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NEGLECTED THREATING HUMAN LEISHMANIASIS: A BRIEF REVIEW

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Abstract

Leishmaniases are a group of heterogeneous vector-borne diseases caused by obligate intracellular flagellated protozoans of genus *Leishmania*. At least 21 species have been proven to be pathogenic to humans. The principal mode of transmission of leishmaniasis is by the bite of an infected female sand-fly. *Leishmania* infections have six clinical forms, defined by the location of the parasite in the infected tissues: visceral (VL), post-kala-azar dermal leishmaniasis (PKDL), cutaneous (CL), diffuse cutaneous (DCL), mucocutaneous (MCL) and mucosal (ML) leishmaniasis. The clinical outcomes of leishmaniais dep- end on factors inherent to the parasite, the vector, and the host. Leishmaniasis is emerging and threatens to become an uncontrollable disease. Most patients live in low-to-middle income countries where governments are faced with limited healthcare budgets and other ailments such as malaria, tuberculosis, and HIV. As a result, little research is dedicated to the diagnosis, management, and control of leishmaniasis. This brief review focuses on recent developments in the diagnosis and treatment strategies of leishmaniasis caused by both Old and New World *Leishmania* species.

Keywords: Leishmaniasis, Leishmania, Cutaneous, Visceral, Neglected Tropical Disease

Introduction

Leishmaniasis is an ancient disease with historical descriptions found in old manuscripts and modern studies on ancient archaeological samples (Akhoundi *et al*, 2016). Muslim scholars in medieval era were the leader chroniclers who reported cutaneous leishmaniasis (CL). The Persian scholar al-Rāzī (1930) in Baghdad, described the prevalence of cutaneous sores. Also, a Persian Physician Avicenna (Aboali Ibn Sīnā, 980-1037) reported the initial representation of an oriental sore; a skin condition with characteristics indication of *Leishmania tropica* (Steverding, 2017). William Leishman found unique organisms, in a spleen of died soldier with distinct chromatin aggregates, a large nucleus, and a kinetoplast (Mann *et al*, 2021).

Review and Discussion

Leishmaniases are a heterogeneous vector-borne ailments caused by intracellular flagellated protozoa of genus *Leishmania*, Class Kinetoplastae and Order Trypanosomatida (WHO, 2020). WHO (2022) considered leishmaniasis as one of the neglected tropical diseases to emphasize its significant impact on public health and societies. It primarily affects underprivileged communities due to deprivation of resources, inadequate houses, population dislocation, malnourishment, and compromised immune systems. Of 200 nations, 99 had endemic leishmaniasis; 71 were endemic for VL and CL, 9 for VL only, and 19 for CL only (Alvar *et al*, 2012). Thirteen countries including Sudan, and Syria account for more than 90% of the newly reported cases (Hayani *et al*, 2015). Leishmaniasis, is considered a tropical regions infectious disease, but dramatically expanded boundaries, epidemiological changes driven by climatic changes, travel, urbanization, and migration (De Vries and Schallig, 2022). Increase in sandflies numbers was due to climate change and insecticide resistance, along with resumption of post-COVID-19 travel may also, spread leishmaniasis (Riebenbauer *et al,* 2024). Nowadays, a total of 54 *Leishmania* species are known of which 21 species are zoonotic (Akhoundi *et al*, 2016).

Leishmania were classified into two main groups: 1- Old World species included *L. (L.) major, L. infantum,* and *L. (L.) tropica*

