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Leishmaniasis: A Neglected Tropical Disease

Abstract *Leishmaniasis is an emerging tropical disease in the world as declared by WHO. At least 89 countries are affected, risking lives of 350 million people resulting in 70,000 deaths annually. It is spread to human beings and animals by bite of sandflies of genera Phlebotomus and Lutzomyia. The disease is more prevalent in the poverty-stricken populations. In Pakistan, the most affected province is KPK. Despite its global occurrence, it is not a very life-threatening disease except visceral leishmaniasis in which death can occur in immunocompromised patients. The only satisfactory treatment is through intravenous antimonials. Vaccine is not yet available but environmental control of sandfly can help in prevention of leishmaniasis. There is a dire need of improving the existing conventional therapy but the neglection of the disease has led to lack of financial support for the development of a novel drug.*

Key Words: *Leishmaniasis, Prevalence, Treatment, Control*

Introduction

Leishmaniasis is one of the neglected infectious diseases among the seven most important emerging tropical and subtropical diseases of the world (WHO, 2017). In humans, it is spread by the bite of female infected sandfly. Out of 900 sandflies known species, 100 are suspected to act as vectors of leishmaniasis of genera *Phlebotomus* and *Lutzomyia* (Gradoni., 2018). Its pathogen is an intracellular protozoan parasite of genus *Leishmania* having at least 20 recognized species causing leishmaniasis in approximately 98 countries of the world (Gyapong and Boatin., 2016). Clinically leishmaniasis is of three types: Cutaneous, Subcutaneous and Visceral leishmaniasis. Among these three types, only VL is life threatening whereas rest of the two are self-healing within few months, however, leading to the cause of secondary infections (Dujardin *et al.*, 2008; Gradoni, 2018). Among the tropical and subtropical areas, it is mostly prevalent in the poverty associated regions. The foremost reason behind its neglect is that it primarily affects the impoverished community living in rural areas, urban slums, remote and conflict zones. These individuals are confined to distinct geographical areas having a weak healthcare infrastructure. Most of the cases reported are from the developing countries Moreover, the surveillance and control of leishmaniasis are also neglected.

In addition, the unawareness about the disease among rural population regarding its prevention promotes spread and contributes to its rapid emergence. The physicians also lack information regarding the disease. Due to the lack of effective and affordable drugs, this lethal disease is left untreated. Since CL is an emerging disease in Pakistan and is self-healing, it heals within 3-18 months (Burza *et al.*, 2018). So, people avoid its treatment which further results in major consequences. Firstly, the lesions formed on the skin act as suitable environment for the growth of several bacteria resulting in secondary infections. Secondly, promoting its anthroponotic spread. Thirdly, healing after secondary infections leaves permanent scars on the skin. There is a crucial

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need to improve patient compliance by the development of cost-effective oral drugs for the treatment of leishmaniasis and it vector control.

Global Prevalence of Leishmaniasis

The disease is spread worldwide. In around 89 countries leishmaniasis is prevalent. On the American continent it's a jungle zoonosis. Almost 350 million people globally are at risk and about 12 to 15 million people are infected. Each year about 1.5- 2 million new cases arise causing demise of at least 70,000 people annually.

Within the duration of 13 years Algeria, Bouchemal and Khezzani declared 4,813 cases of CL according to a report in 2017.The people who were most affected included people 10 to 19 years old i.e., 31.41% and children aged less than 9 years i.e., 25.7%. A large percentage of the people affected included men i.e., 65%.

In Iran annual distribution of CL was shown by 589,913 cases being reported in central areas of Iran and In Autumn the highest incidence rate of lesions was found i.e., 35.14%. at 60,000 new cases of CL are predicted to occur each year. Leishmaniasis is more prevalent in areas at height of about 0 to 1500 m above the sea level where temperature lies above 20 C and annual precipitation between 1500 to 3000mm. In Mexico all types of leishmaniasis have been documented since the Pre-Hispanic period. The clinical presentation mostly perceived were of pure cutaneous and cutaneous chondral types, which how ulcer of the classic chiclero which affects the ear. The main endemic lies in the Neotropical region of the southeast including southern areas of Chiapas, Veracruz, Oaxaca, Quintana Roo and Campeche.

