INVITED ARTICLE

Leishmaniasis

Mradul K Daga [1], Ishan Rohatgi [2], Rashmi Mishra [3]

Abstract

Leishmaniasis is one of the chronic debilitating vector-borne diseases caused by obligate intracellular protozoa. The global burden of disease although not increasing but potential risk of spread is there. At least 20 species of Leishmania are pathogenic to human beings. The transmission is from female sandfly through a blood meal. The disease pathogenesis is dependent on parasite and host mechanism—primarily cell-mediated immunity. The three common forms are visceral, cutaneous, and mucocutaneous. The diagnostic tests are mainly based on aspiration from the spleen or bone marrow. The use of K39 antibodies is the best serodiagnostic test. Antimonial, amphotericin B, miltefosine, and paromomycin are the drugs used to treat leishmaniasis. Amphotericin therapy shows the response within 7 to 10 days in most subjects, and 2 weeks of therapy is sufficient. However, those going into relapse need new treatment regimes. There is a definite benefit of combination therapy. However, there is still no breakthrough on a vaccine for prophylaxis.

Keywords: Amphotericin B; K39 antibodies; Leishmaniasis.

Indian Journal of Critical Care Medicine (2021): 10.5005/jp-journals-10071-23844

Introduction

Leishmaniasis, a vector-borne disease endemic in close to 100 countries but still remains neglected, is caused by obligate intracellular protozoa that have been characterized a century ago. The disease is classified on the basis of the location of replication of the parasite into visceral (VL), cutaneous (CL), and mucosal (MCL) leishmaniasis. Availability of limited chemotherapeutic drugs, significant side effects, and drug resistance warrant the search for alternate modalities, including immunotherapy.

Epidemiology

The global burden of leishmaniasis has remained stable, as approximately 15 million are infected, while close to one-third of a billion, alarmingly, are at the peril of acquiring a new infection. The incidence of VL and CL reaches an estimate of 30,000 and 1,000,000, respectively, with 70,000 mortalities per year as per the latest World Health Organization data.[1,2] The above data are thought to be markedly underreported because of subclinical infections and higher prevalence in the impoverished countries, primarily. The infection has been classified geographically as follows:

1. Old World (OW) leishmaniasis is a disease of the Eastern Hemisphere; endemic in Southern Europe, Asia, and Africa.
2. New World (NW) leishmaniasis is a disease seen in Western Hemisphere, existing in Mexico, Brazil, Argentina, and other South American countries.[3]

Historically, the disease has been restricted to the tropical and subtropical continents, but factors involved in its propagation are:

- Climate change,
- Urbanization,
- Deforestation,
- International travel, and
- Immigration from endemic countries.[4]

At least 20 species of Leishmania are pathogenic to humans, and all of them spread by the bite of sandfly.[5,6] The species vary based on the geographical location as shown in the table below

[1-3]Department of Medicine, Maulana Azad Medical College, New Delhi, India

Corresponding Author: Mradul K Daga, Department of Medicine, Maulana Azad Medical College, New Delhi, Delhi, India, Phone: +91 9990092732, e-mail: drmraduldaga@gmail.com

How to cite this article: Daga MK, Rohatgi I, Mishra R. Leishmaniasis. Indian J Crit Care Med 2021;25(Suppl 2):S166–S170.

Source of support: Nil

Conflict of interest: None

Transmission

Female sandflies (*Phlebotomus*) bite (their blood meal) infected mammals (zoonoses) or human beings (anthroponoses) and acquire the amastigotes. The amastigotes replicate in the sandfly gut and transform into promastigotes. The disease further spreads by infected sandfly during a blood meal (generally after sunset). It injects promastigotes in the subcutaneous tissue to complete the cycle. The parasite by its ability to prevent breakdown and hence binding to the epithelium protects itself from being excreted. This in fact determines its efficacy. Seldom sandflies may not be involved in transmission. VL can rarely occur (infective form: amastigote) via blood transfusion or organ donation. The cutaneous forms can manifest after injury via an infected sharp Fig. 1.[7,8]

Immunopathogenesis

The two key factors governing the pathogenesis are parasite and host mechanisms. The promastigotes enter the macrophages as the initial step of the infection. The progression of the disease is based on maintaining the histiocytes in an inactivated state. The two virulence factors expressed by the parasite are:

- Lipophosphoglycan, which hinders macrophage and dendritic cell function, and
- Surface membrane metalloprotease GP63, which is protective against complement and helps in ingress into the macrophages.

