

Leishmaniasis: Epidemiology, Control and Future Perspectives with Special Emphasis on Egypt

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Abstract

Leishmaniasis is a protozoan disease that is recorded in several Eastern Mediterranean Region (EMR) countries including Egypt. In Egypt, both forms of the disease that are Cutaneous Leishmaniasis (CL) and visceral leishmaniasis (VL) were recorded in several geographical localities. Different Leishmania isolates were isolated from different cases of leishmaniasis where several diagnostic tests that include parasite culturing, patterns of isoenzymes, inoculation of experimental animal models and molecular methods were used to define the underlined causative organism in each case. However, several reports of conflict in these studies make it harder to compile a map of disease distribution, its prevalence, the causative Leishmania species and the type of vector sandfly involved. Another significant problem is the absence of notification system with many of disease cases were just went unreported or been treated from dermatological aspect (CL) or internal medicine aspect (VL). All of these factors contribute to the neglect of the disease by health authorities and hinder the prospective of any control programs that can be deployed to combat it. In this review, we will start by summarizing the most recent data on the epidemiology, disease patterns and the life cycle of the causative organism. Then, in a more specific way, we will move to discuss the history of the disease and its geographical distribution, and to proceed by discussing the currently applied methods of diagnosis, and to finish by highlighting the control and treatment programs that are in act against the disease. All of the above will principally contribute to a better understanding of the disease epidemiology and will provide a foundation upon which more effective control and treatment programs should be developed.

Keywords: Leishmaniasis; Cutaneous leishmaniasis; Egypt; Epidemiology; Diagnosis; RFLP-PCR; Antimony

Abbreviations CDC: Center for Disease Control and Prevention; WHO: World Health Organization; CL: Cutaneous Leishmaniasis; VL: Visceral Leishmaniasis; MCL: Mucocutaneous Leishmaniasis; PCR: Polymerase Chain Reaction, PKDL: Post Kala-azar Dermal Leishmaniasis.

Note for readers, Information in this review article are based on original research articles, bibliographies, other review articles, and from the personal archives of Leishmania's scientists and researchers.

Introduction

What is Leishmaniasis?

Leishmaniasis is an infectious disfiguring vector-borne disease, which is caused by protozoan parasite of genus Leishmania, and in which humans get infected through the bite of infected sandfly of genus phlebotomus [1-3]. The first scientist who reported on protozoan parasite of Leishmania was Surgeon Major Cunningham of the British Indian army; who in 1885 described the organism but failed to connect it to the related disease [4,5]. In 1903, physicians Leishman and Donovan were first to describe the protozoan causative agent of visceral leishmaniasis. They recovered it in stained smears from spleen of affected patients who developed symptoms of malaria-like disease, and later named it Leishmania donovani [6,7].

Epidemiology, disease patterns and life cycle of Leishmania parasites

The disease, Leishmaniasis, prevails throughout different geographical regions of the globe, but occurs more specifically in the

tropics and sub-tropics of Africa, South and Central America, the Middle East, Southern Europe and Asia [2,3]. According to the most recent reports by WHO and CDC, Leishmaniasis affects people in nearly 88 developing and developed countries where about 350 million people are living in these regions. The disease is reported in approximately 12 million people worldwide with recorded incidence of 1.5-2 million new cases each year of cutaneous form and 500,000 new cases of the visceral form of the disease [8]. According to clinical manifestations, leishmaniasis could be broadly classified into three major clinical forms [2]. These are cutaneous leishmaniasis (which is the most common form), mucocutaneous leishmaniasis and visceral leishmaniasis (the most serious form of the disease). The cutaneous form of leishmaniasis occurs on the epidermal layer of the skin and begins with a papule at the site of the vector sandfly bite. The papule then grows in size and after sometime it eventually turns to crust form which may also ulcerates. Unless it is complicated by secondary infections, majority of cutaneous cases will eventually heal on itself within 2-10 months [9]. In the mucocutaneous form of the disease, the incubation period is 1-4 months. The lesions of the mucocutaneous form extend from the skin to the nose, oral cavity and pharynx which associate with difficulties in respiration and eating with considerable risks of mortalities [1]. In the third form, the visceral leishmaniasis, the incubation period

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is extendable from 3 to 8 months with symptoms varying in severity from fever, skin pigmentation (kala azar; black disease) and weight loss to more severe lesions of hepatosplenomegaly, lymphadenopathy, pancytopenia and death [10].

