Chapter

Visceral Leishmaniasis: Asymptomatic Facts

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Abstract

Visceral Leishmaniasis (VL) caused by protozoan parasite *Leishmania* is a vector borne disease and infection is limited not to human but also to animals worldwide. For infection identification and prevalence in both *Leishmania* endemic and nonendemic regions, several serological and genetic techniques are used. Although diagnostic techniques and clinical symptoms can establish illness status, it is extremely difficult to diagnose infection in the absence of symptoms. Asymptomatic are healthy people who have an infection but are unaware of it. The epidemiology of asymptomatic Leishmaniasis is critical for its eradication. Only a small percentage of infected people are clinically suspected of having VL, as the majority of them may not show any symptoms and remain asymptomatic. Some asymptomatic infections may go away after a while, or they may linger for years, or they may develop to illness with clinical signs. Asymptomatic infection varies per endemic location, but almost all of them point to this hidden category of parasite infection. It is now critical to understand many factors such as diagnostic markers, genetic markers, and immunological markers along with different risk factors. All of these criteria, as well as some innovative techniques to diagnosing and controlling asymptomatic leishmaniasis, will be covered in this chapter. The main focus will be on asymptomatic condition of Indian Visceral Leishmaniasis, which is caused by *Leishmania donovani* and spreads via female sand fly *P. argentipes* biting. The numerous criteria that play a role in asymptomatic to symptomatic conversion in a specific time period will also be discussed in this chapter.

Keywords: *Leishmania*, infection, asymptomatic, markers

1. Introduction

Leishmaniasis is a serious public health problem in many countries throughout the world. The illness is caused by numerous intracellular protozoan parasites of the genus *Leishmania*. The most frequent vectors of this neglected infectious illness are phlebotomine sandflies, *Phlebotomus* and *Lutzomyia*, which is most widespread in the tropics and subtropics of Africa, Asia, America, and southern Europe. This illness is the world's second most lethal parasitic killer (after malaria). It's multi-faceted. It can be a deadly murderer in certain forms, or a merciless mutilator who disfigures its victims for life in others. There are now an estimated 12 million cases of leishmaniasis in 98 countries, with 1.5–2 million new cases emerging annually, 1–1.5 million instances of cutaneous leishmaniasis, and 5,00,000 cases of visceral leishmaniasis (VL). VL can create large-scale epidemics with a high case fatality rate. VL (kala-azar) is a latent danger to more than 147 million people residing in the disease-endemic South East Asia region of the Indian subcontinent, which is

caused by *Leishmania donovani*. Out of the five VL-affected nations in the area (India, Bangladesh, Nepal, Thailand, and Bhutan), India accounts for more than 80% of recorded cases, while Bhutan and Thailand have sporadic reports. Bihar is the most VL-endemic state in India, accounting for 90 percent of all VL cases in the country [1]. *L. infantum, L. chagasi,* or *Leishmania donovani* are the parasites that cause Visceral Leishmaniasis (VL) in North Africa and Southern Europe as well as Latin America and East Africa respectively [2]. The transmission of *L. donovani* is usually thought to be anthroponomic. Its prevalence is gradually growing around the world, creating a public health issue in the VL endemic zone. The classic WHO definition of Visceral Leishmaniasis is "a person with clinical symptoms (primarily persistent irregular fever, splenomegaly and weight loss) and serological and/or parasitological evidence." Leishmaniasis Post Kala Azar (PKDL) has been related to Visceral Leishmaniasis (VL). According to WHO, probable PKDL affects individuals from VL endemic areas who have numerous hypopigmented maculae, papules, plaques, or nodules but no corresponding loss of sensitivity. However, these are the circumstances in which *Leishmania* infection manifests as symptoms. Many people in endemic locations are infected with the parasite yet show no symptoms of the sickness, according to previous research. These are classified as asymptomatic stages of *Leishmania* infection. *L. donovani* infection can range from asymptomatic carrier to full-blown symptomatic illness with persistent fever, splenomegaly, pancytopenia, and hypergammaglobulinemia. Visceral leishmaniasis has an asymptomatic incubation period of variable duration [3]. A large majority of those who have been exposed to the parasite are asymptomatic, with just a tiny number of people exhibiting clinical manifestations [4, 5]. A disease is considered asymptomatic if it lacks the visible symptoms that are normally associated with it. Asymptomatic conditions may not be detected unless medical tests are performed. Importantly, doctors lack the tools necessary to tell apart asymptomatic patients from those who are suffering from something more subtle [6]. In a Mexican cutaneous leishmaniasis region in 1953, the term asymptomatic in *Leishmania* infection was first used [7]. Asymptomatic *Leishmania* infection was used initially in 1974 by Pampiglione but the description has remained ambiguous five decades later. At this time, there is no way of knowing who of the asymptomatically infected persons would acquire VL illness and when. Asymptomatic people are those who live in an endemic region and have an immune response to L*eishmania* (either antibodies or a particular cellular response), or who have parasites—or parasite DNA—in their blood, but are otherwise healthy [8]. Escalating asymptomatic leishmaniasis due to distinct *Leishmania* species like *L. donovani* and *L. infantum* is critical for determining trends in the disease's prevalence. This first interaction between parasite and host is known as infection. The parasite can be killed by inherent or acquired immunity in the host, or it might persist by using an effective mechanism that bypasses the host's defenses. If the parasite persists, it may lead to a fascinating dynamic interaction between the host and parasite, where the host becomes an asymptomatic carrier when everything is in balance. Due to co-evolution, it is quite frequent in several parasite illnesses for the number of patients to be less when compared to the vast number of persons with asymptomatic infection (in general, an infected person who is asymptomatic is not necessarily a patient). Because of this, asymptomatic instances of VL are common in regions with the disease [9, 10].