Table 1: Disease patterns and manifestations.

	Old World	*New World*
Visceral leishmaniasis	*L. donovani*	*L. infantum*
	L. major	*L. amazonensis*
	L. tropica	*L. infantum*
Cutaneous leishmaniasis	*L. aethiopica*	*L. Mexicana*
Mucocutaneous leishmaniasis	*L. major* (rare, case reports)	*L.braziliensis* *L. panamensis*

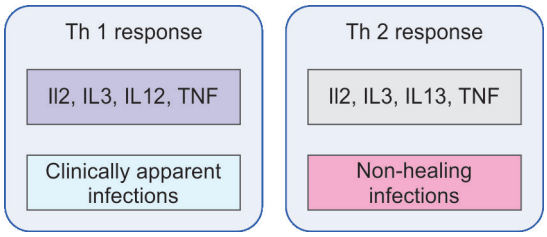

Fig 2: Immune response

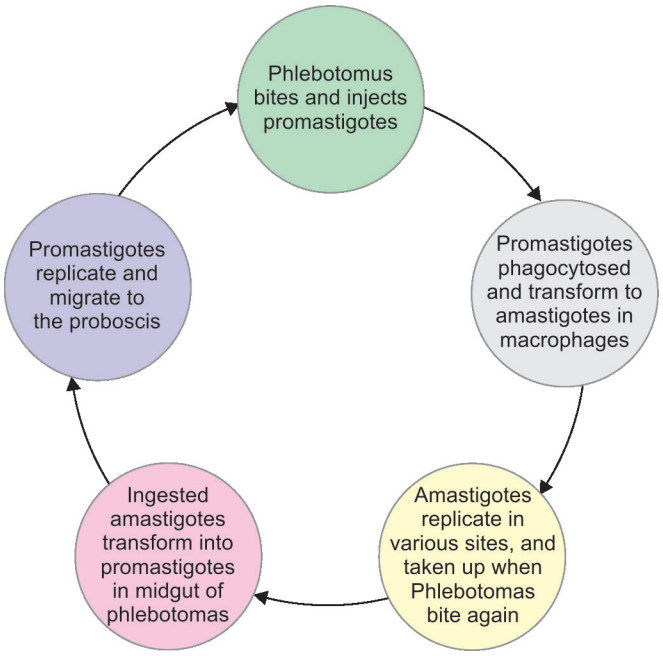

Fig 1: Transmission Process

The A2 gene locus in the amastigotes promotes infectivity and localization to the viscera. The CD4+ cell responses (Th2) are suppressed by the Leishmania-activated C-kinase receptor, and the property has been used to establish experimental infections.[9,10]

The immunocompetent host fights the infection via innate and acquired immune responses, which include delayed-type hypersensitivity and cell-mediated immunity. The clinical picture is decided by these responses and varies from asymptomatic infection to full-blown disease, which may or may not be responsive to treatment. The course of the disease is determined by the primary response mounted by the host, T helper Th1 or Th2 response Fig. 2.

The Th1 response involves the attachment of promastigotes to the reticuloendothelial cells, in turn stimulating CD4 cells to produce inflammatory cytokines IL2, IL3, IL12, and tumor necrosis factor. The activated macrophages phagocytose the promastigotes into vacuoles, which subsequently fuse with lysosomes. The recrudescence of latent, chronic infection is also prevented by the Th1 response, while CD8 cells and memory CD4 cells are involved in vaccine-induced immunity and resistance to reinfection.