The life cycle of *Leishmania* species involves final mammalian host and intermediate sand fly host [11-14]. The sandfly vector acquires *Leishmania* infection in the form of macrophages containing amastigotes during feeding on blood of infected mammal. Once in the midgut of infected fly, the round non-flagellated amastigotes (2-7 μm) transform into elongated flagellated promastigotes (10-20 μm). Promastigotes in turn multiply and penetrate midgut to migrate to salivary gland and proboscis to transform into metacyclic promastigotes. When infected sand fly vector feeds on new host, it injects infective metacyclic promastigotes into wound of affected host. At the site of bite and wound, promastigotes are taken up by macrophages where they get rid of flagella and revert to amastigotes. Infected macrophages then spread locally at the site of bite (in the case of cutaneous leishmaniasis), or they migrate to different internal tissues and organs such as spleen, liver and bone marrow (in the case of visceral leishmaniasis).

History of leishmaniasis in Egypt

Evidence of infection by *Leishmania* organisms was reported among ancient Egyptians around 4000 years ago (during the middle kingdom). This is probably due to the close trading relationship with Nubia where VL was believed to be prevailed during that time [15]. The evidence proof came from successful amplification of *Leishmania donovani*'s DNA from bone tissue samples of ancient Egyptian mummies that originated from pre- to early dynastic era (3500-2800 BC) [15]. Since the mid-to late twentieth century, sporadic cases of CL and VL have been reported among Egyptian people as some of these were imported; while others were indigenous [16] Table 1.

Geographical distribution

In Egypt, most foci of *Leishmaniasis* are confined to Sinai and Suez Canal region, and in some cases (at long extending intervals), infections tend to spread to other parts of the country such as Agamy region in Alexandria North of Egypt. On the other hand, cutaneous leishmaniasis is the most detected form, and with some exceptions such as foci of visceral leishmaniasis in Agamy, Alexandria, the cutaneous leishmaniasis was responsible for more than 90% of the disease epizootics in Egypt. Despite the confined localization, fear due to the possibility of the disease transmission to other parts of the country still exists. Thus, CL currently represents an increasing problem in Egypt especially when combined with other contributing factors such as the problem of non-mandatory notification of leishmaniasis, and the lack of implementing an effective control program of leishmaniasis due to financial problems and the standstill political turmoil [17,18].

Diagnosis of Leishmaniasis

Since treatment of cases of leishmaniasis is species dependent, it is very essential to perform accurate diagnosis of the diseased case. The inherent problem with diagnosis of *Leishmania* infections is the lack of awareness among medical professionals, as both CL and VL cases are suspected to be underreported [16]. In Egypt, it is not mandatory to notify of leishmaniasis occurrence and the disease is not on the list of notifiable diseases by the government authorities. It is believed that import of several leishmaniasis cases from Libya and Sudan may occur on regular basis. Nevertheless, these imported cases might go unnoticed due to the lack of proper quarantine measures and surveillance mechanisms on the border regions with both countries

[16]. In most cases, diagnosis is based on the presented clinical symptoms of the disease, such as nodular and ulcerated lesions of the skin that accompany history of bite by sandfly. However, the widely varied clinical presentation of the leishmaniasis makes the diagnosis of active cases a challenging and tricky procedure. Moreover, in the most active diseased cases of leishmaniasis it is necessary to perform differential diagnosis against other diseases with closely similar clinical spectra such as leprosy, skin cancers and tuberculosis for CL; and malaria and schistosomiasis for VL [19]. Beside clinical manifestations, a number of diagnostic methods are currently applied for identification of leishmanial organisms, and these are summarized in Table 2. For visceral leishmaniasis, the most widely applied method of identification is based on the direct detection of diagnostic stages (amastigotes) in the Giemsa-stained smears from tissue aspirate or biopsy from internal tissues and organs [2]. Cutaneous leishmaniasis, on the other hand, is mostly diagnosed by detection of diagnostic stages in microscopic smears from skin lesions or biopsies that are usually collected by scraping from the edge of lesions. The direct microscopic examination in both cases is rapid and low-cost effective, but has very low sensitivity especially when parasites are low abundant (chronic infection) [1]. Beside the microscopic examination, a number of other diagnostic techniques and tools being used in clinics and research laboratories for detecting *Leishmania* infections, and these are reported in Table 2. In Egypt, some of these diagnostic methods are applied in the identification of causative organisms in suspected leishmaniasis cases or under surveillance programs.