Specific antibodies have been documented in 17% of the general population in serological studies conducted in Becanchén (Southern State of Yucatán). An occurrence and prevalence rate of 2.35 and 9.41 per 100,000 populations were also observed in Tabasco, respectively; it is therefore considered a high-endemic region. These rates are clarified because farmers operate just 10 km from the site where the largest number of reservoirs and transmitting vectors of rodents are located. In 20.42 percent of military personnel serving in this zone, positive serology is observed. *Lutzomyia olmeca* and *Lutzomyia cruciate* are the two primary vectors of this illness in the Yucatán Peninsula. *Leishmania mexicana* is the primary agent in Campeche and Yucatán (Mexico); Leishmania braziliensis predominates in Belize and Guatemala, where cases of *L. mexicana* has also been described.

In a descriptive study conducted by Vita et al., (2016) assisted by data from the Brazilian Information System on Notifiable Diseases (SINAN) and the Brazilian Institute of Geography and Statistics (IBGE), 1,470 cases of CL were analysed; 87.89% were clinically dermal, and higher incidences were found in the white ethnic group (49.72%) and in the 20-39 age group (32.44 percent). The researchers deduced that the number of cases based on a detection coefficient decreased from 1.44 per 100,000 inhabitants in 2004 to 0.20 per 100,000 in 2013.

Prevalence of Leishmaniasis in Pakistan

Species of sandflies in Pakistan were determined by taking an entomological survey in Baluchistan an endemic area in Pakistan. Three species of sand flies that were reported are: *P. sergentii* 63.7% , *P.papatasi*30.5% and *P. sergentomia squamipleuris* 5.8%. former was found to be the most important vector in causing disease (Khosravani *et al.*,2016). Two types of leishmaniasis are prevalent in Pakistan namely ACL and ZCL caused by *L.tropica* and *L.major* respectively. *L.tropica* is observed in urban areas and *L. major* is observed in rural areas. (Afghan, *et al.*, 2011). There are no records available as to when the first case of disease was reported, however severe outbreak occurred in Quetta in 1935 hence no definite area of endemic has been identified (Ozkeklikçi *et al.*, 2017). Pakistan has 4 provinces Punjab, Sindh, Baluchistan and Khaybar Pakhtunkhwa (KPK) along with Azad Jammu Kashmir (AJK). Its spread around the country is majorly due to wars, refugees, deforestation and breeding of animals (Afghan, *et al.*, 2011) In Punjab epidemic was reported in Multan, Lahore and Dera Ghazi Khan. Areas affected in Sindh were Dadu, Larkana and Jacobabad districts (Pandey *et al.*, 2011). Within Baluchistan most cases were reported in Quetta, Ormara and Uthal and the major reason was refugees migrating from war-affected country of Afghanistan during early 1980s to 1990s (Afghan, *et al.*, 2011). Status of this disease is varying in country over years. Khaplu valley was the breeding ground in 1960. Epidemic broke in Multan from 1971-1972.Later in 1974 Kharmang valley was hot bed of the disease. Two cases were observed in Parkuta village in 1975 (Masmoudi *et al.*, 2013). It has emerged as an endemic disease in KPK due to recurrent mobility of internally displaced persons (IDPs) from Waziristan concerning to surgical strikes and military operations against radicals by Armed forces of Pakistan