The counter balancing Th2 response produces IL4, IL10, and IL13 and transforming growth factor that derails Th1-type response by shutting down macrophages. This limits inflammation-mediated tissue injury but promotes intracellular infection. We have both Th1 and Th2 type responses in varying ratios in symptomatic infections, with prominent inflammation responsible for tissue injury at the sites of infection. The nonhealing infections that comprise VL, chronic CL, and post-kala-azar dermal leishmaniasis (PKDL) show a predominant Th2 response. In subclinical infections, effective and synchronized host responses produce limited signs of inflammation.

Interleukin 10 has been tried on an experimental basis for therapeutic purposes, which promotes parasite killing by activation of Th1 responses, and can be used concurrently with chemotherapy in acute infection.[11-13] The organisms have been found in the lymph nodes even after patients are labeled as clinically cured, thus sourcing the origin of recrudescence of leishmaniasis. The relapse has been documented even up to years after the index infection, once the cell-mediated immunity is impaired.[8]

Clinical Features

The disease patterns have been classically described into VL, CL, and MCL. The disease manifestation depends on the:

- Species of Leishmania,
- Zymodeme of the organism, and
- Location of the replication of the parasite in the reticuloendothelial system.

Visceral Leishmaniasis

The disease has an incubation period ranging between 2 and 8 months. The chief complaints include fever, weight loss, loss of appetite weakness, and night sweats. Skin pigmentation may be seen forming the etymological basis of the name kala-azar. On examination, we usually find organomegaly in the form of liver and spleen, lymph node enlargement, and pallor. The disease commonly runs an insidious progressive course and is terminal without treatment.[7,8,14]

Variations of Visceral Leishmaniasis

PKDL, a well-known entity, has been observed in Asia and Africa after the treatment of VL. The interval may be variable from the completion to the onset. The skin lesions range from macular, maculopapular, or nodular, appear initially in the perioral area, and subsequently spread to the other areas of the body.[8]

Cutaneous Leishmaniasis

CL occurs where the sandfly bites, initially as a papule that gradually increases in size, then crusts, and ultimately ulcerates. The incubation period is the differentiating feature between OW (2 weeks to months) and NW (2–8 weeks). In OW CL, we have papulonodular lesions or nodule–ulcers. Ulcerative lesions are common in the disease caused by the NW species. The healing is usually delayed but definitely extends up to 18 months in 90% of cases.[7,8]

Variations of Cutaneous Leishmaniasis

Leishmaniasis recidivans is a variant defined by tuberculoid lesions, which develop around the previously healed scars. They usually have a low parasite count on microscopy and are difficult to treat.

In diffuse CL, skin lesions disseminate secondary to reduced cell-mediated immunity. They commonly have high parasite numbers on microscopy and are majorly associated with the NW Leishmania only.

Mucocutaneous Leishmaniasis (Espundia)

The third form of leishmaniasis is the most debilitating form due to the severity of the lesions. The incubation period is between 1 and 3 months; however, cases do occur even years after the index lesion has been treated. The mucosa is commonly involved in the South American region with common affection on the nasopharynx and oropharynx. Lesions hamper the eating and are prone to secondary infections, which contribute considerably to the mortality.[3,7,8,14]

HIV–Visceral Leishmaniasis Coinfection

Human immunodeficiency virus (HIV) coinfected patients can have similar clinical presentations, but the depletion of CD4 cells induced by HIV infection has its ramifications. The patients have widespread atypical organ involvement, poor response to chemotherapy, and frequent relapses once the treatment is discontinued. With the lack of research in this subset of the population, the consensus of discontinuation of treatment and threshold of relapse is yet to be finalized.[7]

Diagnosis (Fig. 3)

The diagnosis is based on the clinical presentation. Routine investigations will reveal anemia, or even pancytopenia and hypergammaglobulinemia. Visualization of the parasite in the tissue specimens is the gold standard method, with the added advantage of identification of the species.[7,15]