Control and treatment of leishmaniasis

Control: The transmission life cycle of *Leishmania* involves the definitive host (mainly human and dog), the reservoir host which is mainly rodents, and the vector hosts of sandflies. Therefore, a combination of strategies should be applied in the control and treatment of eat leishmaniasis. This is mainly depends on case identification, swift treatment, control of flies and reservoir hosts and the protective vaccination whenever possible.

In addition, the control of the disease relies on the epidemiological map and customs of the people in the targeted area [20]. The vector, sand fly, is very vulnerable to insecticide [21]; hence practices such as spraying houses with insecticide are effective in reducing the cutaneous leishmaniasis especially when the sand flies are endophilic [22,23]. Additionally, pesticide-treated and untreated bed nets have shown efficacy in area where the sandflies are endophagic [24]. For cutaneous leishmaniasis, an alternative control option depends on implementing the insecticide-impregnated materials such as bed nets, clothes and bed sheets [22,25,26] and curtains [22,25,27,28]. Alternatively, applying repellents as a defensive mechanism against bite of sandfly proved itself as an effective control potential especially in regions with poor health facilities [25].

In countries with endemic VL such as Sudan, the incidence has been sharply reduced after a community distribution of insecticide-treated nets [29]. Moreover, there were studies stated that the use of impregnated bed net and going to bed early could result in a high degree of personal protection against VL [30]. Despite the positive attitude, the use of bed nets in Northern and Western Africa as a sandfly control is restricted. This is because of the fact that during the hot season it is considered not too comfy to sleep under the fine-mesh nets because it is too hot with limited ventilation [31]. Moreover, during the dry season people prefer to sleep outdoors and may be refusing to use nets. Finally, the use of house spraying as a vector control in these countries is limited by logistic constraints and the associated high cost [32].

Year	Case	Location	Vector host	References
1982-1985	113 (CL)	East Sinai	P. papatasi	Mansour et al. [86]
1982-1985	27 (VL)	El Agamy area, Alexandria	P. langeroni	Faris et al. [94]
1983-1984	80 (CL)	73 in northern Sinai, 6 in central Sinai and one in southern Sinai	P. papatasi	Fryauff et al. [95]
1987	2 (VL)	El Agamy area, Alexandria	P. langeroni	Awadalla et al. [86]
1988	5 (CL)	Multi-localities	P. papatasi	WHO;Neouimine [50]
1989	6 (CL)	Multi-localities	P. papatasi	WHO;Neouimine, [50]
1990	2 (CL)	Multi-localities	P. papatasi	WHO;Neouimine, [50]
1991	0	Multi-localities	P. papatasi	WHO;Neouimine, [50]
1992	84 (CL)	Multi-localities	P. papatasi	WHO;Neouimine, [50]
1993	12 (CL)	Multi-localities	P. papatasi	WHO; Neouimine [50]
1994	1 (CL)	Multi-localities	P. papatasi	WHO;Neouimine [50]
1995	5 (zCL)	North Sinai governorate	P. papatasi	Morsy et al. [96]
2003	16 (zCL)	Sinai and Suez canal governorate	P. papatasi	Hamadto et al. [96]
2004-2010	37 (CL)	Al-Hussein University Hospital, Cairo, Egypt	P. papatasi	El-Khalawany et al. [92]
2005	229 (CL)	Multi-localities	P. papatasi	Alvar et al. [17]
2006	178 (CL)	North Sinia	P. papatasi (L. major) P. sergenti (L. tropica)	Alvar et al.[17] Shehata et al. [106]
2007	287 (CL)	Multi-localitiesT	P. papatasi	Alvar et al. [17]
2008	47 (CL)	North Sinai		
2008	1 (VL)	Suez region	P. papatasi	Alvar et al. [17]
2009	174 (CL)	Multi-localities	P. papatasi	Alvar et al. [17]
2010	318 (CL)	Multi-localities	P. papatasi	Alvar et al. [17]

Table 1: Summary of Leishmaniasis foci in Egypt. zCL; zoonotic cutaneous leishmaniasis.

