Original Article

Treatment Failure in Cutaneous Leishmaniasis Patients Referred to the School of Public Health, Tehran University of Medical Sciences during 2008–2017

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Abstract

Background: Cutaneous leishmaniasis (CL) is a vector borne disease predominantly found in tropical and subtropical countries, including Iran. For more than 6 decades, pentavalent antimonials have been used successfully worldwide for the treatment of leishmaniasis, but over the past few years, clinical resistance to these medications has increased. In this study, we evaluated CL patients who did not show any desirable responses to the anti-leishmanial treatment within a 10-year period (2008 to 2017).

Methods: All patients from different parts of Iran suspected of having cutaneous leishmaniasis, who were referred to the laboratory of leishmaniosis in Tehran University of Medical Sciences from 2008–2017 were parasitological examined.

Results: During this period, a total of 1480 suspected CL patients were referred to the laboratory of leishmaniosis. Samples from 655 patients (70.8%) suspected of having CL were positive microscopically. The failure rate in patients treated with anti-leishmaniasis medications for a minimum of three complete treatment periods was 1.83% (12 cases). There was no association between the number and size of skin lesions and patient characteristics. Also, the route of drug administration had no significant effect on the number and size of lesions.

Conclusion: In the present study, treatment failure was found in some confirmed CL patients treated with meglumine antimoniate. Over the past few years, it seems that had been increased in resistance to these medications. So, a review of the correct implementation of the treatment protocol and/or a combination therapy may be helpful in preventing an increase in the rate of treatment failure.

Keywords: Cutaneous leishmaniasis; Anti-*Leishmania* drug; Treatment failure; Iran

Introduction

Protozoan parasites of the genus *Leishmania* cause a wide spectrum of clinical manifestations known as leishmaniases. Cutaneous leishmaniasis (CL) is the most common forms of this disease in the world, with more than 350 million people at risk. There is an estimated incidence of 0.9–1.7 million new cases each year (1-2). In Iran, two forms of CL have been reported: zoonotic cutaneous leishmaniasis (ZCL) and anthroponotic cutaneous leishmaniasis (ACL). The main pathogen of ZCL in Iran is *Leishmania major* (*L. major*), whereas ACL is mainly due to *Leishmania tropica (L. tropica*) infections (3). For more than 6 decades, pentavalent antimonial (SbV) compounds, such as meglumine antimoniate (Glucantime®) and sodium stiboglucunate (Pentostam®), have been successfully used as the first-line treatment for all forms of leishmaniasis (4). In Iran, the national treatment protocol for cutaneous leishmaniasis recommends intramuscularly administered 20mg SbV5/kg body weight per

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day. The recommended treatment duration is 14 days for ZCL and 21 days for ACL (5). However, over the past few years, there has been increasing concern about the resistance of parasites to Glucantime® (6). Anti-*Leishmania* resistance and treatment failure is a major challenge in new and old-world countries (7-9). Resistance of *L. tropica* to Glucantime® was first reported in Iran in 2006 (10). Previously identified risk factors for the failure of treatment with Glucantime® are body weight above 68kg, previous anti-*Leishmania* treatment, having ≥ 3 skin lesions, and failure to complete a course of treatment (11). In this study, we evaluated cases of treatment failure in CL patients who were referred to the leishmaniosis laboratory of Tehran University of Medical Sciences (TUMS) between 2008–2017 (10 years) (12, 13).

Materials and Methods

Patients

All patients suspected of having cutaneous leishmaniasis, who were referred to the leishmaniosis laboratory of TUMS between 2008–2017 were considered eligible for inclusion in this study. Parasitological confirmation was performed after gaining accurate information about the place of living of patients. After sterilizing the skin around the lesions/nodules with Ethanol 70%, a small incision was made in the margin of the lesion with a disposable lancet, and some tissue and exudates were removed by scraping. The scrapings from the margins of the lesions were air dried, fixed in absolute methanol, and stained with Giemsa 10%. The specimens were then examined for amastigotes demonstration by light microscopy with high magnification (14).

Design

The following data were obtained and recorded for each patient: age, sex, history of travel to endemic areas of leishmaniasis, number of lesions, size of the largest lesion, duration of infection, and adverse events. In this study, we focused more on CL patients who failed to respond to treatment, and who remained positive for smear prepared from the lesions after receiving at least two complete treatment courses. The necessary criteria for investigating cases of relapse, treatment failure, and clinical resistance were as follows:

Relapse: patients who received a topical or systemic treatment courses, whose outcome was improvement but the symptoms (any active lesion) reappeared in the original site of the lesion (15).