2. Epidemiology

It is essential to understand the global prevalence of asymptomatic leishmaniasis. In addition, it's critical to look at the variables that contribute to asymptomatic

infection. The variation arises because of changes in parasite virulence and host demographic characteristics, as well as from research designs and the tests employed to determine asymptomatic infection. Worldwide epidemiological statistics show that asymptomatic VL can come from both endemic and non-endemic areas. Those who are infected may unintentionally transfer the disease to others. They may go away on their own, or they may develop symptoms at a later time. As per some findings Asymptomatic infections are those who remain seropositive for many (up to 10–12) years without developing into active disease [11, 12], and are more prevalent in VL endemic regions [13]. In the New World, asymptomatic leishmaniasis was considerably more frequent than in the Old World. The higher prevalence might be explained by the greater diversity of leishmaniasis in the New World as a result of the vector's adoption of new hosts and climate change [14]. Asymptomatic leishmaniasis was less common in children than in the general population. This difference, however, was insignificant on a statistical basis. It was hypothesized that the rise in infection prevalence with age was owing to young children's reduced exposure to infectious sandfly bites [15]. Some indicators indicate that in VL endemic locations, the ratio of asymptomatic vs. active VL patients varies: 2.4:1 in Sudan, 4:1 in Kenya, 5.6:1 in Ethiopia, between 4:1 and 17:1 in the Indian subcontinent, and 50:1 in Spain [16]. Reports from the other endemic regions also confirm the existence of parasitic DNA in all VL causing species in asymptomatic individuals.

According to a meta-analysis of original articles reporting asymptomatic leishmaniasis, the prevalence of asymptomatic leishmaniasis was 11.3%, 95% confidence interval (CI) 8.6%–14.4%] in general population, 36.7% [95% CI 27.6%–46.8%] in inhabitants living in the same or neighboring household to the symptomatic patients, and 11.8% [95% CI 7.1–19%] in HIV infected patient. Meta-regression analysis also showed no significant change in the prevalence of asymptomatic leishmaniasis during the last 40 years [17]. From 1982 to 2015, the trend of total leishmaniasis' asymptomatic proportion did not change considerably, according to the meta-regression study [coefficient = 0.0350 (95% CI, −0.0213 to 0.0913), P = 0.2233] [17]. But, while the disease's geographical range is broad, it is not continuous. The study also suggest, for *L. donovani*, the pattern of asymptomatic infection has not altered over time [coefficient = 0.0015 (95 CI, −0.0531 to 0.0561), P = 0.9564]. In contrast, the frequency of asymptomatic *L. infantum* infection has grown with time, although this shift is statistically insignificant [coefficient = 0.0824 (95 percent CI, −0.0126 to 0.1774), P = 0.0892]. According to research on *L. infantum* in the New World, the prevalence has considerably grown over time [coefficient = 0.0908 (95% CI, 0.0321 to 0.1496), P = 0.002]. The frequency of asymptomatic leishmaniasis in children is also rising over time [coefficient = 0.0599 (95% CI, 0.0066 to 0.1133), P = 0.028]. Drought, hunger, and high population density all contribute to the spread of the illness. The infection-to-disease ratio varies from village to village and also changes over time within the same community [18]. Asymptomatic incident *L. donovani* infection is nine times more common than incident VL illness in VL high-endemic foci in India and Nepal [19]. Within the next 18 months, around 1 in 50 of new yet latent infections developed into VL. There is one important asymptomatic category comprises those individuals of endemic regions who turn seronegative in due course of time [19]. These people most likely acquire the required level of immune response following parasite exposure, which protects them from future illness development by efficiently removing living parasites. They are most likely not carrying live parasites and can be called real resistant instances. The spontaneous change of seropositive to seronegative status ranges from 33–86% [19–22]. However, a research conducted in Bangladesh found that this conversion rate drops to as low as 6.3% after a year