(Mubashar *et al.*, 2018). Around 21,000-35,000 cases of both ACL and ZCL are reported. In rural area transmission of ZCL is mainly caused by breeding of wild mammals specifically gerbis such as *Rhombomys opimus* (Khan *et al.*, 2005). In a study in KPK 125 patients were examined and 104 out of them were suffering from leishmaniasis. And 30 individuals were identified to genus level only.74 samples were identified to the specie level and out of them 89.2% were L.tropica , 6.8% were L.major and 4% were *l. infantum*. Two out of three patients suffering from visceral leishmaniasis were army officers and third was a female of sixty years diagnosed at AJK (Khan *et al.*, 2016). 63% of *L.tropica* and 8% of L. major specific bands were observed for north Waziristan. While for south Waziristan 54% of *L. tropica* and 4% of L. major specific bands were observed. Two species of sandflies that are *P.sergentii* and *P.papatasii* are susceptible to carry *L.tropica* and mainly affecting KPK. *L.infantum* was reported only in 2 army personnel stationed in Waziristan. Pervasiveness of cutaneous leishmaniasis in war affected Waziristan during 2013-2015 was 3.61% by PCR (Mubashar *et al.*, 2018).

Types of Leishmaniasis

Different species of leishmania manifest different clinical presentation and can range from severe visceral disease to self-healing cutaneous disease (Burza *et al.*, 2018) The disease is determined by the host factors, vector biology and parasite characters responsible for leishmaniasis. CL is mostly restricted to an ulcer that heals itself in about 3-18 months, but can cause disabilities such as scarring and stigmatization. Only 10% can develop into serious illness depending on the parasite specie (Haq, 1989 and Reithinger *et al.*, 2007) *L donovani* causes visceral leishmaniasis in Africa and Asia whereas in Middle East, Mediterranean basin Central Asia, South America and Central America. *Leishmania Infantum* causes visceral leishmaniasis. It is the most extreme form and is deadly if not treated. (Guerrero *et al.*, 2017). PKDL occurs in people as a skin manifestation after visceral.

Clinical features

There are several species which cause leishmaniasis in human beings.

Visceral Leishmaniasis

The major symptoms include continuous and irregular fever and splenomegaly. Pancytopenia, and hypergammaglobulinemia are the typical laboratory abnormalities. In young children acute malnutrition is due to increased level of parasitemia. In the subcontinent the hyperpigmentation caused by increased cytokine induced production of adrenocorticotropic hormone has lead to its Hindi name kala azar which is translated as black fever. Incubation period is about 2 weeks to 8 months (Burza *et al.*, 2018). If it is not treated and the disease progresses within 2 years cachexia and multisystem failure might occur and superimposed infections can lead to death.

Symptoms might arise again if a person becomes immunocompromised (Guerrero *et al.*, 2017) The Leishmania parasite might persist for decades and becomes reactive as soon a person becomes immunodeficient.

Post Kala Zaar Leishmaniasis

In clinical cases some muco popular rashes around the mouth and trunk and slowly spreads on the entire body. Sensations in the rashes are preserved which differentiates it from leprosy (Burza *et al.*, 2018).

Cutaneous Leishmaniasis

According to clinical manifestation CL can be divided into (LCL), disseminated cutaneous leishmaniasis and mucocutaneous leishmaniasis. It was found that the causative agents for CL were *L.tropica* and *L.maxicana*. It happens by insect bites at the most exposed areas of human body. In the order of increasing frequency the least involved are the ankles, forearms, hands, legs, cheeks, upper lip, nose and the most involved part is ears. Incubation period for CL is 1 to 4 weeks. (Andrade-Narvaez *et al.*, 2013).

The primary sign of disease is an erythema which develops into a nodule and after a coarse of 2 weeks to 6 months it becomes a lesion which is most common in LCL. The severity of these lesions depend on the clinical appearance, time taken to heal and the lymphatic involvement. The lesions of LCL take variable time to heal such as 2-6 months for *L.major,* 3-9 months for *L.mexicana,* or 6-15 months for *L.braziliensis, L.tropica, L.panamesis*. the self-healing can result in lifelong protection but may not be limited to a specific leishmania spp. The patient may suffer trauma from the resulting long-lasting scars after healing (Reithinger *et al.*, 2007).

