Journal of Advanced Zoology

ISSN: 0253-7214

*Volume **45 Special** Issue **02 Year 2023** Page **1733:1743***

A 6-year Cross-Sectional study on Leishmaniasis

Sujatha Tezavat, Syed A Quadri, Sweta Sinha, Madhavi Bodepudi

Assistant Professor[1] Department of Biomedical sciences King Faisal University College of Medicine, Kingdom of Saudi Arabia

Assistant Professor [2], Department of Biomedical sciences King Faisal University College of Medicine, Kingdom of Saudi Arabia

Associate Professor[3] Atal Bihari Vajpayee Institute of Medical Sciences, New Delhi, India

Professor[4] Maheshwara Institute of Medical sciences, Chitkul, Isnapur, Hyderabad, Telangana, India

Corresponding Author: Madhavi Bodepudi, mvutla@gmail.com , mobile 9999775627

Abstract: Leishmaniasis is caused by a protozoan parasite which is transmitted by the bite of infected female phlebotomine sand-fly. Leishmaniasis is more common in poor socioeconomic conditions like poor housing, overcrowding and malnutrition. Asymptomatic Leishmania donovani infections outnumber clinical presentations, however, the predictors for development of active disease are not well known. Asymptomatic persons infected with the parasites causing visceral leishmaniasis (VL) usually outnumber clinically apparent cases by a ratio of 4-10 to 1. Accurate treatment and cure is mainly dependent on the factors like clinical manifestations and diagnosing the causative species among cutaneous Leishmaniasis or mucocutaneous Leishmaniasis and visceral Leishmaniasis.

Aims and objectives: To review the clinico-pathological effects of Leishmaniasis and also to study regarding the epidemiology, Pathophysiology and diagnosis of different types of Leishmaniasis.

Material and Methods: It is a prospective and cross-sectional study done on Leishmaniasis. Study data taken from SGT University (Delhi NCR) from the year 2015 to 2020 from the patients migrated from Bihar state to Haryana and were admitted in the general medicine ward with irregular bouts of fever, weight loss. Splenomegaly, hepatomegaly, anemia and pancytopenia. Elisa, DAT tests done along with bone marrow aspiration and were diagnosed with Visceral leishmaniasis. LD bodies found in the smears. Leishman and Giemsa stains were used.

The data was compared with the Saudi Open Data Portal which is published by the Ministry of Health (MOH) as "Reported cases of Cutaneous Leishmaniasis 2017 to 2022 and tissue sampling procedures were FNAC, scrapping, and punch biopsy. Material collected for Giemsa or H&E staining is from bone marrow aspirate specimens or from FNAC or from buffy coat layer of peripheral blood. Blood samples of the symptomatic patients were collected on filter paper on

two occasions 6-12 months apart, were tested for antibodies against L. donovani with rK39-ELISA and DAT. Seroconverters, (negative for both tests in the first round but positive on either of the two during the second round) and controls (negative on both tests on both occasions) were followed for two years and concluded that the strong association between high DAT and/or rK39 titers and progression to disease among asymptomatic subjects.

Study analysis and Results:

Patients with Visceral leishmaniasis or Kalaazar who attended SGT medical college with irregular bouts of fever, hepatomegaly, splenomegaly anemia and pancytopenia. And the data was compared with Saudi Arabian patients pat with Cutaneous Leishmaniasis by Year, Nationality, Sex, and Age Group. Age ranged from 14 years to 48 years in Indian subcontinent patients who migrated recently from Bihar to Haryana and in Saudi Arabian residents age ranged from less than 1 year to 45 years

The total no of cases who attended the medical ward in SGT Hospital from the Year 2015 to 2020 was 32. On average 5 to 6 patients used to attend the Hospital every year. 98% of them were male patients. Age ranged from 11 years to 48 years. All of them were diagnosed with Visceral Leishmaniasis.

Table 1:

Year wise distribution of Visceral Leishmaniasis cases from 2015-2020

2015	2016	2017	2018	2019	2020
7	8	8	7	4	3

All the patients were Male patients and Age ranged from 11 years to 48 years.