among those with high antibody titers [23]. These people give more tangible proof that a threshold immune response level is required to protect the host against parasites. Despite the fact that the majority of seropositive asymptomatic people go on to become seronegative, these individuals are known to contribute to the spread of disease outside of endemic areas [24, 25].

3. Diagnosis

Diagnosis of VL is done by serological tests and molecular test along with direct parasite identification technique. Direct techniques are *Leishmania* parasite isolation from spleen, bone marrow, or blood for microscopy or culture. Different serological tests are ELISA, direct agglutination test (DAT), immunofluorescence antibody test (IFAT), indirect immunofluorescence (IIF), western blot (WB), rK39 immuno-chromatographic strip test while molecular tests include polymerase chain reaction (PCR), qRT-PCR and k-DNA southern blot whereas immunological test include Montenegro Skin Test (MST)/Leishmania skin test (LST) or Interferon Gamma Release Assay (IGRA). The lack of a good biomarker makes defining *Leishmania* asymptomatic infections extremely difficult. It's also unclear how to distinguish parasite persistence in an asymptomatically infected person from new infections that develop after the first episode, i.e. old parasites eliminated by the immune response followed by new infectious parasite populations that would follow the same destiny. Asymptomatic infections cannot be diagnosed with a single, widely approved test. It cannot be diagnosed using any standard or commercially available methods. Patients who are infected with *Leishmania* do not show any symptoms, but tests such as the polymerase chain reaction (PCR) or leishmania skin test (LST)/Montenegro skin test (MST) are positive regardless of whether they show any symptoms [22]. Population-based demographic and immunological surveys showed high but variable prevalence of leishmanial antibodies in the population of Bihar [26]. Serological test DAT/ELISA can perform in epidemiological studies as is noninvasive. But is indirect methodology and tells antibody response due to *Leishmania* infection. Because of the varying durations between infection and seroconversion (ranging from 3 months to 7 years), serology may not be a useful predictor of infection when employed in cross-sectional research [19]. The most often used techniques include an intradermal skin test that indicates the cellular immune response associated with prior exposure to *Leishmania* and the identification of anti-Leishmania antibodies, a less specific indication of infection or continuing illness [27]. Conventional PCR is direct tool as show presence of *Leishmania* specific DNA in interest of samples (blood, buccal swab, urine). Sensitivity and specificity vary for selection of primers. These above mentioned techniques are in use worldwide in epidemiological study to know prevalence of *Leishmania* infection in healthy individuals. But when data compare is compare with active *Leishmania* cases tough to distinguish healthy infection, i.e. asymptomatic. Using DAT seroconversion as a measure of infection, studies found that asymptomatic infection was nine times more common than acute VL illness in high-endemic foci in Bihar [19]. However, in any longitudinal epidemiological investigation, seroconversion should be the primary criteria for detecting asymptomatic infection. The variation in the ratio of VL cases versus asymptomatic cases in different *L. donovani* and *L. infantum* endemic areas from 2.4:1 in Sudan to 50:1 in Spain [28] reflects variations in parasite virulence and host features, However, this may also be due to variations in research design and the methodologies used to diagnose asymptomatic infection. Cell immunity generally lasts for several years, and in some cases, for the rest of a person's life [29, 30]. Serological indicators, on the other hand, can go from positive