in the Mediterranean basin, the Middle East, the horn of Africa and Indian subcontinent, and 2- New World species that comprised species found in Middle and South America, such as *L. (L.) amazonensis*, *L. (L.) chagasi*, *L. mexicana L*, *L. (viannia) naiffi*, *L. (v.) braziliensis*, and *L. (v.) guyanensis* (Knight *et al*, 2023). Infection is by the infected female sand-fly bites of genera *Phlebotomus* subfamily *Phlebotominae* family Psychodidae in the Old World and *Lutzomyia* in the New World (Maroli *et al*, 2012). Transmission is by about 70 phlebotomines species (Sunter and Gull, 2017). In Egypt, seven *Phlebotomus* species were detected in the Nile Valley (Morsy *et al,* 1900), and *P. langeroni* vector of IVL was found in the North Coastal Zone (Shehata *et al,* 1991). Also, endemic foci of the ZCL were reported in North and South Sinai and Suez Canal Zone (Saleh *et al*, 2017). But, VL was documented mainly in Al Agamy, Alexandria (El-Bahasawy *et al*, 2013). In Egypt, *P. langeroni* is the vector of *L. infantum*. *P. papatasi* is the *L. major* vector from rodents to man. *P. papatasi* is the predominant species all over Egypt and *P. langeroni* was reported in the western vicinity of Alexandria (Morsy and Dahesh, 2023).

Biology and life cycle: *Leishmania* parasites have two major developmental stages; the promastigote forms inhabit sand-fly's gut and amastigotes in mammalian host's macrophages (Giraud *et al*, 2017). Metacyclogenesis is the mechanism transforming *Leishmania* into infectious metacyclic promastigotes within vector's mid-gut (Bates, 2007). Thus, *Leishmania* changes thorugh a number of non-infectious promastigotes; procyclic, nectomonad, leptomonad and haptomonad pro-mastigotes (Dostálová and Volf, 2012). Their function is to colonize and multiply in sand-fly, creating a parasitic clog that move to anterior mid-gut, forcing the vector to regurgitate them while taking its blood meal, releasing filamentous proteophosphoglycan (fPPG) condenses into promastigote secretory gel (Salloum *et al,* 2021). The metacyclic promastigotes are transmitted to the vertebrate host during subsequent blood feeding (Clos *et al*, 2022). Amastigotes are taken up by female sandflies into their digestive tracts with blood meal (De Menezes *et al*, 2016). Significant damage to skin occurs subsequently the dermis and its capillaries rupture, creating a blood pool containing extra cellular matrix (ECM) components derived from tissue and blood, and other cells (Hanson *et al,* 2010). *Leishmania* mostly infects neutrophils in the early stages of infection, but the parasites were not differentiated within them (Gimblet *et al*, 2017).

Pathogenesis: The varied pathologies linked to *Leishmania* infections develop from infected tissues' extravagant inflammatory processes triggered by a complex of interactions between parasites, host immune cells, host skin and gut microbiota of vector, *Leishmania* RNA viruses (Al-Khalaifah, 2022). Parasite can initiate the intracellular parasitism in parasitophorous vacuoles of macrophages (Jain and Jain, 2018). *Leishmania* sp. has evolved with a plethora of membrane-bound or secreted virulence factors, to breach host immune barrier (Gupta *et al,* 2022). Primary structural glycocalyx on surface of *Leishmania* is lipophosphoglycans (LPGs). By the formation of apoptotic bodies, these PMNs aid in safe transfer of promastigotes into macrophages. Trojan horse manner of transfer is a well-known technique in LPG is essential (Raj *et al*, 2019).

Biochemical characterization: *Leishmania* evolved unique signaling pathways to perceive environmental changes and trigger stage differentiation for survival and host infection (Olivier *et al*, 2005). Cell differentiation, proliferation, stress regulation, and apoptosis rely on MAP kinase (MAPK). (Tsigankov *et al*, 2013). The signaling proteins play a role in both extracellular and intracellular signal transduction, which causes the parasite to differentiate into distinct stages and multiply (Tsigankov *et al*, 2014). The signaling proteins are promising targets for therapeutics. Beginning with the phosphorylation of MAP kinase (M3Ks), which induces MAP kinase