Treatment failure: cases in which the lesion remained active after four weeks of complete topical or systemic treatment course (15).

Clinical resistance: cases of relapse and treatment failure, in which active lesions persisted for weeks after at least two complete courses of systemic treatment (15).

According to the criteria above, 40 patients overall did not show any desirable therapeutic response to the following regimen of anti-*Leishmania* drugs:

- Glucantime®: systemic injection (20mg SbV5/kg for two weeks): (No= 8)
- Glucantime®: local injection 1–2ml of Glucantime® intralesionally injection around each skin lesion weekly for 4–6 weeks): (No= 0)
- Glucantime®: systemic and local injection (20mg per kg for two weeks): (No= 3)
- Miltefosine®: oral (2.5mg per kg daily for 28 days) and Glucantime®: (No= 4)
- Antibacterial compounds(Antibiotics): (No= 25)

Data analysis

The data were analyzed using SPSS software version 24. Chi-square and Fisher's exact test were used, and $p \leq 0.05$ was considered significant for differences between groups.

Results

During the 10-year study period, 1480 individuals suspected of having CL were referred to the leishmaniosis laboratory. Of

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these, 655 cases were confirmed positive for leishmaniasis by microscopic examination of samples (amastigotes were seen in microscopic examination). The maximum number of positive cases was recorded in 2018 (116 patients, 17.7 %), whereas the minimum number of positive cases was recorded in 2013 and 2016 (45 cases in each year, 6.9%) (Fig. 1). Out of the 655 positive cases, 464 (70.8%) were males and 191 (29.2%) were females. The youngest patients with confirmed infection was 2 months old and highest age among the positive cases was 80 years old (Fig. 2). About the nationality of the patients, 572 patients (87.3%) were Iranians and 83 (12.7%) were from Afghanistan. Overall, the majority of the patients, 397 individuals (60.6%), lived in Tehran but had a history of trip to endemic regions. Karaj (the capitol of Alborz Province) had the second highest number of cases (48 patients, 7.3%). On the other hands, the Highest number of lesions were one to four (Fig. 3) and also the most common site of the lesions was on the hands with 42/3 % (304) (Fig. 4). Among the patients who were referred to the leishmaniosis laboratory, 139 cases (21.2%) had relapse and were referred to the laboratory for re-examination. In the next step, for follow-up and understanding the phase of the disease, the patients were followed up. In 2008 and 2009, 20 patients with positive microscopic test were lost to follow-up due lack of contact information.

During 2010–2017, 40 cases were initially identified as treatment failure, but only 34 patients could be followed up due to lack of contact information. Out of the 34 patients who were followed up, 22 patients improved by modifying the treatment regimen, such as reinjection of Glucantime®, use of supplementary drugs such as Miltefosine® capsule, use of ointments, and other traditional drugs. Among the remaining 12 cases, even with modification of diet and increase in drug dosage, 7 cases did not show any desirable response to treatment (58.3%) and five cases had treatment failure (41.7%) (Table 1, Fig. 5). Among these 12 individuals with treatment failure, the youngest and oldest were 16 and 62 years old, respectively. Among the patients with treatment failure, the shortest duration of disease was six and the longest was 312 months, respectively.

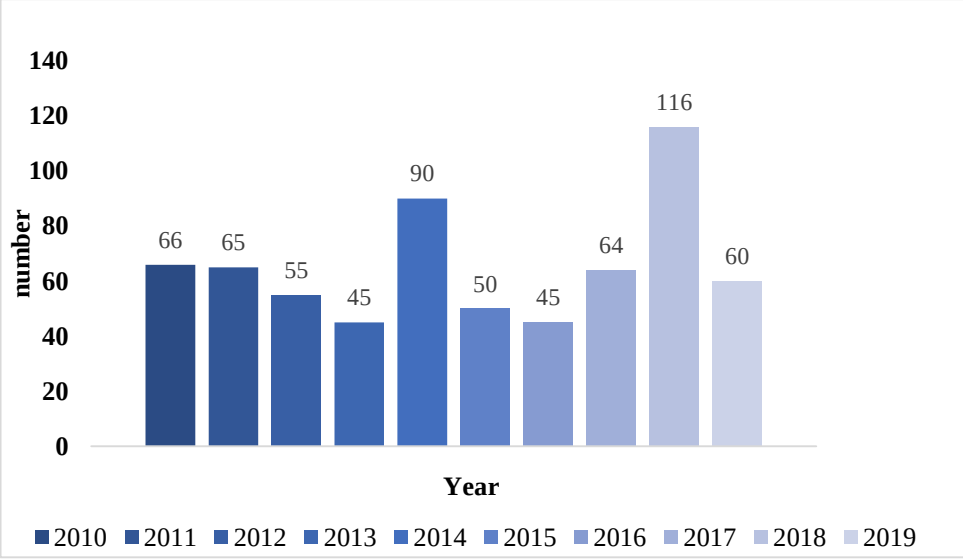

Fig. 1. Distribution of confirmed CL cases referring to leishmaniasis laboratory Tehran University of Medical Sciences during 2008–2017