to negative in as little as four months after the initial sample is examined [22]. In endemic locations where mean parasitemia levels are low or intermittent, serology is generally unreliable for identifying silent Leishmania infection [6]. Cytokine release assays are useful for detecting asymptomatic individuals among immunocompetent subjects in VL-endemic areas; they can also detect the same among immunosuppressed subjects following solid organ transplantation [31]. When compared with the reference test SLA-lymphoproliferative assay, IL-2 appears as a new, 100% sensitive and specific marker for asymptomatic individuals with a positive cellular response (compared with 100% and 84.78%, respectively, for IFN-γ) [32]. Some laboratory tests, including SLA-stimulated PBMC assay, may be difficult to perform under certain conditions. In contrast, the WBA holds much promise as a test at the point-of-care level [33]. The WHO recently recommended screening healthy populations for leishmaniasis infection using SLA-stimulated blood. There are ways to diagnose those who have asymptomatic *Leishmania* infection by whole blood stimulation with the soluble *Leishmania* antigen (SLA), followed by plasma cytokine and chemokine measurements. Combining these diagnostic tests with molecular studies might assist in estimating the real scope of the *Leishmania* outbreak in the endemic region. CXCL10 and CXCL9 DPS were shown to be reliable indicators for identifying asymptomatic individuals in *L. infantum* and *L. donovani* endemic regions. In distant areas, it makes samples more accessible and reduces the cost of epidemiological and epidemic investigations [34].

The immunological determinants such as Adenosine deaminase (ADA), Interferon gamma (IFN-γ), Tumor Necrosis Factor alpha (TNF-α) and Interleukin 10 (IL-10) were examined to predict probable biomarkers for conversion to symptomatic VL. Asymptomatic cases were also earlier reported to harbor the parasite in their blood [35, 36]. Many immunological methods such as direct agglutination test (DAT) and lateral flow immune-chromatographic tests, such as rK39 and rkE-16 have been introduced to screen large number of individuals in endemic areas [37–39]. *Leishmania infantum/chagasi* infection is endemic in Sicily. Approximately 47% of residents live in areas at risk of infection. The prevalence of asymptomatic carriers is unknown. In asymptomatic subjects, IFAT showed sensitivity (30.1%) higher than rK39-ELISA (26.3%) for the detection of cryptic infection, even though a lower specificity was reported (63.4 vs. 76.3%).

Molecular methods are the most suited due to the lack of a gold standard and the limitations of conventional diagnostic procedures, where parasitology is ethically impractical for persons without symptoms and serological tests do not discriminate between past and present illness. Recent molecular methods, such as conventional polymerase chain reaction (PCR) and quantitative real time PCR assay (qPCR), have made considerable advances in screening, diagnosis, and post-therapy follow-up, allowing for better sensitivity than prior serological assays. Quantitative PCR (qPCR) is now a days promising tool for detection and quantification of Leishmania and able to describe threshold as well as reference value for asymptomatic infection.

There are several types of molecular methodologies, and the choice of use should be based on what results are expected to be achieved. While in the conventional Polymerase Chain Reaction (cPCR) the results are only qualitative, quantitative products can be obtained in the Real-Time technique (qPCR), such as the levels of parasitic DNA circulating in the blood [40]. The sensitivity of the assays may vary according to the types of targets and samples used. The most used amplification targets are: kinetoplast DNA (kDNA) [41, 42], internal non-coding spacer region (ITS-1) [43] and the smaller ribosomal subunits (SSU - rRNA) [44–46]. Sudarshan et al. [35] when performing a qPCR, analyzed the level of circulating parasites to differentiate a possible disease progression. They obtained a minimum level of detection of 0.001 parasitic genomes/mL of blood and 34.79% of positive samples by the technique,