For VL, aspirates from the spleen (95%), bone marrow (55–90%), or lymph node (60%) can show the amastigote form in decreasing order of sensitivity.[15] The tissue aspirate can be cultured in Novy–McNeal–Nicolle medium (N-N-N medium) or Evans biphasic medium with the sensitivity reaching up to 85%.[3] The prevalence of the disease locally determines the efficacy of serum anti-Leishmania IgG values in the diagnosis. The use of anti-K39 antibodies has made field serodiagnosis with the help of a finger prick and has higher sensitivity (90–100%) in patients with classical symptoms and signs.[15] Detection of urinary Leishmania antigens is a recent update in the diagnostic modalities.[16] Polymerase chain reaction (PCR) technology is still limited to high-end laboratories, but it rapidly identifies the species with a sensitivity of 97 to 100%.[17]

In cases of CL, impression smears, edge biopsy, or scrapings are used to identify the parasite with the highest yield obtained from the ulcer base.[18] Combining microscopy with culture increases the sensitivity. PCR once again is the most sensitive method for identification and speciation, but limited availability curtails its use in developing countries.

The Leishmanin skin test is akin to the commonly done Mantoux test for tuberculosis. The basis of this test is delayed hypersensitivity-type reaction. Here, we use phenol-killed promastigotes, which are injected intradermally, and an induration exceeding 5 mm after 3 days indicates prior exposure to the organism. However, it is not diagnostic of active disease. Patients with leishmaniasis will have a positive reaction and stay positive for life.[19]

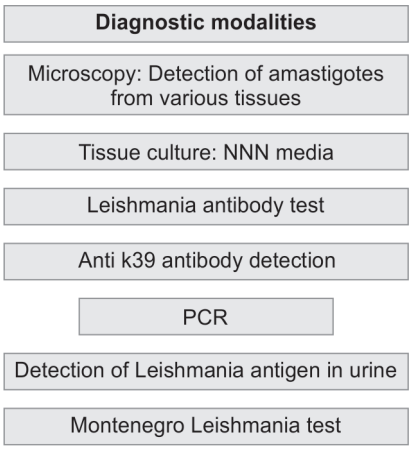

Fig 3: Diagnostic Modalities

Treatment

Chemotherapy has been central to the management of leishmaniasis as it is the only curative option. However, we are still fraught with side effects, high costs, and drug resistance. Systemic therapy is suggested for all cases of VL, while for CL, even local therapy may suffice. In cases of CL, one needs to start treatment immediately in:

- Immunocompromised patient,
- More than three lesions,
- Lesions greater than 2.5 cm, and
- Lesions on the joints, face, hands, or feet.[14]

Conventional Therapies

The following drugs are in use for the treatment of leishmaniasis:

- **Antimonials** (sodium stibogluconate IV and meglumine IM) have been in use for more than half a century and still remain the first-line therapy except in India where drug resistance has developed to it. The antileishmanial mechanisms of Sb^{3+} are related to its interaction with sulfhydryl-containing biomolecules, including thiols and enzymes. The dosing regimen is 20 mg/kg/d IV or IM for 3 to 4 weeks with remission achieved in more than 90%. Significant side effects include elevated lipase, leukopenia, thrombocytopenia, and cardiotoxicity. For CL, intralesional therapy has the benefits of low dosage, lower systemic side effects, rapid action, and lower costs.[20,21]
- **Amphotericin B** is the therapy of choice in the Indian subcontinent where resistance to antimonials is prevalent, while elsewhere its use is limited in view of serious adverse effects, prolonged hospitalization, and higher costs. This drug binds to membrane ergosterol, forming complexes that arrange into ion channels and increase membrane permeability, resulting in cell death. The liposomal amphotericin B was introduced in view of lesser side effects and is the treatment of choice in pregnant women and HIV coinfection. Liposomal formulations are preferentially taken up into the reticuloendothelial tissue, which is also the site of disease in VL. The beneficial effect is derived from its lengthier stay, thus allowing administration of higher doses of the drug over a small number of doses. The second benefit is less nephrotoxicity due to its localization limited to the reticuloendothelial system. The dosage of L-AmB is 3 to 5 mg/kg for 5 days, while conventional amphotericin B requires therapy for at least 15 days.[14,20,21]