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Table 1. The characteristics of patients with no responses to anti-*Leishmania* drugs referring to leishmanisis laboratory Tehran University of Medical Sciences during 2008–2017

No	Age (year)	Living place	Travel place during two past years	Duration of persistence of lesion	Site of lesion	Type of administered drug and the type of injection
1	62	Tehran	Firoozkuh	3 years	leg	21 systemic Glucantime®
2	22	Iranshahr	Unknown	6 years	leg, ankle, toes	200 systemic Glucantime® 60 local Glucantime® 56 oral Miltefosine®
3	47	Hesarak Karaj	Qom	4 years	hand	76 systemic Glucantime®
4	30	Qom	Kashan	3 years	hand	100 systemic Glucantime®
5	27	Neyshabour	Unknown	13 years	Face, nose	300 systemic Glucantime® 100 local Glucantime® 70 oral Miltefosine®
6	36	Tabriz	Isfahan	3 years	Ear, eyes	25 systemic Glucantime® 25 local Glucantime® oral Miltefosine®
7	30	Dehloran	Unknown	13 years	back	23 systemic Glucantime®
8	20	Karaj	Afghanistan	6 years	face	200 systemic Glucantime® 100 local Glucantime®
9	16	Mashhad	Unknown	9 years	In nasal mucosa	84 systemic Glucantime® 90 oral Miltefosine®
10	25	Tehran	Afghanistan	26 years	The entire body	21 systemic Glucantime®
11	16	Tehran	Afghanistan	1 year	face	21 systemic Glucantime®
12	43	Unknown	Pakistan	6 months	face	42 systemic Glucantime®

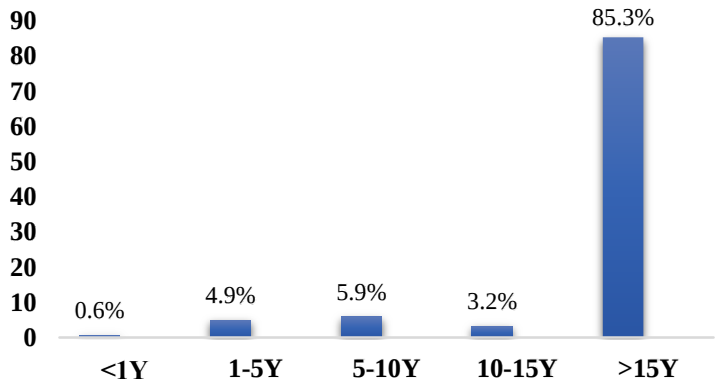

Fig. 2. Frequency of confirmed CL patients referring to leishmaniasis laboratory Tehran University of Medical Sciences by age groups during 2008–2017

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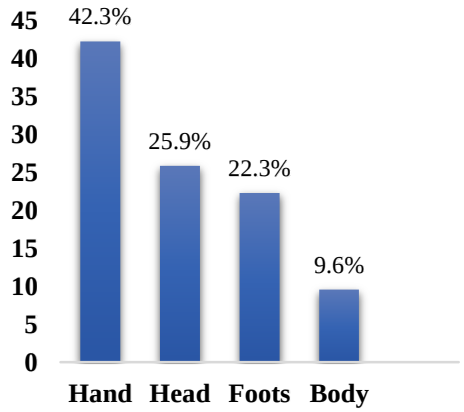

Fig. 3. Distribution of skin lesions on the bodies of the confirmed CL cases referring to leishmaniasis laboratory Tehran University of Medical Sciences during 2008–2017