Chart 1: Year wise distribution of Visceral leishmaniasis cases

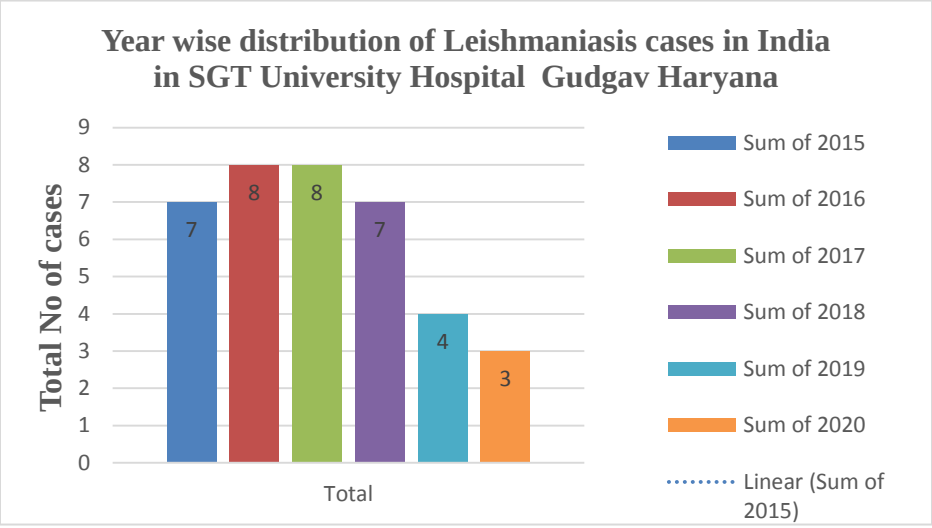
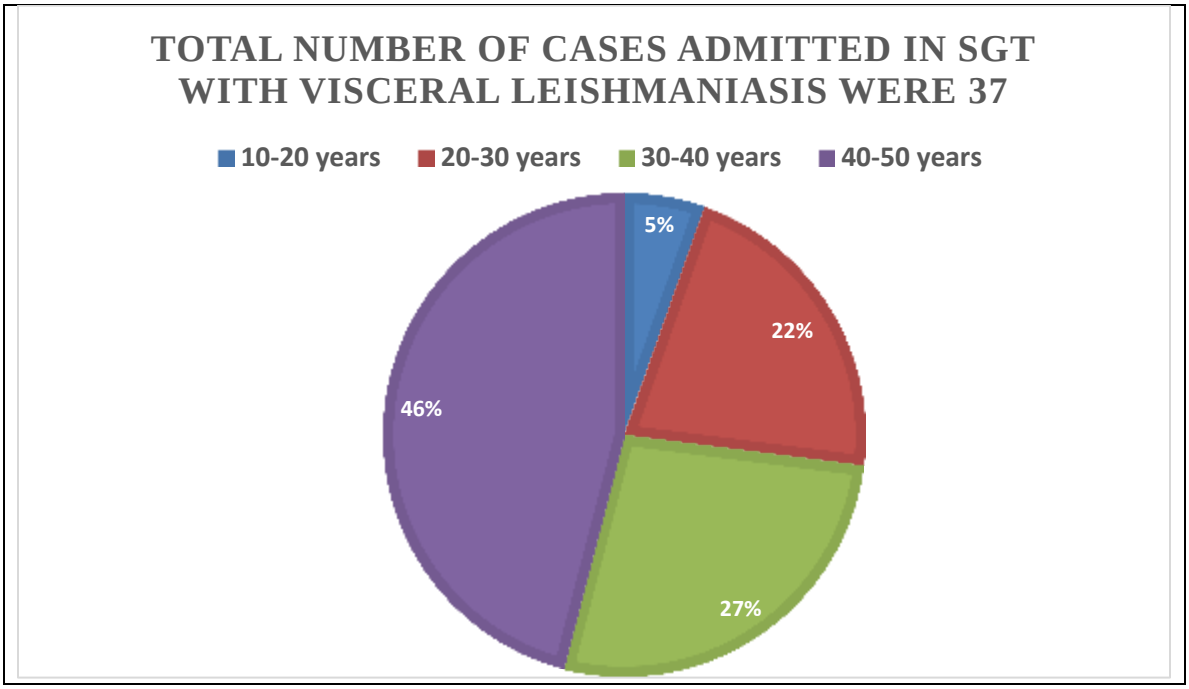


Table 2 : Age wise distribution: Out of 37 patients attended 2 patients belong to pediatric age group and the other patients were adults

10-20 years	20-30 years	30-40 years	40-50 years
2	8	10	17

Chart 2: Age wise distribution of Visceral Leishmaniasis cases



Leishmaniasis cases in Saudi Arabia

Total number of cases reported in 2017 are **1007**. Out of 1007 Male patients are **820** and Female patients are 187. Age ranged from less than 1 year to 45 years.

Nationality: **441** are Saudi-Arabian people and **435** are Non-Saudi people 950 are Saudi residents and **57** are non-residents.

Total number of cases reported in 2018 are **921.** Out of 921 Male patients are **734** and Female patients are 187.