using the kDNA and hydrolysis probes of the TaqMan type, as a target and method of visualizing the products. Likewise, Kaushal et al. [6, 47] (S. Das et al., 2014; Kaushalet al., 2017, [6, 47] (S. Das et al., 2014; Kaushal et al., 2017) (Das et al. 2014, Kaushal et al. 2017, ([6, 47], when carrying out a study to detect asymptomatic individuals, obtained an amount < 5 parasites/mL of blood and a positive sample rate of 21.54%, using kDNA as a target and SYBR Green I as a result detection system. Sudarshan et al. [48], affirm that Leishmania DNA may be used as a marker of infection since it is detected before the seroconversion of antibodies. Individuals can be diagnosed as seronegative when they are tested before the development of immunity or when it is in a very low quantity, not being identified by serological methods. Similar data were also suggested by Costa et al. [27] and Bhattarai et al. [49], wherein asymptomatic infections detected by molecular methods have been observed in seronegative people. This demonstrates that possibly due to the limitations of serological methods, molecular tests are more suitable for the identification of asymptomatic cases. Although parasitic DNA is considered the first infection marker before immunological conversion [35], there are controversies regarding its use. The limitation of the use of DNA as a target is found in a possible detection of the genetic material of the parasite when it is already dead, although this is discussed, the half-life of the nucleic acid in the body is around 24 hours, which can cause flaws in distinguishing viable parasites from detecting fragments of lifeless parasites. Lack of standardization of a methodology still becomes a gap that can lead mainly to problems and delays in detection of the cases. Moreover, the use of nanoparticle techniques represents a trend for diagnosis, immunotherapy, and programs to eliminate VL. These methodologies bring a new approach with new forms of diagnosis and drugs, where improvements in efficacy and less toxicity can be observed. There will be continuous and significant improvements to all their current roles in diagnostics and will also provide multiple roles in terms of recognizing other DNA or materials, using fluorophores or other active molecules. it is reasonable to have a lower value of serum hemoglobin, hematocrit, and albumin among symptomatic patients. So, they would be considered as a marker of symptomatic diseases rather than a risk or protective factor. Studies are going on to define asymptomatic as yet there is no or very less agreement between different markers. Although Gold standard for Leishmaniasis detection is parasitological confirmation by microscopy which need splenic aspirate. But for asymptomatics it is not possible as ethical issues are very high because of invasive nature of samples. As the use of spleen or bone marrow aspirate is not ethical in asymptomatic subjects, the negative predictive value (NPV) cannot be exactly evaluated.

4. Immunology

Asymptomatic cases differ considerably from VL patients, and it is assumed that a mixed profile is crucial not only for the management of parasite replication but also for the preservation of these people' immune state. The increased number of cells expressing different cytokines demonstrates this. However, in VL-endemic areas, the clinical form is frequently asymptomatic, followed by protective immunity with a predominant type 1 T-cell response [50]. Asymptomatic seemed to have mixed profile having an increase of IFN-γ + neutrophils/eosinophils and NK cells, of IL-12+ eosinophils/monocytes, along with increase of IL-4+ neutrophils and NK cells and IL-10+ eosinophils/monocytes [51]. Despite earlier findings of a constant type 1 T-lymphocyte immune response during asymptomatic VL it was recently shown that asymptomatic patients' PBMC generated significant amounts of IL-10 when stimulated with *L. infantum* recombinant antigens [52].