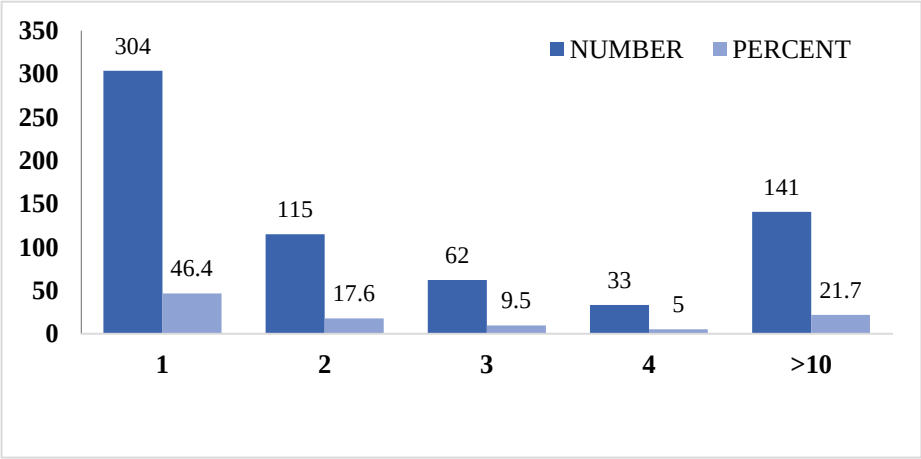

Fig. 4. Distribution of the number of lesions in confirmed CL patients referring to leishmaniasis laboratory Tehran University of Medical Sciences during 2008–2017

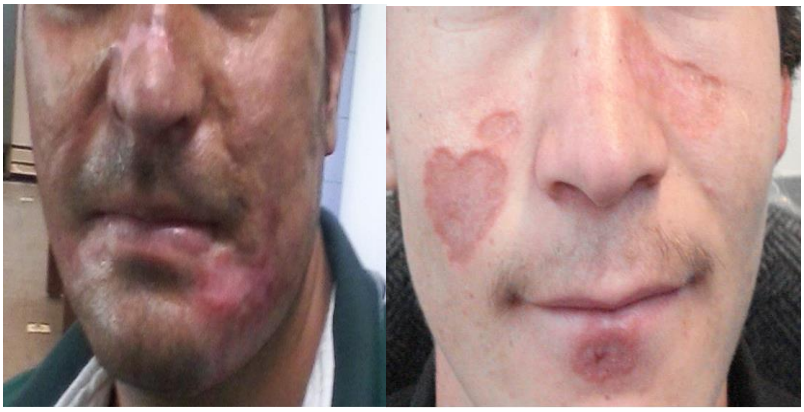

Fig. 5. Skin lesions with no responses to anti-*Leishmania* drugs in two CL patients referred to leishmaniasis laboratory Tehran University of Medical Sciences

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Discussion

In this study, 40 cases were initially confirmed as treatment failure, but only 34 could be followed up. Out of this, 22 improved by changing therapeutic regimen such as reinjection of Glucantime®, use of supplementary drugs such as Miltefosine® capsule, use of ointments, and other unknown drugs (12, 13, 15). However, in the remaining 12 cases, even by changing the therapeutic regimen and raising the drug dose, they did not show any desirable response and treatment failure was observed (Table 1).

Furthermore, in the present study, among treatment resistant patients, the longest course of the disease was 312 months (26 years), highlighting the importance of factors such as immune system of the patient, drug kinetics, and existence of resistant strains (11). There have been various reports on the incidence of drug resistance in different foci of leishmaniasis in Iran. Specifically, treatment failure rate in anthroponotic cutaneous leishmaniasis due to *L. tropica* infection has been reported to be 10.8 % in Mashhad and 11.1% in Bam. In Isfahan, one of the main foci of ZCL, drug resistance was reported as 11.6% in 2005 and in 2013, 3.7% failure rate for topical injection, 4.7% for systemic injection, and 3.4% for concurrent use of both treatment methods were reported (14, 17).

One of the mechanisms leading to the reduced response clinical forms of leishmaniases to the antimony pentavalent compounds is the development of acquired resistance to the drug. Although the antimony pentavalent compounds have been used for several decades as the first-line treatment for all clinical forms of leishmaniasis, unfortunately the therapeutic effect of these compounds has been jeopardized with the emergence of resistance strains in most endemic regions (16). Specifically, in India, Sudan, Latin America, Europe, and Middle East, drug resistance is an important threat to effective treatment of the clinical forms of Leishmaniasis. For example, in India, over 60% of cases of visceral leishmaniasis caused by *Leishmania donovani* do not respond to treatment, which can be due to different reasons such as the development of drug resistance by the parasite, immunologic changes of the patient, ineffective treatment regimen mainly due to lack of patient compliance (23, 24).

In this regard, different studies have corroborated the idea that the resistance to the antibiotics could be acquired. To investigate the existence of acquired drug resistance in Bihar region in India, Lira et al. isolated *L. donovani* from drug responsive patients and those who were confirmed as treatment failure. Using the presence of intra-macrophage amastigote (in-vitro) in isolates as criteria for resistance, they found that the isolates from patients who had responded to treatment were three times more sensitive to Sodium Stibogluconate drug in comparison to drug-resistant isolates. These results confirm the existence of acquired resistance in India (25).