- **Miltefosine** is an anticancer drug, whose antileishmanial activity was discovered in 1980s and till date remains the only efficacious oral agent available. The drug seems to inhibit the cell membrane synthesis and interferes with the signaling pathways, thus promoting cell apoptosis. The dosing is convenient in comparison (2.5 mg/kg/days for 4 weeks), and cure rates up to 90% were reported after its initial introduction. However, high cost and increasing resistance have limited its use to only combination regimens.[21,22]
- **Pentamidine** has been used in patients unable to tolerate antimonials, with cure rates reported ranging from 35 to 90%. The dosing regimen includes 2 to 4 mg/kg every alternate day for seven doses. Significant side effects significantly limit its use (cardiotoxicity, nephrotoxicity, hepatotoxicity, and hyperglycemia). It is contraindicated in pregnancy.[14,21]
- **Paromomycin** is an aminoglycoside, used parenterally for VL, and topically and parenterally for CL. It was introduced as a monotherapy for treatment in India with considerable cure rates but lagged behind when tried in Africa and South America. Topical application has been approved for both OW CL and NW CL after successful outcomes in a meta-analysis of 14 randomized control trials.[3,21]

Alternative therapies have been tried, including azithromycin, allopurinol, azoles, delamanid, and imiquimod, but with inconsistent results in different geographical regions.[14]

Treatment Response and Prognosis

Commonly patients show signs of clinical improvement by 7 to 10 days of initiation of therapy. Nearly 90% of treated individuals become afebrile or have a decline in splenic size, but 10% of patients do not respond to the treatment and eventually succumb to the illness. Pancytopenia improves by the end of the treatment, while the spleen becomes nonpalpable within 6 months. The relapse commonly occurs within 6 months after the treatment; thus, it is naïve to label a complete response unless 6 months have passed uneventfully. Relapse of the disease has to be treated with a different regimen than the previous one.[7,22] Treatment failure can be defined as follows:

- No response, that is, the persistence of symptoms and
- Relapse, that is, fresh symptoms after initial apparent cure.

In either scenario, clinical symptoms should be verified by parasitological means. Studies have concluded that the relapses usually occur within a year of treatment, therefore, following the patients for the said duration before labeling them as treated seems justified.[23]

The success of combination therapy for tuberculosis, malaria, and HIV was an indication to try the same for Leishmania. The advantages of combination therapy would be lower dosage, shorter duration of therapy, lesser individual side effects, and less chance of drug resistance. The combination of stibogluconate and paromomycin was a serendipity and has been used since to great effect except in India.[24] In a study, the activity enhancement index of different drugs *in vivo* demonstrated the maximum potentiation of miltefosine with amphotericin B and PM. Keeping this in mind, three arms of L-AmB monotherapy, L-AmB + PM, and L-AmB + MIL underwent a trial in India, Sri Lanka, and Pakistan and displayed excellent outcomes with cure rates of 98.1, 99.4, and 94.4%, respectively.[25]

Local Therapy

In patients with CL, and with no evidence of systemic spread, local therapy is warranted. Thermotherapy is based on the premise that Leishmania parasites do not multiply in temperatures beyond 39°C. It is effective against both OW and NW species, and sessions vary from single sitting to up to 7 days.[26,27] It can be combined with topical paromomycin as well. It is less costly, heals with minimal scarring, and has no systemic side effects. Cryotherapy (liquid nitrogen at –195°C) has been used once or twice weekly on the lesion for a duration of 6 weeks, has shown >90% efficacy, and has been used in OW CL as chances of progression to mucocutaneous form are very minimal.[28] CO_2 laser is another modality that lyses the infected tissue with minimal effect on the normal mucosa. However, it may cause hyperpigmentation.[29]