Different findings point to the idea that IL-10 is a key immunomodulator in asymptomatic people, dampening host defense mechanisms and favoring immune response regulation following parasite elimination. Furthermore, CD4+ T cells from asymptomatic patients infected with *L. infantum* have been shown to generate significant amounts of IL-5 [53]. In immunocompetent people, leishmania infection is typically asymptomatic, although the percentage of HIV+ people infected with the parasite who stay asymptomatic is unknown. HIV+ individuals might still have a Th1-type cellular response to Leishmania despite their weakened immune system. These people may be identified using cytokine release tests, which identify IFN-γ in the supernatants of SLA-stimulated PBMC and IFN-γ and IL-2 in SLA-stimulated whole blood. These biomarkers appear to be 100% reliable for detecting asymptomatic immune responders to Leishmania among HIV+ patients [33]. Analyses of cytokine responses in symptomatic and asymptomatic VL patients' peripheral blood mononuclear cells (PBMCs) indicated that the production of Th17 cytokines was highly linked with the asymptomatic status [54, 55]. It was discovered by Carvalho et al. that peripheral blood mononuclear cells (PBMCs) from people with subclinical or asymptomatic infection (positive serology and skin test for Leishmania antigen) react to stimulation with Leishmania antigen by producing IL-2, IFN- and IL-12 [50]. An Indian study found that active disease elicited a mixed IFN-/IL-10 response, but asymptomatic infections (IFN- release assay [IGRA]–positive endemic healthy controls) did not trigger an antigen-driven whole-blood IL-10 response [56]. Surprisingly, the frequency of CD4+ T cells is higher in people with asymptomatic infections who have positive LST [57], Furthermore high levels of IFN- are produced by CD8+ T cells isolated from asymptomatic patients, which implies that CD8+ cells play a role in human resistance to Leishmania infection. Furthermore, researchers discovered that in asymptomatic people, a distinct subpopulation of CD4+ cells that produced both IFN- and IL-5 was important for infection management [22, 53]. A longitudinal study conducted in Sudan recently suggested that Th17 cells may play a protective role in human VL, and it was found that *L. donovani* stimulates the production of IL-17 and IL-22 by exposed PBMCs from healthy and resistant subjects who did not develop VL before or after cytokine response testing. In addition, elevated levels of IFN-γ, C reactive protein, nitric oxide, and IL-12 in the blood have also been reported to offer resistance in asymptomatic patients [58]. Disease resistant endemic individuals have an immune response that shields them against pathogenesis in response to an insect bite which are quite similar to those of VL immunology, and as a result, they cannot be utilized to provide a clear picture of protective immune parameters in asymptomatic patients. Besides the protective immune response, it appears that these people either possess a large number of long-lived memory B cells that continuously secrete antibodies or they are continually exposed to *Leishmania* but do not acquire VL as a result of the protective immunological response [59]. The quantity of antibodies in blood has been connected to the incidence of asymptomatic to symptomatic VL conversion. Although it will be difficult to track these instances, they may aid in the discovery of host immunological mechanisms that influence disease susceptibility and resistance. Strong cell mediated immunity, a broad repertoire of memory T and B cells, and short-lived plasma cells may be linked with the immune biology of resistant asymptomatic infections (they do not show seropositivity for a longer period). Focused research on these people might disclose the characteristics of protective immunity that are needed to build a preventive vaccination candidate. In asymptomatic patients, the levels of ADA, IL-10, and IFN-γ were continuously high, with ADA and IL-10, but not IFN-γ, remaining higher as clinical symptoms progressed into active VL. ADA and IL-10 might be used as a biomarker in the transition from asymptomatic to symptomatic VL [60]. Due to their high innate cellular immunity, many asymptomatic individuals become seronegative without acquiring

VL. IFN- γ became high in asymptomatic infection but dropped after conversion, but TNF-α levels did not alter much at either stage of illness. The cytokine profile might be utilized to better treat VL patients with autoimmune diseases, as well as to identify and protect individuals with asymptomatic infection who are at risk of developing illness. Assays for cytokine release are already being utilized to detect asymptomatic individuals [31, 61]. Cytokines, which operate on macrophages, are receiving a lot of attention these days because of their ability to alter the immune response. Studies on the function of cytokines in asymptomatic infections and/or subclinical VL cases are scarce in the literature, and these investigations generally assess cytokine levels just once, before any clinical symptoms arise. Research into immune responses shows that patients with low levels of IL-10 production have additional flaws. In VL, IL-10 mRNA expression is highly expressed, and this cytokine's involvement in reducing T cell responses in these individuals has been well established. According to the finding, the balance between the production of IFN-γ and IL-10 may be a significant factor in determining whether or not patients develop illness after contracting *L. chagasi* infection even while they are asymptomatic [62].

5. Genetics

For many years, there has been speculation that, in VL, the Leishmania genotypic differences involve in asymptomatic or symptomatic forms of the disease. There was findings that the Leishmania Internal Transcribed Spacer 1 (ITS1) from symptomatic VL and asymptomatic cases has significant genetic differences in southern Iran [63]. Several investigations have shown that the genotypic characteristics of symptomatic and asymptomatic VL patients might differ [64, 65]. Researchers identified significant genetic diversity between *Leishmania* species isolated from asymptomatic and symptomatic patients, particularly those with HIV/VL coinfection, in a study done in southern France. The study also discovered that asymptomatic isolates had a modest polymorphism in their parasite genome [64]. In Southern France, MLMT showed parasite genotype appear to differs in *Leishmania* patients compared with asymptomatic related carriers [66].