Diffuse Cutaneous Leishmaniasis (DCL) is more difficult to treat as compared to LCL. The lesions do not self-heal. They arise from non-ulcerative nodules and can promulgate from the site of bite to the whole body. Lymphadenopathy, Lymphedema, and fever

are typically seen. When mucus membranes of nose or pharynx are involved the sore lesions can cause obstruction of airways. (Guerrero *et al*., 2017).

Mucocutaneous leishmaniasis also known as "espundia" is a complication of LCL usually 5 years after healing when person becomes immunocompromised. The specie including *L. guyanensis, L. braziliensis,* and *L. panamensis* attacks and infiltrates the nasal mucosa slowly and the injury to mucosa remains unnoticed which then results in swelling and mild pruritis. The lesions from the nasal mucosa spread into the mucosa of orophanyx and the lips and cause discomfort but in severe cases it can spread to the whole oesophagus, pharynx and larynx. In initial stages the ulceration is superficial while in later stages the lesions becomes necrotic and painful. The uvula and tonsils can be completely ruined.

Life Cycle of Leishmania Parasite

There are two stages in leishmania parasite's life cycle i.e the sandfly stages and the human stages. The type of leishmaniasis depends on the specie involved. The transmission takes place through the sandfly either it can be anthroponotic or zoonotic. (Mansour., 2014) The cycle starts when the blood meal is taken by sandfly and and injects the parasite at promastigote stage into the body. The promastigotes are taken up by macrophages and phagocytosed which causes them to shed their shells and become amastigotes. These amastigotes then divide in the infected cells and other tissues depending on the type of parasite specie such as *Leishmania donovi* moves through reticuloendothelial system and produces visceral leishmaniasis whereas *Leishmania major* mainly causes cutaneous leishmaniasis (Khan., 2005) *Leishmania braziliensis* infects the oral mucosa and may remain dormant for years and when reactivated causes destructive mucocutaneous form, espundia (Kalter., 1989) now again when a healthy sandfly bites the infected host and takes a blood meal, the amastigotes are taken up by the sandfly. Then in the gut of sand fly these amastigotes converted into promastigotes.The promastigotes divide in the sandfly which is mainly done by binary fission. (Killick-Kendrick., 1990) The cycle starts again when an infected sandfly injects parasite in the promastigote stage into a healthy individual.

Diagnosis

The base of diagnosis is on concurrent and clinical epidemiological context. Protozoan is present in scraping of mucosal and cutaneous ulceration and in non-ulcerated lesions. Biopsy has been obtained as diagnostic tool in the active border of lesions (Guerrero *et al*.,2017). The diagnosis is done by the laboratory, clinical and epidemiological characteristics. Serological tests or direct methods are used for confirmation of leishmaniasis.

PCR

The characteristics are linked to biochemical criteria (electrophoresis of isoenzymes) as well as on genetic criteria using different molecular methods including PCR and monoclonal antibody technique with specific panel. PCR is an expensive procedure and only used in high centers.

Smear

The samples were obtained from scratches of lesion through sterile surgical blades and then parasites was isolated (scalpel, vaccinostyle). Aspirated punctures by the use of disposable syringe.

Culturing

In NNN/schneider medium 3 to 10 days are required for the culture to grow. Specimen should never be removed until they are negative for 4 weeks. Different immunological techniques like direct agglutination test, enzyme linked immunosorbent assay and etc are used for diagnosis (Singh.,2006).

Treatment

It is self-healing process in case of cutaneous leishmaniasis within 3-18 months. But drawbacks include that it leads to secondary infections, anthroponotic spread and healing after secondary infection leads to permanent scars on skin. No specific oral treatment is available. Only treatment is through injections that are highly painful and expensive.

Pentavalent Antimonials: This is the only one type of treatment against all type of leishmaniasis with major satisfying antimicrobial results For antimonials, it is considered to have board spectrum of action. When enters the host cell antimony (SbV) , this drug pass through the phagolysosomal membrane and has converted to trivalent antimony (Sblll). Then it acts on amastigotes by comparing the cells thiol redox potential by creating efflux of intracellular thiols and the it inhibits trypanothione resuctase (TR). SbV may also destroy parasite by indirect mechanism like by the increase of cytokine levels. They can also function at DNA level and can indue DNA damage in

vivo, and lead to inhibition of topoisomerase I (Freitas *et al.*,2012).