Vaccines

Inadequate, protracted, and with significant side effects, chemotherapy should not be the mainstay of management. Centuries before Leishmania was identified, the disease was described and the concept of vaccination was introduced; Kurdish tribes practiced voluntary exposing their babies' buttocks to the Phlebotomus as they observed that it prevented facial lesions in adulthood.[30,31] This actuality was a primitive and crude form of Leishmanization (inoculation of virulent parasites as a whole). In the late 1980s, a refined method of Leishmanization was used, which resulted in successful inoculation in the majority but was plagued with the development of large uncontrolled lesions and immunosuppression.[20] The various species of Leishmania mostly share a common pool of antigens; thus, a universal vaccine would suffice. The practice of vaccination with live attenuated organisms was halted abruptly due to safety concerns, even though it leads to protection against subsequent infection. The subset of immunocompromised patients or possible immunosuppression in the future was at the maximum risk because the parasites persist lifelong after the vaccination.[22]

Prevention of leishmaniasis with an effective vaccine has, to date, not been materialized. The side effect profile of both the vaccines has been in question. Fresh inputs about the genome will definitely help in the development of newer vaccines.[32] A new approach in the form of recombinant DNA-derived antigen vaccines is in the pipeline. It is a peptide-based vaccine based on an immune response generated from an antigenic epitope. They have the added advantage of lower costs, lower antigen complexity, and absence of potentially damaging materials. A major drawback is an inactivation or degradation by the immune system, so the search is on for adjuvants that enhance the antigen immunogenicity.[33] Lastly, nanotechnology has been incorporated in the development of peptide vaccines, as they help stabilize and prevent degradation by the immune system.[34]

Conclusion

Leishmaniasis remains an age-old, tropical, and subtropical endemic disease with significant mortality and morbidity. The geographical speciation and the causative organisms in different parts of the world have been well characterized. The Th1 helper response is the cornerstone in wading off the infection, albeit depletion of CD4+ cells as in HIV coinfection leads to extensive disease. Chemotherapy for leishmaniasis is limited, with tedious courses and morbid side effects. Combination therapy and local

therapy have also been tried, but with restricted success. Despite significant advances in the formulation of vaccines, we still await an effective candidate that can provide us better prophylaxis than the existing vector control measures.

Orcid

Mradul K Daga https://orcid.org/0000-0001-7774-7602
Ishan Rohatgi https://orcid.org/0000-0001-5333-1581
Rashmi Mishra https://orcid.org/0000-0003-2621-6515