Aside from the parasite genotype, the host's genetic background may have a role in determining whether VL is asymptomatic or symptomatic [67]. Study also linked symptomatic VL to a gene that codes for a receptor for transforming growth factor beta (TGF-β) whereas the asymptomatic is connected to gene encoding II-a receptor for the Fc fragment of IgG [67]. However, the association between SNP/HLA genotyping and progression from asymptomatic or seroconversion to VL overt disease has been insignificant [68]. Polymorphism at SLC11A1 has been shown to be linked [69, 70] and associated in regulating susceptibility with human VL in Sudan. However, no evidence of such an association was found in an Indian population [71]. Few studies indicate that host genetic association and development of clinical symptoms is linked to NRAMP1, TNF-α, IL-4 and IFN-γ receptor (IFNGR1)], TGF β, IL-8, C-X-C chemokine receptor 1 (CXCR1) and C-X-C chemokine receptor 2 (CXCR2), IL-2R, Delta-like1 (DLL1), and mannan binding lectin genes [48, 69, 72, 73]. In one of the recent most studies on asymptomatic VL, were able to link several HLA-DRβ allele groups with the progression of VL [68].

6. Other risk factors

Besides, serological methodologies performed on individuals without symptoms may have low sensitivity due to a weak humoral response [74]. Risk factors have

been analyzed by some studies, taking into account that contact with the parasite is necessary, but it is not sufficient for the development of the active disease. These characteristics can play an important role in the cycle of asymptomatic individuals [6]. The male gender is one of the individual factors that demonstrate a positive association with asymptomatic infection [75]. Although other hosts and parasite variables may be additional causes, the conversion of asymptomatic infections to symptomatic VL also indicates the survival of parasites in these people [76]. The extrinsic variables such as age and nutritional state, as well as a weakened host immunological system, are thought to be significant in the progression from asymptomatic to symptomatic infection. Poor dietary status has been linked to an increased chance of developing clinical VL in addition to hereditary risk. The relationship between malnutrition and the course of VL at the cellular level is poorly understood. A better understanding of these mechanisms might open new opportunities for prevention or therapeutic dietary intervention [16].

There were evidences that suspected individuals living in households with family history, were at particularly high risk of infection. Although the cohort studied did not contain population-specific genetic markers, the addition of such factors might help predict outcomes when molecular diagnostics and serodiagnostic testing are combined. Even if they have a competent immune response, persons who have come into touch with the parasite do not inevitably acquire the symptomatic version of the condition [16]. Age, genetic, immunological, and dietary features, the existence of other diseases, and vector density are all potential risk factors for the disease's development [75], and type of "asymptomatic" definition applied to the study [28]. Despite being practical and easy, methodologies handling have some limitations: (i) do not differentiate past disease from recent [75, 77] (ii) there is the possibility of cross-reactivity with other related parasites [78]. Asymptomatic infection is usually observed in family members or in direct contact to clinical VL cases, suggesting that family members are at risk of infection. In a research from India, it is discovered that family members of VL patients had 1.8 times the risk of becoming infected as compared to those who did not have VL in the house. Kala-azar patients were younger ($P < 0.001$) and reported lower red meat consumption ($P < 0.01$) than asymptomatic seropositive individuals. Retinol and zinc levels were lower in current kala-azar patients and those who later developed kala-azar compared with uninfected and asymptomatically infected subjects. The characteristics that help determine whether an infection leads to overt disease appear to include age and dietary factors such as intake of iron- and zinc-rich red meat [79, 80]. Kala-azar patients were younger ($P < 0.001$) and reported lower red meat consumption ($P < 0.01$) than asymptomatic seropositive individuals. In comparison to uninfected and asymptomatically infected people, active kala-azar patients and those who later acquired kala-azar had decreased retinol and zinc levels. In contrast with different groups, kala-azar patients had greater CRP values. A population at increased risk of symptomatic illness may have a low red meat intake and low zinc and retinol levels.

7. Conclusion

Control efforts for leishmaniasis (especially asymptomatic VL), particularly in endemic regions, need a detailed understanding of *Leishmania* ecology and epidemiology. Those infected with viscerotropic *Leishmania* species, on the other hand, may remain asymptomatic, which is the most typical result of infection in endemic regions. However, it is impossible to offer reliable estimates of the number of infected vs. those at risk. The number of persons infected but asymptomatic is far greater than the number of people infected and presenting with clinical disease.