kinases (M2Ks) and in turn activates MAP kinases (MAPKs) to regulate multiple cellular processes, including cell division, proliferation, stress response, infectivity, and apoptosis (Cargnello and Roux, 2012). Leishmanial MAPKs are critical to the parasite's growth and ability to survive inside cells. The different MAPKs, such as *L. mexicana's* MAPK1 and MAPK2, are necessary for parasites to survive in blood stream (Ashutosh *et al*, 2012). Also, LmxMAPK1 is essential for antimony drug resistance (Erdmann *et al*, 2006). *L. mexicana* MAPK3 (LmxMPK3) and MAPK9 (LmxMPK9) control the flagella length in promastigotes (Morales *et al*, 2010). Besides, LmxMPK4, LmxMPK7, and LmxMPK10 were crucial in stage differentiation (Morales *et al,* 2007). Each of these MAP kinases is crucial to *L. mexicana's* ability to survive and spread infection (von Freyend *et al*, 2010). Studying *Leishmania* kinome helps understand how the parasite adapts to harsh host conditions for intracellular and extracellular survival during infection. (Ballart *et al*, 2021).

Clinical spectrum: *Leishmania* infections have six clinical forms, defined by the location of the parasite in the infected tissues: visceral (VL), post-Kala-azar dermal leishmaniasis (PKDL), cutaneous (CL), diffuse cutaneous (DCL), mucocutaneous (MCL) and mucosal (ML) leishmaniasis (Akhoundi *et al*, 2016). The clinical outcomes of the disease depend on factors inherent to the parasite, the vector, and the host. CL patients should be diagnosed and treated even if CL lesions may self-resolve in up to 70-80% of cases, as they can cause a high degree of morbidity, social stigmatization, and sometimes cases evolve to MCL (Ponte-Sucre *et al,* 2017). Tegumentary leishmaniasis (TL) has two different clinical presentations: ulcerative skin lesions (CL) or a destructive mucosal inflammation (MCL) that affects oral-nasal-pharyngeal cavities. MCL usually appears after a CL episode but both clinical forms can present together. Infections remain asymptomatic in many cases (Sundar *et al*, 2024).

Cutaneous leishmaniasis: CL is the most common form of the disease. CL is characterized by the development of slow-healing skin sores in or near the areas of infected sand fly bite. Initially presenting as small red papules, they progress to painless nodules, eventually rupturing to distinct well-circumscribed ulcers with a raised violaceous border (Handler *et al*, 2015). Ultimately, this leads to secondary depressed atrophic scars, disfiguration and stigmatization that continue even after treatment . Lesions commonly occur on well-exposed areas of the face and extremities) (Mann *et al*, 2021). CL occurs in the Old-World forms (*L. tropica, L major, L. aethiopica* and less commonly *L. infantum* and *L. donovani*) as well as the NW forms (*L. mexicana* , *L. amazonesis, L. venezuelensis, and L. viannia* subgenus including *L. V. braziliensis*, *panamensis, guyanesis*). There are a variety of atypical cutaneous manifestations, however, including sporotrichoid, disseminated, psoriasiform, verrucous, zosteriform, eczematous, and/or erysipeloid (Handler *et al*, 2015 and Aronson *et al*, 2016). All patients with CL should be evaluated for evidence of mucosal lesions through a naso-oropharyngeal exam. *Leishmaniasis recidivins* (associated most commonly with *L. tropica*) occurs as satellite lesions surrounding old scars (Burza *et al*, 2018).

Diffuse cutaneous leishmaniasis: Diffuse cutaneous leishmaniasis (DCL) starts as a painless nodule but may progress to involve the entire cutaneous surface. Sites of predilection are face, ears, and extensor surfaces such as the knees and elbows (Morsy and Faris, 1991). Invasion of the nasopharyngeal and oral mucosa may occur in up to a third of patients. Organisms most associated with DCL are *L. aethiopica* in the Old World and *L. mexicana* in the New World disease (Ortiz-Flores *et al*, 2015).

Mucocutaneous leishmaniasis: Mucosal di-

sease is due to either hematogenous or lymphatic spread, occurred after resolution of cutaneous lesions within two years or may delay decades (Ahluwalia *et al*, 2004). *L. braziliensis* accounts for the majority of mucocutaneous disease although other organisms can be implicated. It usually involves the oral and nasal mucosa although ulcerative involvement may extend to vocal cords and tracheal cartilage, but bony structures are uninvolved. Mucosal disease may be severe and life-threatening (Handler *et al*, 2015). Patients usually report chronic nasal symptoms including secretions, epistaxis, pain, and bleeding. Unlike cutaneous disease, mucosal disease does not show spontaneous healing (Marsden, 1986).The cartilaginous septum inside the anterior nares is commonly involved and perforation of the nasal septum can occur. Other complications include collapse of the anterior nose and destruction in the nose and mouth (Walton *et al*, 1973).