Nationality: 486 are Saudi-Arabian people and 435 are Non-Saudi people. 879 are Saudi Residents and 72 are Non-residents. Age ranged from less than 1 year to 45 years.

Total number of cases reported in 2019 are **1096** out of total cases male patients are 889 and Female patients are 207.

Total number of cases reported in 2020 are **1067** out of total cases male patients are 879 and Female patients are 188.

Total number of cases reported in 2021 are **600** out of total cases male patients are 465 and Female patients are 135.

Total number of cases reported in 2022 is **528** out of total cases male patients are 387 and Female patients are 141.

Figure 1: show Promastigote forms, which were cultured on NNN medium (Novy. -MacNeal -Nicolle)

Figure 2 and Figure 3 showing Amastigote forms seen in macrophages and in polymorphonuclear granulocytes obtained from skin lesions of cutaneous leishmaniasis and from Peripheral blood smear. Sample obtained from cut lesions and were stained with Geimsa stain. In Figures 4 and 5 LD bodies (Leishman stain on 40x) seen in Macrophages and also in Bone marrow aspirate.

Figure 1: Promastigote forms, which were cultured on NNN medium (Novy. -MacNeal - Nicolle)

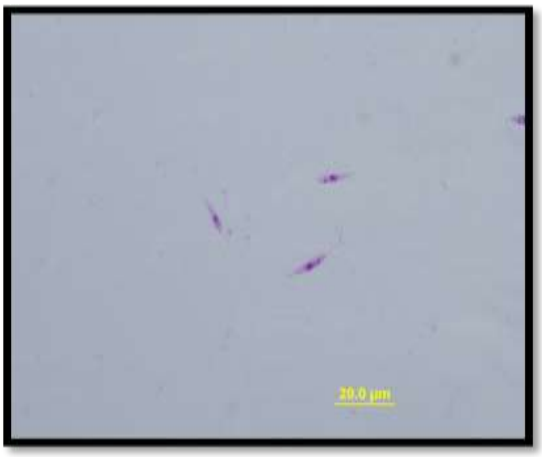
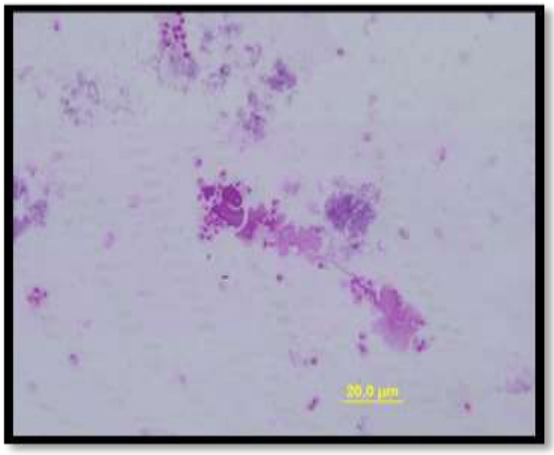
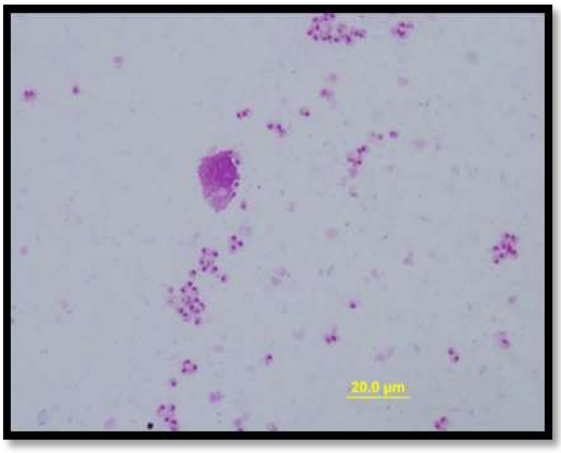
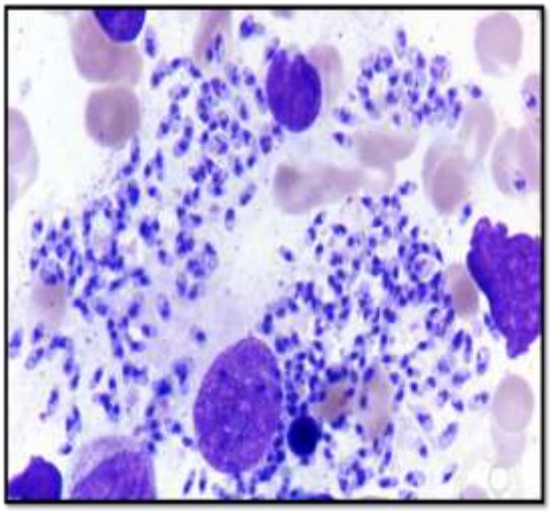


