phlebotomus perniciosus. 1987;44(3):375–7.
27. Saberi R, Moin-Vaziri V, Hajjaran H, Niyyati M, Taghipour N, Kheirandish F et al. Identification of leishmania species using N-acetylglucosamine-1-phosphate transferase gene in a zoonotic cutaneous leishmaniasis focus of Iran. 2018;55(1):14–9.
28. Saberi R, Fakhar M, Hajjaran H, Ataei-Pirkooh A, Mohebali M, Taghipour N et al. Presence and diversity of leishmania RNA virus in an old zoonotic cutaneous leishmaniasis focus, Northeastern Iran: haplotype and phylogenetic based approach. 2020;101:6–13.
29. dos Santos Meira C, Gedamu LJM. Protective or detrimental? Understanding the role of host immunity in leishmaniasis. 2019;7(12):695.
30. Kaufmann SH. Heat shock proteins and the immune response. Immunol Today. 1990;11:129–36.
31. Bifeld E, Lorenzen S, Bartsch K, Vasquez J-J, Siegel TN, Clos J. Ribosome profiling reveals HSP90 inhibitor effects on stage-specific protein synthesis in leishmania donovani. Msystems. 2018;3(6):00214–18. https://doi.org/10.1128/msystems.
32. Yao C, Donelson JE, Wilson ME. The major surface protease (MSP or GP63) of leishmania Sp. Biosynthesis, regulation of expression, and function. Mol Biochem Parasitol. 2003;132(1):1–16.
33. Chan A, Ayala J-M, Alvarez F, Piccirillo C, Dong G, Langlais D, et al. The role of leishmania GP63 in the modulation of innate inflammatory response to leishmania major infection. PLoS ONE. 2021;16(12):e0262158.
34. Brochu C, Haimeur A, Ouellette M. The heat shock protein HSP70 and heat shock cognate protein HSC70 contribute to antimony tolerance in the protozoan parasite leishmania. Cell Stress Chaperones. 2004;9(3):294.
35. Folgueira C, Cañavate C, Chicharro C, Requena J. Genomic organization and expression of the HSP70 locus in new and old world leishmania species. Parasitology. 2007;134(3):369–77.
36. Folgueira Fernández C, Carrión J, Moreno J, Saugar JM, Cañavate C, Requena JM. Effects of the disruption of the HSP70-II gene on the growth, morphology, and virulence of leishmania infantum promastigotes. 2008.
37. Mottram JC, Coombs GH, Alexander J. Cysteine peptidases as virulence factors of leishmania. Curr Opin Microbiol. 2004;7(4):375–81.
38. Descoteaux A. Leishmania cysteine proteinases: virulence factors in quest of a function. Parasitol Today. 1998;14(6):220–1.
39. Cysne-Finkelstein L, Silva-Almeida M, Pereira BAS, dos Santos Charret K, Bertho ÁL, Bastos LS, et al. Evidence of subpopulations with distinct biological features within a leishmania (Viannia) Braziliensis strain. Protist. 2018;169(1):107–21.
40. Black JA, Reis-Cunha JL, Cruz AK, Tosi LR. Life in plastic, it's fantastic! How leishmania exploit genome instability to shape gene expression. Front Cell Infect Microbiol. 2023;13:1102462.

Publisher's note