Visceral Leishmaniasis: VL (Kala-azar) is the gravest form of the disease (Steverding, 2017). It is usually associated fever, splenomegaly, hypergammaglobulinemia, and pancytopenia, caused by *L. donovani, L. infantum,* and/or *L. chagasi* (Tofighi *et al*, 2014). All these species cause broadly similar diseases, but *L. infantum* and *L. chagasi* predominantly affect children with a greater tendency to produce lymphadenopathy (Shirian *et al*, 2014). Subjective symptoms are fatigue, abdominal pain, and weight loss in patients with HIV and is considered an opportunistic infection,up to 25-70% of HIV co-infections in Europe (Morales *et al*, 2007).

The Post Kala-azar dermal leishmaniasis (PKDL): This is applieed to cutaneous involvement that manifest after treatment of the visceral disease as a papular rash on the face and upper extremities. These skin lesions are non-disfiguring and self-limited (Handler *et al*, 2015).

Diagnosis: In the endemic areas, diagnosis of CL is often relying on epidemiologic and lesion characteristics, but confirmation is done by microscopic demonstration of the parasite (scraping, fine-needle aspiration, and touch smears), in the tissue and/or by sample culture to avoid potential misdiagnosis (Reimão *et al*, 2020). While these techniques are highly specific, they are insufficiently sensitive. Moreover, they do not allow species discrimination *Leishmania* (Thakur *et al*, 2020). As regard to VL, parasitological methods remain the gold standard diagnostic techniques and are inevitable in epidemiologic research. The most frequently taken samples are bone marrow or splenic aspirates. However, amastigotes can also be identified in other samples like buffy coat of peripheral blood, lymph nodes, and liver biopsies (Sakkas *et al*, 2016). Amastigotes (also, called as Leishman-Donovan bodies), with round or oval bodies, 2-4μm in diameter, with characteristic nucleus and kinetoplast, which specificity is high, but the sensitivity varies according to the examined aspirates, splenic aspiration sensitivity ranges from 93 to 99% (Elmahallawy *et al*, 2014).

Culture techniques: Clinical samples can be cultivated in either diphasic (Evans modified Tobie's medium and Novy-McNeal-Nicolle medium) or monophasic (Schneider's insect medium, M199, or Grace's medium). The culture techniques are labour-intensive, protracted processes that need complex laboratory settings, and they carry a risk of contamination (Reimão *et al*, 2020 and Elmahallawy *et al*, 2014).

Immunological tools: The immunological procedures have been employed for screening and for the definitive diagnosis of diverse parasitological illnesses because of their easiness and accuracy. For leishmaniases, these are largely applied, being, in some routine protocols, the single diagnostic principle, before anti-*Leishmania* drug prescription (Dutta *et al*, 2024). Specific serological diagnosis is based on the presence of a specific humoral response. Current serological tests are based on four formats: indirect fluorescent antibody (IFA), enzyme-linked immunosorbent assay (ELISA), western blot, and direct agglutination test (DAT)

(Pinnegar *et al*, 2021). Because of their simplicity and accuracy, immunological methods have been used for both screening and final diagnosis of a wide range of parasitic diseases. These are largely applied in leishmaniases, and in certain standard protocols, they serve as the only diagnostic criterion prior to the prescription of anti-*Leishmania* drugs (Dutta *et al*, 2024). The existence of a particular humoral reaction serves as the basis for a specific serological diagnostic. Four formats serve as foundation for serological tests are western blot, ELISA, direct agglutination test (DAT), and indirect fluorescent antibody (IFA) (Pinnegar *et al*, 2021).