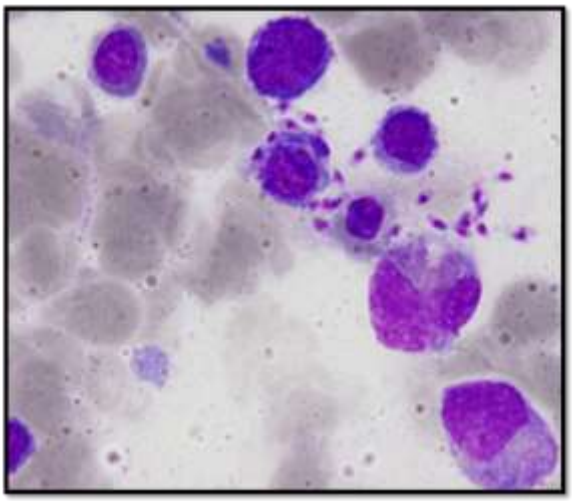
Figure 2 & 3: Amastigote forms seen in Neutrophils and in Macrophages (Giemsa stain-40x)





Figures:4 and 5: LD bodies seen in Macrophages (Fig4 40x Leishman stain) and in Bone marrow aspirate(Fig5 40x Leishman stain) show Kinetoplast.





Case analysis: Charts 1,2,3 and 4 show total number of Leishmania cases from 2017 to 2022 in Saudi Arabian and Non-Saudi Arabian patients. with Year wise, age wise distribution and sex wise distribution.

Number of cases were low in Covid 19 pandemic years (2021 and 2022 years) compared to non-Covid season.

Male patients were affected more compared to female patients. Maximum number of cases were noted in the Year 2020. Cases were more in Saudi Arabian residents compared to Non-Saudi Arabian residents.

More cases were reported in the age group between 15 to 45 years of age. In infants (< 1 year) and in Pediatric age group (1-15 years) less no of cases were reported.

Chart No 3: Total no of Leishmaniasis cases reported per year (From Year 2017 to 2022)

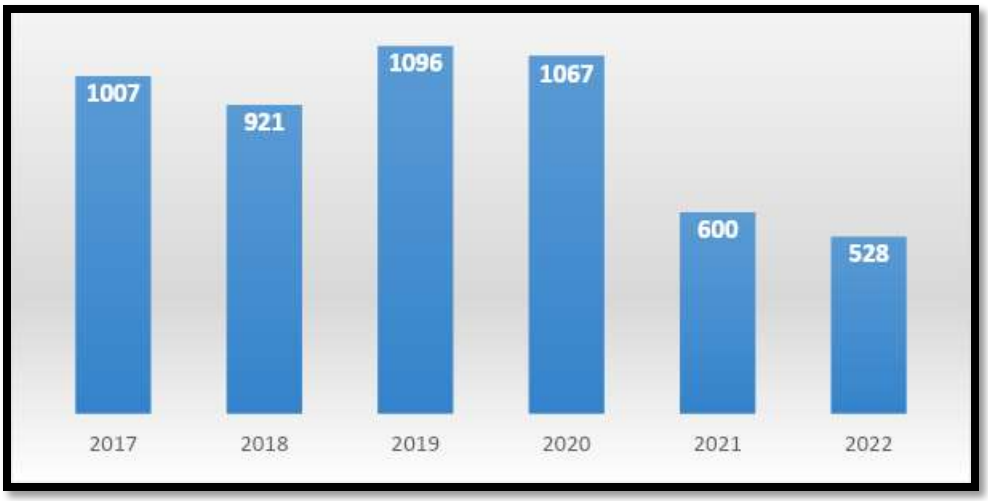


Table 3: Year wise Distribution of Leishmaniasis cases in Saudi-Arabia from 2017-2022

Study Year	Total no of Leishmaniasis cases reported	Nationality-Saudi Arabians	Nationality-Non-Saudi Arabians	Saudi residents	Non-Saudi residents
2017	1007	441	435	950	57
2018	921	486	435	879	72
2019	1096	556	540	879	42
2020	1067	493	574	1035	32
2021	600	323	277	550	50
2022	528	321	207	503	25

Chart No 4: Year wise distribution of Leishmaniasis among Saudi and Non-Saudi Population