Regarding the control of the animal reservoir hosts, L major zoonotic and localized cutaneous leishmaniasis (LzCL) foci were controlled by destroying burrows of the rodents, which is the main LzCL reservoir [33]. In another example, in Tunisia, a significant reduction of the incidence of cutaneous leishmaniasis among the local human population has been achieved by the destruction of Psammomysobesus burrows by deep ploughing, removal of chenopods and planting of trees in a 2-3 kilometer zone surrounding human settlements. Furthermore, application of deltamethrin-impregnated dog collars was found to be an effective in protecting domestic dogs against VL infection. The approach was successful in reducing the risk of L. infantum infection in Iranian children; and in offering a good replacement of the controversial dog culling programs in several countries [34].

In Egypt, the use of insecticide spraying to control vector flies including the sandfly is regularly done, nonetheless it showed no efficacy. Additionally, some control programs to eliminate or reduce reservoir hosts were applied but it is not specifically targeted for leishmaniasis [17].

In spite of the successful trials of the different programs of vector and reservoir control, and their possibility to reduce risk of leishmaniasis, different health authorities still rely mainly on the treatment especially during the disease outbreaks. Currently, a number of chemotherapeutic agents are being applied in the treatment of both forms of leishmaniasis (Table 3). Despite the wide access and use, most of these chemotherapeutics have drawbacks such as they are non-specific, costly, difficult to administer (require extended treatment durations) and have adverse side effects. More recently, several studies are initiated with the sole purpose of identifying novel anti-Leishmania drugs that can be effective, cheap and with shorter treatment durations. Under most cases, the use of anti-leishmanial medicines are more effective than the vector and reservoir control programs which necessitate some environmental management strategies that are expensive, labor-

intensive and in many cases because of their logistic constraints.

Vaccination: A vaccine against leishmaniasis is theoretically possible since most individuals that were once infected become resistant to clinical infection when later exposed [35]. More interestingly, leishmaniasis is considered unique among parasitic diseases because a single vaccine could successfully protect against the infection with more than one species [36]. Leishmania is an intracellular pathogen since it requires strong cell mediated immunity to be controlled. However most of vaccines trials provide humoral response which is not adequate against Leishmania.

The century-old practice of “Leishmanization”, which is based on the deliberate inoculation of virulent Leishmania from the exudate of a cutaneous lesion, is the oldest vaccination procedure against cutaneous leishmaniasis. The practice was applied for centuries but later becomes increasingly unacceptable due to its serious side effect [37]. Therefore, the attention was drawn to the live attenuated and killed vaccines [38]. In 2008, the first generation vaccine was developed and was based on the whole killed parasite, and was tested against CL and VL with an average clinical efficacy as low as 54% [39]. However, and long before that, there was a field vaccination trial against human Leishmania infection that was performed in Sudan by using an autoclaved L. major vaccine with BCG and it achieved 43.3% vaccine efficacy [40]. Leish-111f is the only single product as a therapeutic vaccine that entered phase II of clinical testing in human against visceral leishmaniasis [36,41]. It is composed of fusion of the three relatively conserved L. major proteins that is formulated with MPL-SE. Nonetheless, there is no single vaccine that is licensed for prophylaxis against VL in human [42]. In 2013, Guha et al. demonstrated the potential of hemoglobin receptor (HbR) as a vaccine candidate against visceral leishmaniasis where they found that HbR is conserved across various strains of Leishmania and can potentially induce a complete protection against virulent Leishmania donovani infection challenges in both mice and hamsters [43].