Pentamidine: About 2-4mg/kg/day dose is usually given on alternate days with two or four applications. Most frequently it may lead to anorexia, abdominal pain, nausea, fatigue, headache, metallic or bitter taste, hypotension.

Amphotericin B: Its dose is 18mg/kg and lead to erythema and mild dyspnea in some patients (Oliveira *et al.*,2011).

Miltefosine: It is an oral antineoplastic agent and is alkylphospholipid and is mostly used to treat leishmaniasis. It is approved first oral treatment of leishmaniasis in some countries. It is affective against visceral and cutaneous leishmaniasis and for antimony-resistant infections.

Sitamaquine: It is and oral treatment used for visceral leishmaniasis. Dose is 2mg/kg/day. Abdominal pain, renal damage, headache was reported.

Paromomycin: It is an aminoglycoside used for both visceral and cutaneous leishmaniasis and can be treated with antibiotic. This antileishmanial drug having poor oral absorption (Tiuman *et al.*,2011).

Prophylaxis

Measures to prevent the spread of disease, effective vaccination and vector control strategies all contribute to the prophylaxis of the disease. Since the disease is vector-borne, the eradication of the vector sandfly from the environment is important in the prevention of leishmaniasis. This can be achieved by use of insecticides, insect repellents, sanitation, stagnant water elimination and using suitable clothing having long sleeves (Guerrero *et al.*, 2017). Moreover, the use of insecticide treated nets has proved to be effective for controlling CL and VL. However, mosquito nets commonly used are not considered effective for the control of sandfly as its size is smaller than mosquitoes. the WHO is working for the formation of effective vaccines development against all types of leishmaniasis (Guerrero *et al.*, 2017).

Future Perspectives

The recent advancement in the field of molecular parasitology and its application which is concerning with parasitic infection like leishmaniasis brings new methods of disease diagnosis and control. So Several molecular methods have been successfully used and evaluated in the diagnosing of Leishmaniasis. In which the molecular techniques of PCR is the main tool of diagnostic techniques in research and as well as in health facilities. PCR is hundred percent useful in the identification of causative leishmania parasite when it is compared with other diagnostic procedures which are used for the identification of leishmania species. However, the implementation of PCR based diagnosis in the health facilities and in clinical practice need technical expertise which is unfortunately not existing commonly in developing and under developing countries. Therefore, more focus in the future is required to provide the PCR techniques attainable, easy and inexpensive in the areas in the areas particularly with high disease endemics.

It is accepted that chemotherapy has adverse outcomes but still it is considering the significant strategy in curing and controlling the leishmaniasis. Moreover, as the infection rate of leishmaniasis reach to the unbeaten point then the chemotherapy is no more accepted as significant cure strategy for the eradication of leishmaniasis and the disease reach to the level where there is need for the novel vaccines development. Currently, the development of vaccine remains a difficult procedure due to antigen diversity and the fact that the parasite has diagnostic life cycle in two divergent host which is human and sand fly vector. However, a combination of current efforts with genetically modified leishmania trial could lead us to at least applicable vaccines (Bessat *et al.*, 2015).

Drug resistance scenario of the 1st and 2nd line therapeutic regimen of anti-leishmanial and its severe side effects has coerced the scientific community to develop and design the new and improved anti-leishmanial that has high level of efficacy and less side effects. But unfortunately, the disease is neglected so financial support for the development and design of novel drug is unsatisfactory. Therefore, in this environment, scientific community has started working on a strategy to improve the existing conventional therapy and it has resulted in promising therapeutic efficacy. The specific enzymes or structures of leishmania parasites that is involve in the resistance of anti-leishmaniasis drugs (Yasinzai *et al.*, 2013).

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