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Letter to the Editor

Investigation of Leishmania RNA Virus 2 (LRV2) in Cutaneous Leishmaniasis Strains Isolated from Hatay, Turkey

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Received 18 Jul 2022
Accepted 26 Jul 2022

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Dear Editor-in-Chief

Leishmania RNA virus (LRV) is divided into two main groups LRV-1 in New World Leishmaniasis and LRV2 in Old World Leishmaniasis. There is an important link between leishmaniasis and the virus. In chronic cases, in patients who do not respond to treatment and have mucosal involvement, the virus should be considered (1, 2).

In Hatay, the CL causative species in general are *L. infantum/donovani* and *L. tropica*. A small number of cases of *L. major* have been shown to be a causative species (3).

We aimed to investigate LRV2 in strains isolated from the lesions of CL patients in Hatay, Turkey.

Twenty CL isolates in the liquid nitrogen tank (Biyobank) in the Deparment of Parasitology HMKU, Faculty of Medicine, were selected, and their demographic characteristics were recorded. All samples consisted of isolates belong to positive and who received no treatment. The information of five patients could not be reached (Fig. 1).

	City of origin	Age	Gender	Lesion location	Lesion duration	Smear P/N	T/S
1	Altınözü	53	M	Left arm	6 mounth	P	T
2	Hassa	50	F	Right arm	2 mounth	P	T
3	Altınözü	48	M	Right arm	1 year	P	T
4	Apaydın	15	M	Left cheek	7 mounth	P	S
5	Altınözü	55	M	Left leg	2.5 mounth	P	T
6	Antakya	7	F	Left arm	1-1.5 year	P	T
7	Antakya	30	M	Forehead	2.5 mounth	P	T
8	Altınözü	5	F	Throat	3 mounth	P	T
9	Antakya	7	M	Forehead	1 year	P	T
10	Altınözü	17	M	Right arm	1 year	P	T
11	Altınözü	70	M	Right hand	1.5 year	P	S
12	Antakya	44	M	Mouth/Nasal	15 mounth	P	S
13	Apaydın	53	F	Right arm	6 mounth	P	S
14	Reyhanlı	5	M	cheek	2 mounth	P	S
15	Altınözü	37	M	Shoulder	2-3 mounth	p	T

Fig. 1: Demographic characteristics of the isolates in the study
(Smear P: Positive, N: Negative; T/S: T:Turkish, S:Syrian; Gender: F: Female, M: Male)

The samples were removed from the liquid nitrogen tank, then inoculated into an NNN medium. After reproduction, it was transferred to the liquid medium. DNA isolation was performed of samples reaching the logarithmic phase, and isolates were typed by the RT-PCR method targeting an ITS-1 gene region (4).

RNA extraction was performed from samples. Total RNA extraction is performed according to the protocol contained in the RNeasy Mini Kit (Qıagen, Hilden, Germany) kit.

Complementary DNA (cDNA) synthesis was performed using the QuantiTect Rev Transcription kit (Qiagen, Hilden, Germany). PCR was then applied using primers to detect the presence of LRV2 in the *Leishmania* parasite. The primers used for LRV2: F-ATGCTGATAACTTGAAACAGGAG and R-CAT CATTGCCTGTAAGTGAGTAG. As the Internal Control, it was used a KMP 11 primer pair (F-AGATGCAGGAACAGAACGCC and R-TGCTTGAA GTGCTCCGAGTG) amplified a 160-bp length region (2).

For conventional PCR; PCR mix was prepared with a total volume of 20 µl and it was performed on a Thermo Fisher SimpleAmp Thermal Cycler, USA. The resulting products were visualized in 1.5 agarose gel and displayed under UV light.

Of the 20 samples, 17 were typed as *L. tropica*, two as *L. major* and one as *L. infantum/donovani* by the ITS-1 RT-PCR method. No positivity was found in any sample of 20 isolates at PCR performed with specific LRV2 primers. The samples were evaluated by comparing with KMP11, an Internal control (Fig. 2).