Method	Disease	Comment	References
Microscopic examination of Giemsa-stained lesion biopsy smears (CL); or lymph node, bone marrow, and spleen aspirates (VL)	CL and VL	High specificity (gold standard) because its availability especially in endemic area. In some cases, the sensitivity of microscopy is a disadvantage.	Herwaldt [1]
In vitro culture in combination with multilocus isoenzymes electrophoresis	CL and VL	Allows for identification and characterization of parasite species. It requires a wealth of technical expertise and is time-consuming. Data from different experiments tend to be highly variable (low specificity).	Herwaldt [1]
Detection of K39 antibodies by using rK39 antigen. (commercially available as immunochromatographic dipstick tests)	VL	High sensitivity and specificity. Are easy to use, and require minimal technological expertise and laboratory setup.	Chappuis et al. [89] Singh et al. [107]
The direct agglutination test (DAT)	VL	Semi-quantitative, it was validated in several endemic areas and is being used for diagnosis of visceral leishmaniasis in countries such as Sudan	Chappuis et al. [89] Allain & Kagan [82]
Montenegro Skin Test (MST)	CL	Easy to use, highly sensitive, specific but requires culture facilities to produce the MST antigen and in the same time does not distinguish between past and present infections	Weigle et al. [115] Asilian et al. [84]
A latex agglutination test (KATEX) for the detection of leishmanial antigens in the VL patient's urine	VL	100 % specificity, with a range of sensitivity between 68-100%	Attar et al. [85]
Detection of Leishmania DNA	VL and CL	Highly sensitive, specific, and more rapid than the currently available methods—namely, serological tests, the MST, and microscopic examination of lesion biopsy stained with H&E staining or immunostaining. 100% specificity was recorded for PCR detection of Leishmania DNA in bone marrow aspirates. Also, PCR has been proved to be the most important tool for diagnosis of chronic cutaneous leishmaniasis.	Martin-Sanchez et al. [101] Tavares et al. [113] de Oliveira et al. [91] Singh [107]
Isolation by experimental animals inoculation and histopathologic examination of biopsy samples	CL and VL	Without identification of amastigote form in biopsy samples, the technique is rarely good enough to make a specific diagnosis	Singh [107]
Indirect Fluorescent Antibody (IFA) test to detect anti-leishmanial antibody, while the Direct Fluorescent Antibody (DFA) test to detect the antigen (amastigotes).	CL and VL	IFA is useful in very early stages of infection, sensitivities are variable. DFA test is more useful in the diagnosis of CL, MCL and PKDL	Singh et al. [108] Boelaert et al. [88] Iqbal et al. [97]

Table 2: Different diagnostic methods of leishmaniasis.

More recently, the control program of leishmaniasis with vaccination has been drawn toward the use of genetically modified live attenuated vaccine, where the concept of vaccination with genetically modified *Leishmania* parasite is stimulation of protective immunity by dissociation of virulence from the parasites. This has mainly been done by intervening with the *Leishmania* virulence genes, and in which a mutated parasite is obtained that lacks the virulence potential. In 2009, *L. donovani* centrin null mutants (*LdCen-/-*) has been found to protect BALB/c mice and Syrian hamsters against homologous as well as heterologous infectious challenge [44]. Nevertheless and despite many trials, very limited numbers of genetically modified live-attenuated forms of *L. donovani* exist as vaccine candidates. Another development in the anti-*Leishmania* vaccination is the use of non-pathogenic species to protect against subsequent challenge by more pathogenic types. The best example was demonstrated by the use of *L. tarentolae* which showed a potent immune -protection against *L. donovani* challenge in BALB/c mice [45].

Despite its promising experimental results, the use of live *Leishmania* vaccines, whether non-pathogenic or genetically modified, has several limitations with the most notable example in the immunocompromised

patients due to the potential of conversion of attenuated strains to more pathogenic types. Moreover, the role of sandflies transmission in safety blocking of the vaccine strain is still needed to be further studied. Finally, the presence of antibiotic resistant genes in the attenuated parasite strains antagonizes their use in clinical studies [46].

Although much effort was spent in developing a vaccine (from “Leishmanization”, till killed and modified genetic vaccines), and the wealth of biological and genetic information of *Leishmania*, there is no vaccine that is currently licensed to protect against human leishmaniasis [19].

Treatment: Since no licensed commercial vaccine against leishmaniasis is available until now, the main control strategy is depending on therapeutic (chemotherapeutic) treatment approach. In treating different leishmaniasis cases, outcomes vary and this depends on species, the geographic localities, and the clinical presentations. Therefore, a species-specific approach should be considered when treating the diseased cases [47,48]. Some control programs cannot provide anti-leishmaniasis drug free of charge therefore, leishmaniasis patients have to look for the drugs in the markets which are costly and often out of reach of patients especially of the low socioeconomic