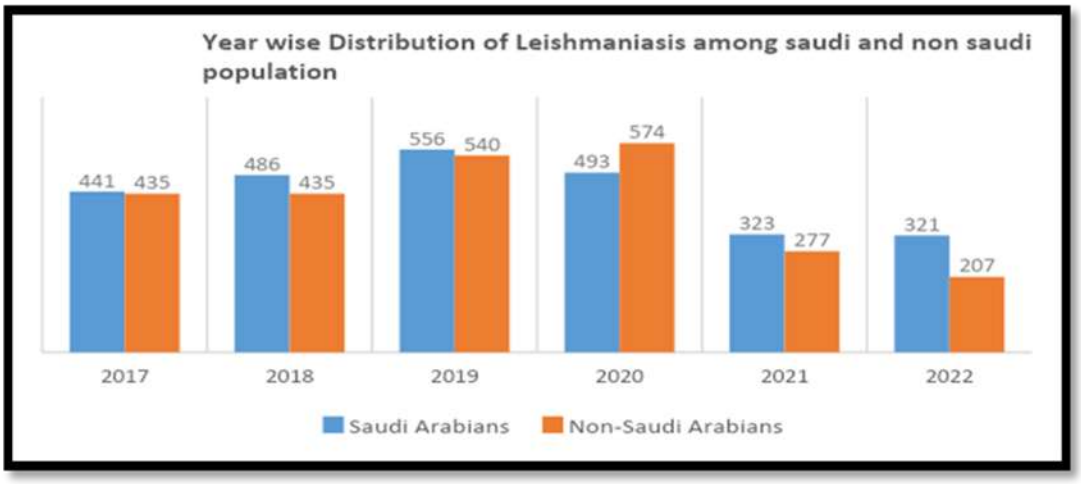


Table-4 Sex wise distribution of Leishmaniasis cases in Saudi Arabia

Study year	Total no of patients	Male	Female
2017	1007	820	187
2018	921	734	187
2019	1096	889	207
2020	1067	879	188
2021	600	465	135
2022	528	387	141

Chart No 5: Year wise and sex wise distribution of Leishmaniasis in male and female

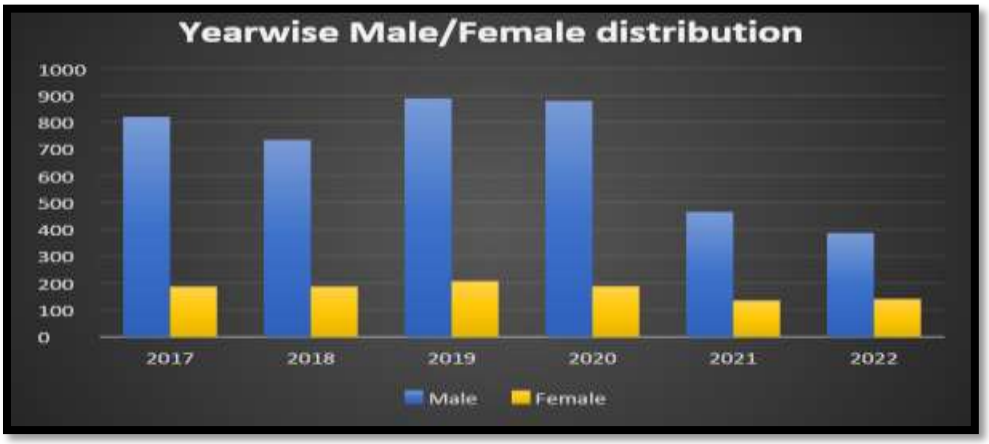
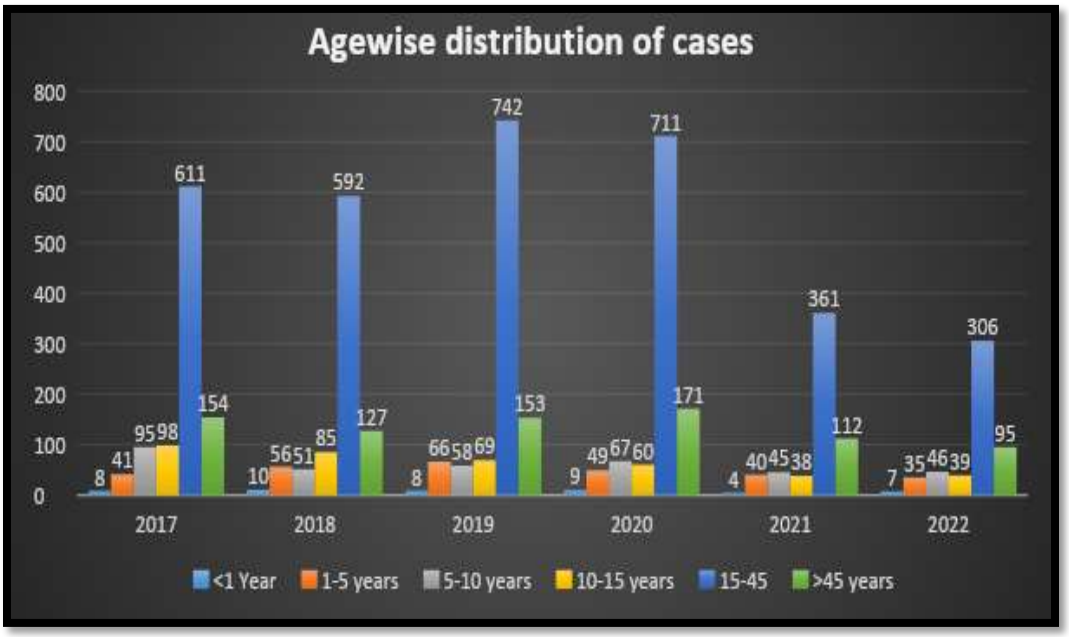