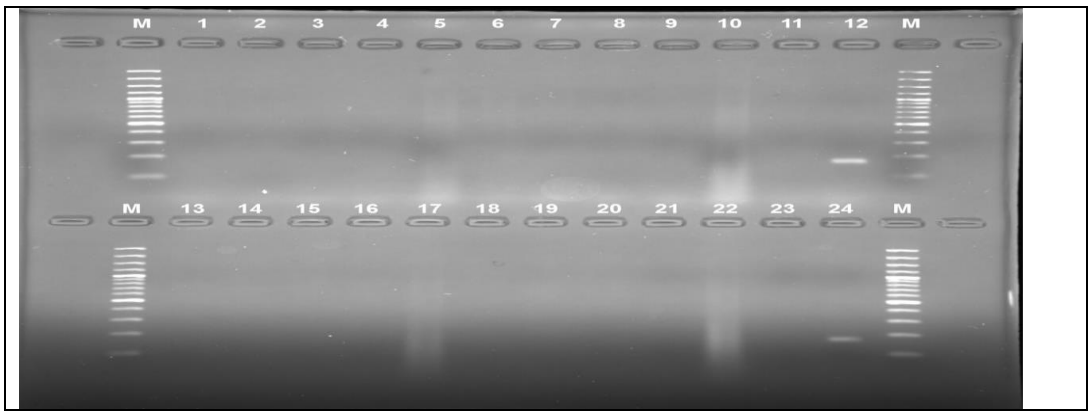

Fig. 2: (Marker: 100bp, 1-10: Leishmania samples, 11: Negative control, 12: Internal Control (KMP11), 13-22: Leishmania samples, 23: Negative control, 24: Internal Control (KMP11)

There are in many protozoon, endosymbiotic double stranded RNA (dsRNA) viruses. In some studies related to New World *Leishmania* species, these viruses have been responsible for CL treatment failure, relapse and aggressiveness of lesions (1, 2, 5).

In Uzbekistan, they found LRV2 positivity in two *(L.major)* of 10 *Leishmania* isolates (6). Hajjaran et all reported LRV in two samples of a total of 50 isolates, one *L. infantum*, and the other *L. major* (5). In Iran, isolates obtained from 85 CL patients were typed 83 as *L. major* and two as *L. tropica*. They found LRV2 positive in a total of 59 samples, 58 L. major and one *L. tropica* strain. The study also reported for the first time that they reported LRV2 in the *L. tropica* strain in Iran (7).

There are still a limited number of studies related to LRV in Turkey. Kurt et al. found LRV2 in a sample isolated from the lesion of patient in Manisa, and was typed as *L. major*. The study was the first LRV positive report in a CL case in Turkey (2). Nalcacı et al. found LRV positivity in 10 of the 29 *Leishmania* isolates. Seven LRV positive samples were *L.tropica*, and three were *L. major* (three CL) isolates. They also reported that it was the first study to detect LRV2 in *L. tropica* strains (1).

In the study, LRV positivity could not be detected. The study is the first to investigate LRV2 in CL isolates in Hatay. Since CL is endemic in Hatay, it was concluded that working with more isolates would be more meaningful in detecting LRV positivity.

Acknowledgments

This study was carried out with the permission of Hatay Mustafa Kemal University (HMKU) Tayfur Ata Sökmen Medical Faculty Non-Invasive Clinical Research Ethics Committee dated 06/07/2020, and it was supported by HMKU Scientific Research Projects Coordination Unit (Project number:20.M.049).

References

1. Nalçacı M, Karakuş M, Yılmaz B, et al. Detection of *Leishmania* RNA virus 2 in *Leishmania* species from Turkey. Trans R Soc Trop Med Hyg. 2019;113(7),410-417.
2. Kurt, O, Mansur, N, Cavus, I, et al. First report and in silico analysis of *Leishmania* virus (LRV2) identified in an autochthonous *Leishmania major* isolate in Turkey. New Microbiol. 2019. 42,64-67.
3. Çulha G, Kaya T, Dogramaci AÇ. Genotyping of Cutaneous Leishmaniasis Cases Detected Before and After Migration with Real-Time Polymerase Chain Reaction in Hatay. Turkish J Parasitol. 2020;44(1),48-52.
4. Toz SO, Culha G, Zeyrek FY, et al. A real-time ITS1-PCR based method in the diagnosis and

species identification of Leishmania parasite from human and dog clinical samples in Turkey. PLoS Negl Trop Dis. 2013;7:e2205.

5. Hajjaran H, Mahdi M, Mohebali M, et al. Detection and molecular identification of *Leishmania* RNA virus (LRV) in Iranian Leis*h*mania species. Arch Virol. 2016. 161(12),3385-3390.
6. Kleschenko Y, Grybchuk D, Matveeva N S, et al. Molecular Characterization of *Leishmania* RNA virus 2 in *Leishmania major* from Uzbekistan. Genes. 2019;10(10),830.
7. Saberi R, Fakhar M, Hajjaran, H., et al. 2020. 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.101,6-13.