Molecular tests: Polymerase Chain Reaction (PCR) techniques remain complex and expensive, and in most VL-endemic countries, they are restricted to a few teaching hospitals and research centers (Sundar *et al*, 2024). Due to the shortcomings of traditional methods, researchers are looking substantially at molecular diagnostics as an alternative to improve leishmaniasis diagnosis. Using various target regions and samples, PCR technology and its variants, such as nested-PCR (nPCR), semi-nested-PCR (snPCR), and quantitative real time PCR (qPCR), have been widely used for the optimisation of new diagnostic assays. qPCR can be used to evaluate the parasitological burden in various specimen types. *Leishmania* species characterisation is another significant PCR application that has received a lot of attention recently (Sakkas *et al*, 2016 and Elmahallawy *et al*, 2014). However, PCR methods are still costly and difficult, and they are only available in a small number of research facilities and teaching hospitals in the majority of VL-endemic nations (Sundar *et al*, 2024).

Imaging: Imaging modalities can be used in diagnosis of VL as ultrasonography, computerized tomography (CT), and magnetic resonance imaging (MRI) (Zanoni *et al*, 2019). PET/CT is not used in routine diagnosis or screening for VL due to its high costs and limited availability. However, FDG-PET/CT could be used as a new alternative noninvasive method for diagnosis of VL, or in patients' fever of unknown origin with hepatosplenomegaly that are suspicious to have VL (Valencia *et al*, 2013).

Treatment: *Leishmania* has an intricate life cycle, and the amastigote, dwells within immune cells of the mammalian host, which makes it more difficult for specific drugs to access parasite (Croft and, Olliaro, 2011). Chemotherapy is still the most effective therapeutic option because its goal is to eradicate the intracellular parasites (Sundar *et al*, 2024). Curiously, the approaches to treat leishmaniasis are mostly very broad and not species-specific, in spite of all clinical diversity and of the great deal of correlation between species-specific determinants of clinical disease patterns (Moore and Lockwood, 2010). First line treatment for localized cutaneous disease includes intralesional forms of Pentostam®, sodium stibogluco-nate and meglumine antimoniate (Sebai *et al,* 1975). Others include systemic miltefosine, amphotericin B, Pentamidine isethionate, Paromomycin, or Granulocyte macrophage colony-stimulating factor (GM-CSF). Heat or cryo-therapy to treat cases with < 5 lesions (Morsy *et al*, 1989). For MCL patient, meg-lumine or stibogluconate gave as high as 95% with high doses, although low doses only decrease the side-effects. Azoles (i.e., fluconazole, ketoconazole, itraconazole) can be used in isolation and in combination with amphotericin B. Their use is limited by regional variants of the disease; resistance and having a minimal additive benefit to already effective amphotericin. Agents established with good cure rates (90%) include amphotericin B (li-posomal or colloidal dispersion), amphotericin B deoxicholate, and pentamidine (Moore and Lockwood, 2010). CL is treated by cost-effective CO_2 laser and thermotherapy to damage the parasites, but more successful (94%) than combined cryotherapy or intralesion injections (Aronson *et al,* 2016). For VL, pentostam (East Africa), amphotericin (India), pentamidine (Africa &

South America), and oral miltefosine are used (Mann *et al*, 2021). Management of VL must involve the malnutrition, skin infection, fever that may need hospitalization, but mild case could be treated as outpatient (Kumari *et al,* 2021). New therapies have been developed for VL alternative to chemotherapy as targeted therapy, host-directed therapies, and combined therapy, or the host directed therapies includes use of host enzymes like histone lysine as targets for the new drugs (Zumla *et al,* 2016).. The targeted therapy includes inhibitors against KDM6B and ASH1L, HTS09796, GSK-J4 & AS-99 as anti-leishmanial agent with high efficacy for promastigotes and amastigotes if were combined with amphotericin-B and/or miltefosine (Dutta *et al*, 2024).

Conclusion

Leishmaniasis is still a risky health problem worldwide. Several factors hinder the production of a proper vaccine. Its life cycle is complex, and parasite is intracellular in the host immune cells.

Shedding spotlight on biochemical characterization of *Leishmania* spp. may open the door for high throughput research studies in this critical field of therapy. No doubt, early diagnosis and proper treatment are indicated.

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