References

1. Mokni M. Cutaneous leishmaniasis. Ann Dermatol Venereol 2019;146(3):232–246. DOI: 10.1016/j.annder.2019.02.002.
2. Leishmaniasis [Internet]. [cited 2021 Feb 23]. Available from: https://www.who.int/health-topics/leishmaniasis#tab=tab_1.
3. Chakravarty J, Sundar S. Current and emerging medications for the treatment of leishmaniasis. Expert Opin Pharmacother 2019;20(10):1251–1265. DOI: 10.1080/14656566.2019.1609940.
4. Bueno-Marí R, Jiménez-Peydró R. Global change and human vulnerability to vector-borne diseases. Front Physiol 2013;4:158. DOI: 10.3389/fphys.2013.00158.
5. Gradoni L. A brief introduction to leishmaniasis epidemiology. Leishmaniases Old Neglected Trop Dis 2018;1–13. DOI: 10.1007/978-3-319-72386-0_1.
6. Wilhelm TJ. Visceral leishmaniasis. Chirurg 2019;90(10):833–837. DOI: 10.1007/s00104-019-0994-1.
7. Murray HW, Berman JD, Davies CR, Saravia NG. Advances in leishmaniasis. [Review] [199 refs]. Lancet 2005;366(9496):1561–1577. DOI: 10.1016/S0140-6736(05)67629-5.
8. Piscopo TV, Mallia AC. Leishmaniasis. Postgrad Med J 2006;82(972):649–657. DOI: 10.1136/pgmj.2006.047340corr1.
9. Joshi PB, Kelly BL, Kamhawi S, Sacks DL, McMaster WR. Targeted gene deletion in Leishmania major identifies leishmanolysin (GP63) as a virulence factor. Mol Biochem Parasitol 2002;120(1):33–40. DOI: 10.1016/s0166-6851(01)00432-7.
10. Kelly BL, Stetson DB, Locksley RM. Leishmania major LACK antigen is required for efficient vertebrate parasitization. J Exp Med 2003;198(11):1689–1698. DOI: 10.1084/jem.20031162.
11. Gasim S, Elhassan AM, Khalil EAG, Ismail A, Kadaru AMY, Kharazmi A, et al. High levels of plasma IL-10 and expression of IL-10 by keratinocytes during visceral leishmaniasis predict subsequent development of post-kala- azar dermal leishmaniasis. Clin Exp Immunol 1998;111(1):64–69. DOI: 10.1046/j.1365-2249.1998.00468.x.
12. Bourreau E, Preévot G, Gardon J, Pradinaud R, Launois P. High intralesional interleukin-10 messenger RNA expression in localized cutaneous leishmaniasis is associated with unresponsiveness to treatment. J Infect Dis 2001;184(12):1628–1630. DOI: 10.1086/324665.
13. Gomes R, Oliveira F. The immune response to sand fly salivary proteins and its influence on Leishmania immunity. Front Immunol 2012;3:1–9. DOI: 10.3389/fimmu.2012.00110.
14. Kevric I, Cappel MA, Keeling JH. New world and old world leishmania infections: a practical review. Dermatol Clin 2015;33(3):579–593. DOI: 10.1016/j.det.2015.03.018.
15. Sundar S, Sahu M, Mehta H, Gupta A, Kohli U, Rai M, et al. Noninvasive management of Indian visceral leishmaniasis: clinical application of diagnosis by K39 antigen strip testing at a kala-azar referral unit. Clin Infect Dis 2002;35(5):581–586. DOI: 10.1086/342057.
16. Islam MZ, Itoh M, Mirza R, Ahmed I, Ekram ARMS, Sarder AH, et al. Direct agglutination test with urine samples for the diagnosis of visceral leishmaniasis. Am J Trop Med Hyg 2004;70(1):78–82. DOI: https://doi.org/10.4269/ajtmh.2004.70.78
17. Fissore C, Delaunay P, Ferrua B, Rosenthal E, Del Giudice P, Aufeuvre JP, et al. Convenience of serum for visceral leishmaniasis diagnosis by PCR. J Clin Microbiol 2004;42(11):5332–5333. DOI: 10.1128/JCM.42.11.5332-5333.2004.
18. Ramirez JR, Agudelo S, Muskus C, Alzate JF, Berberich C, Barker D, et al. Diagnosis of cutaneous leishmaniasis in Colombia: the sampling site within lesions influences the sensitivity of parasitologic diagnosis. J Clin Microbiol 2000;38(10):3768–3773. DOI: 10.1128/JCM.38.10.3768-3773.2000.