Table 5: Age wise distribution- Age group (years)

Study year	<1 Year	1-5 years	5-10 years	10-15 years	15-45 years	>45 years
2017	8	41	95	98	611	154
2018	10	56	51	85	592	127
2019	8	66	58	69	742	153
2020	9	49	67	60	711	171
2021	4	40	45	38	361	112
2022	7	35	46	39	306	95

Chart 6: Age wise and Year wise distribution of Leishmaniasis cases



Results: Results were compared with Saudi Arabian cases data published by Ministry of health between the years 2017 to 2022. In Saudi Arabia Cases were more in The State of Bihar where there are endemic areas like Vaishali district with the case load of more than 600 cases/ year. But with effective preventive and eradication programs the case load is very low now. In India, the cases are Visceral leishmaniasis and in Saudi Arabia cases were Cutaneous leishmaniasis type predominantly. Occasionally Visceral leishmaniasis cases were reported. Male predominance is noted in Visceral leishmaniasis in India. In Saudi Arabia also there is male predominance and cutaneous leishmaniasis is more common.

Introduction: Leishmania is a vector-borne, obligate intracellular, protozoan parasite causing cutaneous, mucocutaneous, and visceral disease in the Old and New World [(1)]. Leishmaniasis is a tropical disease infecting the people of low and poor socioeconomic groups in over 90 countries throughout Asia, Africa, the Middle East, and Central and South America**.** [(1)] Risk factors for Leishmaniasis include poverty, population migration, malnutrition, poor hygiene, and an immunocompromised state [(1)] the sandflies are found across the globe and tropical species can complete the lifecycle throughout the year. In subtropical areas, however, species can only complete their life cycles during warmer months. Sand flies are most active at night; the sandflies can also fly silently. [(1)] Over 20 species of the *Leishmania* parasite have been characterized and are transmitted from approximately 70 different types of phlebotomine sand flies and again subdivided into old word and New world. [(2)]

There are 22 species belonging to the genus *Leishmania* that has been further subdivided into the subgenera Leishmania and Viannia based on development of the organism in the digestive tract of the sand-fly. Each parasite species has specific geographical predilections, host factors, and symptom characteristics. For example, *L. donovoni* presents as VL in South Asia (Bangladesh, India, and Nepal) and East Africa (Sudan, Somalia, Ethiopia, and Kenya) and often predominates among younger individuals while sparing older adults due to acquired immunity. [(3)]

L.infantum (same species as L. chagasi in Latin America), on the other hand, also can present as VL, but is usually found in the Mediterranean, Middle East, Pakistan, Iran, and Brazil. In Texas, all endemic cases of Leishmaniasis were found to be caused by L. Mexicana CL. [(4)]

Leishmaniasis is classically thought of as an imbalance of TH1 vs TH2 CD4+ helper cells [5,6.] Those with a primary TH1 response have excellent parasite control with low levels of parasitemia; however, they are primed toward mucocutaneous disease as a result of overactive cellular immunity and cellular destruction [(6, 7).] Those with a TH2 response have increased parasite load as antibody neutralization is ineffective against the intracellular parasite [(6)]**.** TH2 responders are more likely to develop disseminated disease, which leads to visceral disease and, in the New World, results in disseminated cutaneous leishmaniasis (DCL) [(6).]