Disease pattern	Drug	Dose	Comments	References
Visceral leishmaniasis	Pentavalent antimony (SbV) Sodium Stibogluconate or Meglumine Antimonate	20 mg / kg I/M or I/V for 28 days, depending on species or the clinical syndrome	Increasing resistance to antimonials is a major problem. Reducing the treatment period for 10 days course may minimize the resistance effect. The cure rate is 90% to 95%	Murray et.al. [2] Wortmann et. al. [116]
	Liposomal form of Amphotericin B	3 mg / kg per day I/V for 5 days plus an additional 3 mg / kg dose on the tenth day	It is the preferred treatment method in Southern Europe Highly effective, less toxic and tend to be costly expensive, although shorter course duration may result in improving cost benefits. The cure rate is up to 98%.	Piscopo and Mallia Azzopardi [103] Sundar et.al.[112] Murray [2] Davidson et al. [90]
	Miltefosine	2.5 mg / kg per day for 28 days	It is the first effective orally active drug. It has the benefit of a very good safety profile. The potential is high for using this drug in poor areas with limited health access. The cure rate ranges from 95% to 100% after 3 or 4 weeks of continuous application. Treatment outcomes vary based on age of patients.	Jha et. al. [99] Sundar et al. [112] Sundar et.al. [112] Murray et.al. [2] Bhattacharya et. al. [87]
	Pentamidine	300 mg dose as I/V injection	The drug use is now very limited due to its substantial toxicity.	Jha, [63]
	Paromomycin (aminosidine)	16–20 mg / kg per day I/M for 21 days	Cost saving. High potential developing nephrotoxicity or ototoxicity. Needs further evaluation. The cure rate is 93-97%.	Rosenthal and Marty [104] Jha et.al. [98]
Cutaneous leishmaniasis	Paromomycin	Two ointments per day for 10–30 days	Mainly for <i>L. major</i> while less effective in the case of <i>L. tropica</i> lesions. The cure rate varies from 74% to 86%, and it is higher with repeated application.	El-On et al. [93] Asilian et.al. [73] Ozgoztasi and Baydar [68]
	miltefosine	2.5 mg / kg per day orally for 28 days	In Colombia, it is used for <i>Leishmania viannapanamensis</i> . In Guatemala, is used for <i>L. v. braziliensis</i> and <i>L. mexicanamexicana</i> . Also, it is useful oral agent against cutaneous leishmaniasis due to <i>L. v. panamensis</i> in Colombia but not against leishmaniasis due to <i>L. v. braziliensis</i> in Guatemala. The cure rate varies and it is 91% in Colombia, and 53% in Guatemala.	Soto et.al. [110]
	Pentamidine isethionate	4 mg / kg pentamidine-base	Used to treat cutaneous Leishmaniasis in France. It is highly recommended to use the minimal or lower doses of this medicine.	
			For <i>L. braziliensis</i> . In this case the cure rate is less than 50%.	Andersen et.al. [83]
	Pentavalent antimony (SbV)	Applied as intralésional as 1 mL per lesion every other day for 8-15 injections OR used as 1 mL per lesion every other day weekly for 8-11 injections (on average)	For <i>L. major</i> . The cure rate is equal or more than 75%.	Salmanpour and Nouhpsheh [105]
			<i>L. tropica</i> . The cure rate is equal or more than 90%.	Uzun et.al. [114]

Table 3: Commonly treatment regimens of leishmaniasis

class. Additionally, the high cost of medicines result in interruption of treatment with possibility of development of chronic and debilitating form of the disease [49]. Also, some considerations are to be taken into account when treating cutaneous rather than visceral form of leishmaniasis.

Visceral leishmaniasis: The first line drugs against visceral leishmaniasis are pentavalent antimonials [50]. These drug classes have been used over the past 70 years and are available in two formulations, methylglucamine antimoniate and sodium stibogluconate [51,52]. Despite the long-term use, these compounds have drawbacks such as long duration of treatment, relatively high cost. Moreover several indications of cardiac, renal, hepatic and pancreatic toxicity have

severely limited their use in the elderly people, pregnant woman, and individuals with cardiac diseases and liver alterations [50,53]. The second-choice drugs are the different formulations of amphotericin B due to its high efficacy and low toxicity [54]. In the Indian subcontinent and Europe, liposomal amphotericin B has been used as a first-line drug to treat VL due to the increasingly developed resistance to antimonials in some areas [55]. Miltefosine is the second drug of option to treat VL in the Indian subcontinent since it showed high efficacy in treating moderate cases of VL cases [56,57]. At the time of its first use, Miltefosine has been a breakthrough because it was the first effective orally active drug against leishmaniasis that showed a cure rate between 95% and 100% [58]. However, in other parts of the world, miltefosine is stated to have low efficacy than those of anti-monials.

Another alternative drug of choice to treat VL is systemic paromomycin where it produced high efficacy in the treatment of VL in the Indian subcontinent [59,60], whereas this efficacy is low in the East African region and therefore, it is recommended that this drug is used in high doses or in combination with pentavalent antimonials [61]. The imidazoles (ex. Ketoconazole) and the structurally related triazoles (ex. fluconazole and itraconazole) were firstly introduced as antifungal drugs but afterward were found to have an anti-leishmanial activity. These drugs have the advantage of oral administration and the low adverse effects, but are only effective against some *Leishmania* species [47]. Therefore, again in some cases the causative species determine the type of necessary treatment.