19. Arana BA, Roca M, Rizzo NR, Mendoza CE, Kroeger A. Evaluation of a standardized leishmanin skin test in Guatemala. Trans R Soc Trop Med Hyg 1999;93(4):394–396. DOI: 10.1016/s0035-9203(99)90129-3.
20. Taslimi Y, Zahedifard F, Rafati S. Leishmaniasis and various immunotherapeutic approaches. Parasitology 2018;145(4):497–507. DOI: 10.1017/S003118201600216X.
21. Roatt BM, de Oliveira Cardoso JM, De Brito RCF, Coura-Vital W, de Oliveira Aguiar-Soares RD, Reis AB. Recent advances and new strategies on leishmaniasis treatment. Appl Microbiol Biotechnol 2020;104(21):8965–8977. DOI: 10.1007/s00253-020-10856-w.
22. Ghorbani M, Farhoudi R. Leishmaniasis in humans: drug or vaccine therapy? Drug Des Devel Ther 2018;12:25–40. DOI: 10.2147/DDDT.S146521.
23. Ponte-Sucre A, Gamarro F, Dujardin JC, Barrett MP, López-Vélez R, García-Hernández R, et al. Drug resistance and treatment failure in leishmaniasis: a 21st century challenge. PLoS Negl Trop Dis 2017;11(12):1–24. DOI: 10.1371/journal.pntd.0006052.
24. Davidson RN, Seaman J, Pryce D, Sondorp HE, Moody A, Bryceson ADM, et al. Epidemic visceral leishmaniasis in Sudan: a randomized trial of aminosidine plus sodium stibogluconate versus sodium stibogluconate alone. J Infect Dis 1993;168(3):715–720. DOI: 10.1093/infdis/168.3.715.
25. Sundar S, Sinha PK, Rai M, Verma DK, Nawin K, Alam S, et al. Comparison of short-course multidrug treatment with standard therapy for visceral leishmaniasis in India: an open-label, non-inferiority, randomised controlled trial. Lancet 2011;377(9764):477–486. DOI: 10.1016/S0140-6736(10)62050-8.
26. Cardona-Arias JA, Darío Vélez I, López-Carvajal L. Efficacy of thermotherapy to treat cutaneous leishmaniasis: a meta-analysis of controlled clinical trials. PLoS One 2015;10(5):1–15. DOI: 10.1371/journal.pone.0122569.
27. Bumb RA, Prasad N, Khandelwal K, Aara N, Mehta RD, Ghiya BC, et al. Long-term efficacy of single-dose radiofrequency-induced heat therapy vs. intralesional antimonials for cutaneous leishmaniasis in India. Br J Dermatol 2013;168(5):1114–1119. DOI: 10.1111/bjd.12205.
28. López-Carvajal L, Cardona-Arias JA, Zapata-Cardona MI, Sánchez-Giraldo V, Vélez ID. Efficacy of cryotherapy for the treatment of cutaneous leishmaniasis: meta-analyses of clinical trials. BMC Infect Dis 2016;16(1):1–11. DOI: 10.1186/s12879-016-1663-3.
29. Shamsi Meymandi S, Zandi S, Aghaie H, Heshmatkhah A. Efficacy of CO_2 laser for treatment of anthroponotic cutaneous leishmaniasis, compared with combination of cryotherapy and intralesional meglumine antimoniate. J Eur Acad Dermatol Venereol 2011;25(5):587–591. DOI: 10.1111/j.1468-3083.2010.03781.x.
30. Alvar J, Croft SL, Kaye P, Khamesipour A, Sundar S, Reed SG. Case study for a vaccine against leishmaniasis. Vaccine 2013;31(Suppl. 2):B244–B249. DOI: 10.1016/j.vaccine.2012.11.080.
31. Khamesipour A, Dowlati Y, Asilian A, Hashemi-Fesharki R, Javadi A, Noazin S, et al. Leishmanization: use of an old method for evaluation of candidate vaccines against leishmaniasis. Vaccine 2005;23(28):3642–3648. DOI: 10.1016/j.vaccine.2005.02.015.
32. Handman E. Leishmaniasis: current status of vaccine development. Clin Microbiol Rev 2001;14(2):229–243. DOI: 10.1128/CMR.14.2.229-243.2001.
33. De Brito RCF, Cardoso JMDO, Reis LES, Vieira JF, Mathias FAS, Roatt BM, et al. Peptide vaccines for leishmaniasis. Front Immunol 2018;9(May):1043. DOI: 10.3389/fimmu.2018.01043.
34. Akbari M, Oryan A, Hatam G. Application of nanotechnology in treatment of leishmaniasis: a review. Acta Trop 2017;172(January):86–90. DOI: 10.1016/j.actatropica.2017.04.029..