Leishmaniasis can present with a variety of different clinical manifestations, the main three phenotypic categories of disease are cutaneous (CL), mucosal (ML), and visceral leishmaniasis (VL). These categories can be subdivided further to encompass the spectrum of clinical disease to include ML of the Americas, VL, CL of the New World, CL of the Old World, post-kala-azar

CL, Leishmania recidivante, diffuse CL, and disseminated CL [7] In some individuals, infection can remain asymptomatic or subclinical, but it can also present as acute, sub-acute, or as a chronic disease. The transmission of Leishmania sp. is predominantly driven by symptomatic infection and post-kala-azar dermal leishmaniasis (PKDL), as asymptomatic cases are thought to not infect sandflies [8, 9] In some areas, humans are required to maintain the lifecycle (anthroponotic transmission), which is characteristic of L.tropica responsible for CL in the New World and L. donovoni responsible for VL in India [10, 11] Animals, however, can maintain the lifecycle and may or may not exhibit signs or symptoms of disease. Dogs, rodents, marsupials, monkeys, and edentates are among the susceptible hosts. Visceral leishmaniasis (VL), is a vector-borne disease caused by replication of parasites in macrophages, mononuclear phagocytic system. It is caused by the *Leishmania donovani* complex, which includes *L. donovani* and *L. infantum*. It is endemic in large areas of the tropics, subtropics and the Mediterranean Basin. VL is a systemic disease and is fatal if left untreated. The transmission characteristics of VL differs in different geographical regions; in the Mediterranean Basin, Brazil and parts of Africa, the dog is the main reservoir and VL is zoonotic; while in the Indian subcontinent and parts of Africa, it is anthroponotic.

Post kala-azar dermal leishmaniasis (PKDL) is a sequel of VL, characterized by a macular, maculo-papular or nodular rash and is frequently observed in Sudan and the Indian subcontinent

Parasitological diagnosis: Restricted to endemic area where clinician are familiar with sign and symptoms and for culture sophisticated laboratories are required. Sensitivity: Splenic aspirate 93.1-98.7%, Bone marrow aspirate 52–85%; Lymph node aspirate 52–58 % Specificity: 100%

Less commonly, leishmaniasis can also be transmitted through organ transplantation, blood transfusion, intravenous drug use, or congenitally. CL lesions typically occur at the sand fly bite site (commonly on well-exposed areas of the face and extremities) as a solitary non-suppurative papule, although multiple lesions can occur. 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 New World forms (L. mexicana, L. amazonesis, L. venezuelensis, and L. viannia subgenus including L. V. braziliensis, panamensis, guyanesis) [12,13]. Over weeks to months, the papules progress to painless ulcers with heaped-up borders, which can spontaneously heal over months to years, or cause scars and disfigurement [14]. There are a variety of atypical cutaneous manifestations, however, including nodular, sporotrichoid, disseminated, psoriasiform, verrucous, zosteriform, eczematous, and/or erysipeloid [15]. Other atypical presentations include small satellite lesions outside of the plaque/ulcer (nodular lymphangitis). All patients with cutaneous leishmaniasis should be evaluated for evidence of mucosal lesions through a naso-oropharyngeal exam [16]. Leishmaniasis recidivins (associated most commonly with L. tropica) occurs as satellite lesions surrounding old scars and is often confused for cutaneous tuberculosis [17]

In the subcontinent of India Bihar area there are few districts which are endemic for Visceral leishmaniasis. Visceral leishmaniasis (VL) or Kala-azar has been a major public health problem in Bihar, India, for several decades. A few VL infected districts including Vaishali have reported >600 cases annually. Although India has made substantial progress in the elimination of the

disease since 2012, VL remains a stable public health problem in four middle-eastern states including Bihar. Bihar contributes >61% of the total Indian cases annually, and a few districts of the state have reported more than 600 cases annually. Visceral leishmaniasis is endemic in >80 countries. In the Indian subcontinent (ISC), VL is pure anthroponotic and parasites are spread to humans by the bite of an infected female sand fly species *Phlebotomus argentipes* (Diptera: Psychodidae). [(18)] Visceral leishmaniasis (VL or Kala-azar) is a deadly tropical disease caused by the protozoan parasite genus *Leishmania*. The clinical manifestation of the disease is characterized by irregular bouts of fever, weight loss, splenomegaly, hepatomegaly, and anemia. Elimination and preventive measures include epidemiological analysis, active case detection, vector control using the indoor residual spraying (IRS) of chemical insecticides, awareness campaigns, human resource development, the close monitoring of control activities, and active epidemiological surveillance and entomological monitoring can achieve the elimination target in the highly endemic region of $_{\text{Bihar}}$ [(18)]. Invasive and risky techniques involving demonstration of the parasites in stained preparations from splenic and bone marrow aspirate is still the gold standard for VL diagnosis. Serological tests using rK39 in ELISA or rapid immunochromatographic format, Direct Agglutination Test (DAT), immunoblotting have issues related to a significant proportion of asymptomatic individuals being positive with these tests and their inability to diagnose relapses as these remain positive for several months to years after cure. PCR is the most common molecular technique successfully used for diagnosis and differentiation of species. [(18)]

Conflict of Interest: There are no conflicts.