In other studies conducted in France, acquired resistance in clinically resistant *Leishmania infantum* isolates was confirmed in drug resistance tests under in-vitro conditions (26). Also, studies in Latin American countries such as Columbia using in-vitro tests indicated that some cases of treatment failure in new-world cutaneous and mucocutaneous leishmaniasis have been due to development of resistant strains (24-28). Similarly, in Iran, studies in 2004 and 2006 on 185 patients with ACL in Mashhad reported treatment failure in 20 cases (10.8%).

In these studies, drug resistance of isolates was confirmed under in vitro conditions through the culture of macrophages. The isolates which were clinically resistant to Glucantime® were also resistant in drug sensitivity tests, and required higher doses for their elimination. In the anthroponotic form of the disease, the probability of spread of drug resistant *L. tropica* strains

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across human populations is very high, and it's of significant public health importance (17).

Also, acquired resistance of *L. infantum* and *L. major* with zoonotic cycles have been reported in Iran, and have been confirmed under in-vitro conditions. The progression of antimonial resistance in anthroponotic forms such as in India resulting from *L. donovani* and in Iran due to *L. tropica* infections suggests that in the future, resistance to other anti-leishmaniasis drugs such as Miltefosine® and Amphotericin B may also develop in case of extensive usage (11).

Considering the fact that drug resistance is one of the major challenge in the successful treatment of the disease, identification of the mechanisms involved in drug resistance can be useful in improving therapeutic strategies. Furthermore, identification of suitable markers for monitoring and detecting drug resistant cases and predicting the course of development of resistance in endemic regions could be helpful in this regard. Among the most important reasons of resistance are genetic, protein, and enzyme factors, as well as intracellular factors such as signaling pathways and apoptosis. The initial mechanisms in the development of drug resistance include reduction of drug concentration in the parasite through a decreased uptake or increase in drug excretion through cell pumps, deactivation of drug, and inhibition of drug activation in the cell. A recent research indicated that other than these typical mechanisms, other factors such as apoptosis and signaling pathways are also involved in the development of natural drug resistance. Accordingly, today various methods such as real-time RT-PCR, microarray, and proteomic methods are used to detect the factors and genes affecting clinical resistance phenomenon (27, 28).

In Iran, a study conducted by Kazemi Rad et al. (2013) on *L. tropica* to detect genes whose expression is different between sensitive and resistant *L. tropica* using cDNA-AFLP technique confirmed that 13 genes play a major role, the most important of which were as follows: Aqua Glycero Porine (AQP1), affects drug uptake; Multi Drug Resistance Protein A (MRPA), involved in entrapping the drug; Phospho Glycerate Kinase (PGK), involved in carbohydrate metabolism; ubiquitin, involved in degrading oxidized proteins; Amino Acid Permease (AAP3), involved in uptake of arginine amino acid; protein kinase (PK), involved in signaling pathways; mitogen activated protein kinase (MAPK); and protein tyrosine phosphatase (PTP), involved in phosphorylation pathway. Also, in their study, using Quantitative Real Time PCR (QRT PCR) method, it was found that in resistant isolates, there was an increased expression of AAP3, ubiquitin, PGK, PTP, and MRPA, whereas AQP1 and MAPK had diminished expression (29, 30).

In another study conducted by Zaeran et al. (2015) on *L. major* resistance and sensitive to Glucantime® using two-dimensional electrophoresis method performed for determining and comparing expression of proteins, it was found that out of 2967 protein points, 89 points in resistant *L. major* had altered expression compared with sensitive *L. major*; 60 proteins had increased and 29 proteins had diminished protein expression. Also, they found that 11 protein points which did not exist in the sensitive *L. major* were expressed by resistance *L. major*. These changes of expression may be one of the major causes of resistance in *L. major* (31).

Conclusion

Considering the increased rate of drug resistance cases and numerous reports in this regard in different endemic regions of Iran, complete or hybrid treatment should always be taken into consideration by the treatment team in both private and governmental healthcare centers in order to tackle the acquired resistance of the parasite and relapse of disease. On the other hand, researchers should seek to develop new drugs with high efficacy in order to combat problems such as incomplete treatment

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and the presence of resistant strains in the indigent population. There are several reports on different aspects of leishmaniasis in the country. These reports will provide a guideline for disease control (32-66).

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The authors declare that there is no conflict of interests.

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