Other drugs used in treating leishmaniasis have also been effective in the case of VL. Pentamidine is another agent that could be used in antimonial-resistant patients with visceral leishmaniasis [62]. However, and due to its substantial toxicity, its use is very limited and requires close inpatient monitoring [63].

Cutaneous leishmaniasis: Antimony has been used for years in the treatment of cutaneous leishmaniasis in the shape of systemic (intramuscular or intravenous) or intra-lesion. The use of these drugs is species and clinical presentation dependant [8]. Local infiltration with pentavalent antimony has been used in the Old World localized cutaneous leishmaniasis. Intra-lesion antimonials were found to be less effective in the case of cutaneous leishmaniasis due to *L. tropica* [64]. Another intra-lesion therapy, hypertonic sodium chloride solution or zinc sulphate, was shown to be very effective as a local therapy and was as effective as local sodium stibogluconate in few cases of cutaneous leishmaniasis in Iraq [65,66].

Topical formulations present several advantages over systemic therapy, such as the ease of administration, fewer side effects and the cost effectiveness [47]. Paromomycin, an aminoglycoside antibiotic has been used systemically against both visceral and cutaneous leishmaniasis. Likewise, it has been applied topically as an ointment for treating cutaneous leishmaniasis. It was found that combination of paromomycin with methylbenzethonium was more effective than the combination with urea, but it causes more local inflammatory reactions [67]. Also, paromomycin seems to be less effective in case of *L. tropica* lesions [68].

Imiquimod, a topical immunomodulator showed a leishmanicidal activity during the in vitro infection assay and in infected mice by inducing the expression of the inducible nitric oxide synthase (iNOS) gene and the release of nitric oxide [69]. Recently, imiquimod was used in the form of 5% cream plus meglumine antimoniate. This combination presented a rapid cure in CL-affected patients than those of meglumine antimoniate only with vehicle control [70]. Nevertheless, the drug by itself was ineffective in treating the Old World cutaneous leishmaniasis when it applied topically [71].

Other treatment forms against cutaneous leishmaniasis include the use of temperature whether by using low temperature or high temperature. Cryotherapy has been used for the treatment of Old World cutaneous leishmaniasis. For instance, around 90% healing rate from *L. tropica* infection in turkey was reported after one session of cryotherapy in combination with liquid nitrogen [72]. Another example for using of cryotherapy was reported in two studies in Iran, but this time in combination with local antimonials [73,74]. In the first study, high recovery rates from cutaneous leishmaniasis were reported with cryotherapy combined with intra-lesion antimonial, than antimonial by itself. In the second temperature-based treatment

approach, localized controlled heat treatment was applied in which heat (50°C for 30 seconds) is directed to the lesion from the thermosurgery localized current field radiofrequency generating device (ThermoMed® ThermoSurgery Technologies, Inc, Phoenix, Arizona). It was reported that localized controlled heat treatment was as effective as meglumine antimoniate in the Guatemalan *L. mexicana* induced cutaneous leishmaniasis. Heat treatment was also successfully used to cure 26 soldiers suffered from *L. major* induced cutaneous leishmaniasis during their deployment in Iraq [75]. Similarly, comparable results to intra-lesion antimony were obtained with the localized heat treatment of *L. tropica* induced cutaneous lesions in Kabul, Afghanistan [76]. Nonetheless, and despite the ease of use, the localized controlled heat is less acceptable to patient and has some drawbacks. These include painful distress, the slower re-epithelization of lesion than those that are treated by meglumine antimoniate, and the risk of local infection [77]. Also, the heat-based treatment did not provide satisfactory remedies in *L. braziliensis* infected patients. This is mainly because of the spread of infection to distant mucosal tissue beside localized cutaneous lesions [77,78].

Lasers have been used for treatment of several skin diseases since 1970, and carbon dioxide (CO₂) lasers are now being used for treatment of leishmaniasis [79,80]. Vaporization of local lesions by CO₂ laser (both wet and dry types) was proved effective for treatment of cutaneous leishmaniasis caused by *L. major* [80] and *L. tropica* [81]. The use of laser treatment stimulate wound healing rapidly and produce better aesthetic results with no recurrences for around 7 years.