References:

1. Sarah Mann, Katherine Frasca, Sara Scherrer, Andrés F. Henao-Martínez, Sabrina Newman, Poornima Ramanan & José A Suarez *Current Tropical Medicine Reports* A Review of Leishmaniasis: Current Knowledge and Future Directions. **volume 8**, pages121–132 (2021)
2. Boelaert M, Sundar S. 47 - Leishmaniasis. In: Farrar J, Hotez PJ, Junghanss T, Kang G, Lalloo D, White NJ, editors. Manson's Tropical Infectious Diseases (Twenty-third Edition). London: W.B. Saunders; 2014. p. 631-51.e4.
3. Bern C, Amann J, Haque R, Chowdhury R, Ali M, Kurkjian KM, et al. Loss of leishmanin skin test antigen sensitivity and potency in a longitudinal study of visceral Leishmaniasis in Bangladesh. Am J Trop Med Hyg. 2006; 75(4):744–8.
4. McIlwee BE, Weis SE, Hosler GA. Incidence of endemic human cutaneous leishmaniasis in the United States. JAMA Dermatology. 2018; 154(9):1032–9.
5. Romagnani S. T-cell subsets (Th1 versus Th2). Ann Allergy Asthma Immunol. 2000; 85(1):9–18 quiz, 21.

6. Scott P, Novais FO. Cutaneous leishmaniasis: immune responses in protection and pathogenesis. Nat Rev Immunol. 2016; 16(9):581–92.
7. John E. Bennett RD MBJB. Mandell, Douglas, and Bennett's principles and practice of infectious diseases: 8th ed. Philadelphia: Elsevier/Saunders, [2015]; 2015.
8. Mondal D, Bern C, Ghosh D, Rashid M, Molina R, Chowdhury R, et al. Quantifying the infectiousness of post-kala-azar dermal leishmaniasis toward sand flies. Clin Infect Dis. 2019; 69(2):251–8.
9. Le Rutte EA, Zijlstra EE, de Vlas SJ. Post-kala-azar dermal leishmaniasis as a reservoir for visceral leishmaniasis transmission. Trends Parasitol. 2019; 35(8):590–2.
10. Burza S, Croft SL, Boelaert M. Leishmaniasis. Lancet. 2018;392(10151):951–70. **A concise overview of the major aspects of caring for persons with leishmaniasis.**
11. Davidson R, Croft S. Visceral leishmaniasis in Africa. Afr Health. 1992;14(5):18–9.
12. John E. Bennett RD MBJB. Mandell, Douglas, and Bennett's principles and practice of infectious diseases: 8th ed. Philadelphia: Elsevier/Saunders, [2015]; 2015.
13. Herwaldt BL. Leishmaniasis. Lancet. 1999; 354(9185):1191–9.
14. González C, Wang O, Strutz SE, González-Salazar C, Sánchez-Cordero V, Sarkar S. Climate change and risk of leishmaniasis in North America: predictions from ecological niche models of vector and reservoir species. PLoS Negl Trop Dis. 2010;4(1):e585.
15. Bern C, Amann J, Haque R, Chowdhury R, Ali M, Kurkjian KM, et al. Loss of leishmanin skin test antigen sensitivity and potency in a longitudinal study of visceral leishmaniasis in Bangladesh. Am J Trop Med Hyg? 2006; 75(4):744–8.
16. Aronson N, Herwaldt BL, Libman M, Pearson R, Lopez-Velez R, Weina P, et al. Diagnosis and treatment of leishmaniasis: clinical practice guidelines by the Infectious Diseases Society of America (IDSA) and the American Society of Tropical Medicine and Hygiene (ASTMH). Am J Trop Med Hyg 2017; 96(1):24–45. **One of the primary guidelines about leishmaniasis.**
17. **Burza S, Croft SL, Boelaert M. Leishmaniasis. Lancet. 2018; 392(10151):951–70. A concise overview of the major aspects of caring for persons with leishmaniasis**
18. **Vijay kumar, Rakesh Mandal, Sushmita das, Shreekant Kesari, Divakersingh Dinesh et. al Kala-azar elimination in a highly-endemic district of Bihar, India: A success story May 4, 2020 ttps://doi.org/10.1371/journal.pntd.0008254**