In Egypt, CL cases are treated with antimonials with satisfactory remedy outcomes, however the big limitation is that the drug is not provided by the Ministry of Health or private pharmacies with the only available source is WHO [17]. Liposomal amphotericin B (AmBisome, Gilead) is registered in Egypt, but no available data of its use in combating *Leishmania* infection. In 2008, WHO donated antimonials for the topical treatment of less than 100 patients, but the records of reported CL were 471 cases. Therefore, not all cases were subjected to drug treatment. Such shortage of the available treatments made nomads from North Sinai region to treat themselves by extinguishing cigarettes on their lesions or use substances such as vinegar and bleach as topical remedies. VL cases, when reported, are only diagnosed and treated in specialized medical clinics.

Conclusion

Leishmaniasis is an obligate intracellular vector born parasitic disease caused by kinetoplastid protozoan parasites of the genus *Leishmania* and transmitted by vector host of sandflies. It causes considerable morbidity and mortalities in several countries over the world with reports of 1.5-2 million new cases each year of CL form and 500,000 new cases of VL form of the disease. Therefore, the need of less expensive and effective treatment of Leishmaniasis is required particularly when an effective vaccine to protect against human disease is not in the near sight future. Several medications are available to treat different forms of the disease, albeit with several registered side effects such as toxicity and high expenses especially for the low socioeconomic class and people of developing countries. Now with the emergence of newly developed oral drugs, which might change the way this disease is managed. Contiguously with the treatment, accurate diagnosis is a very critical part in the discovery and treatment course of the disease. The treatment in many cases is species-dependent and requires differential diagnosis from closely related diseases, and all these factors improve the

prognosis course of the disease.

Future Perspectives

The recent advances in the field of molecular parasitology and its applications regarding protozoan diseases including Leishmaniasis brings into horizon new approaches of the disease diagnosis and control. Several molecular methods have successfully been used and evaluated in diagnosis of Leishmania. One of these methods is the PCR-based assay. Nowadays the molecular technique of PCR constitutes one of the main diagnostic techniques in research and health facilitates. Several PCR derivative assays are currently under evaluation in the diagnosis of leishmaniasis. PCR allows a highly sensitive and specific (up to 100%) detection of the Leishmania parasite, and it has been very useful in the identification of the causative Leishmania species. When it is compared with conventional methods such as microscopy and cell culture for the identification of Leishmania species, PCR is very sensitive and highly species specific. Nevertheless, the technique still mainly available and applicable in the research facilities, and its implementation in the health facilities and clinical practices requires technical expertise which is not commonly available in poor and developing countries. In the future, more efforts will be required to make from the PCR technique as much as feasible, easy and cost-effective method of diagnosis particularly in areas with high disease endemics.

Despite its widely accepted drawbacks, chemotherapeutics remain up to date the most significant strategy in treating and controlling leishmaniasis. However as the disease spreads globally, and its transmission rates remain unbeaten, the sole dependence on chemotherapy is no more acceptable and there is the disease situation unquestionably demands the development of an efficient vaccine plus other control strategies. Currently, developing an effective, human-safe vaccine remains a challenging procedure owing to the antigenic diversity and the fact that the parasite has a digenetic life cycle in two divergent hosts (human and the sand fly vector) plus the complicated fact of various reservoir animals. However, there is a semi-agreement that development of vaccine is a long term goal in both human and veterinary medicine. Several trials are in more or less advanced stages but none up to date is clinically confirmed and showed adequate efficacy and become available for the public implementation. Nevertheless, a combination of the current efforts with genetically modified Leishmania trials could lead us to at least one applicable vaccine. Most of the current efforts are focusing on bridging between cellular and molecular components that will hopefully lead to the delivery of a safe, feasible vaccine.

Regarding Egypt, leishmaniasis is not notifiable disease and owing to the lack of awareness, many clinical cases might pass undetected especially when much of attention goes to other more public-alarmed diseases such as cancer and hepatitis. The current available diagnostic methods are mostly conventional, which depends on demonstrating the diagnostic stages in the clinical lesions. With the market unavailability of specific anti-leishmania medicines with except of WHO supplies, there is an increase in leishmaniasis cases in the recent years. More future efforts are required particularly in raising the public awareness of the disease and problems it causing. This is definitely will be fortified by the availability of suitable disease surveillance system, a sustainable source of treatment, and ultimately the activation of a specific vector control program.

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