As a result, it is critical to understand how many infected people will acquire illness and how they may be identified before clinical symptoms appear. Different studied found that utilizing anti-rK39 ELISA to screen family members and contacts might be a very reliable technique for early diagnosis and planning preventive treatment of latently infected asymptomatic carriers in order to eradicate kala-azar. Although there have been isolated instances of parasite circulation in the peripheral blood of asymptomatic individuals with *L. donovani* and *Leishmania tropica* infection. Various study findings explain the immune response as tracked prospectively and its diagnostic value in predicting the fate of latent infection in a relatively large number of patients. Serologically positive state a relatively transient increase in serum antibodies caused by recent infection that lasts for months, whereas LST positive thought to indicate long-term cell-mediated immunity after asymptomatic infection or clinical cure of kala-azar. A favorable LST result may take months to years to manifest after effective kalaazar therapy, but it lasts for decades after exposure. There are few data on risk factors for asymptomatic leishmaniasis, and its epidemiology is unknown. Such knowledge is critical for efforts to prevent and control visceral leishmaniasis, such as the eradication programs. In a Brazilian research, sand flies fed on kala-azar patients were sick in 25% of cases, while none of the sand flies fed on asymptomatically afflicted individuals became infected. Seroepidemiologic data from disease-endemic areas of India are scarce and based on small sample sizes. Serologic status is not a good predictor for conversion to clinical VL. Studies confirmed that 33% persons were serologically positive, only 3.48% seropositive persons showed disease conversion. However, 2.57%seronegative persons at baseline showed disease conversion also [11]. Human instances of transfusion-transmitted visceral leishmaniasis (VL) have been reported in both endemic and non-endemic locations, with clinical characteristics and outcomes comparable to those of natural infection [81].

When innate immune cells from asymptomatic carriers were stimulated with antigens in vitro, they exhibited a regulated rise in cytokine production that differed from that seen in non-infected subjects. This implies that using more than one diagnostic approach makes it easier to identify a substantial proportion of asymptomatic carriers. One often mentioned flaw in these research is the difficulty in identifying those who are briefly and quietly infected with the parasite. A recent study of asymptomatic people' innate and adaptive immunity revealed that a mixed cytokine profile is crucial not just for parasite replication control, but also for the preservation of these individuals' immune state [51]. Only 20% of those infected with *L. chagasi* in endemic regions of VL develop classical VL, according to research. Even before extremely sensitive molecular diagnostic technologies became available, it was established that the majority of infected people living in an endemic region have asymptomatic self-cured illnesses [82]. Nonetheless, despite the PCR assays' excellent sensitivity and specificity in identifying *Leishmania* DNA in clinically sick patients' peripheral blood, a proportion of asymptomatic persons with positive serological tests had no detectable circulating *Leishmania* DNA. As a result, the co-positivity between the PCR assay and different serological assays was astonishingly low. It's conceivable that these apparent differences might be explained by how an illness develops in people. The parasite can be identified in the peripheral blood at a certain point (providing a positive PCR assay), followed by adaptive-mediated immunity with the generation of antibodies and a T lymphocytes-mediated immune response. Because these biological moments are never-ending, it's possible to analyze certain people when they are in a state of transition. Other non-invasive and extremely sensitive techniques, such as PCR in peripheral blood, have recently been available. This has made it possible to identify

asymptomatic carriers of *Leishmnia* who otherwise would not have been detected using just serological methods [83]. Research findings suggest that the method used to diagnose an infection may have an impact on infection-related variables including risk factors and treatment results [61]. At the moment, it is impossible to determine who among the asymptomatically infected persons will acquire VL illness and when. A research from Bangladesh further indicates that about 80% of asymptomatic individuals contribute to disease transmission, compared to 8–10% of VL and PKDL patients [13].

The question of why just a few exposed asymptomatic people acquire full-blown illness symptoms but not all remains unsolved. The major immunological variables that promote the conversion of asymptomatic patients to symptomatic stage of visceral leishmaniasis have been attempted to explicate in several research. It is crucial to note that the use of five diagnostic techniques as a regular strategy would be impractical in endemic situations. Before a particular recommendation can be made, more research is needed to confirm the optimal diagnostic method [61]. The important checkpoints for determining disease resistance or susceptibility are cytokines that control cellular immunity [84, 85]. Despite substantial understanding of host–parasite interactions and immunobiology, reliable protective immunity criteria have yet to be discovered. Asymptomatic instances of human VL can be diagnosed using qPCR using RNA targets. There are also questions about whether or not using RNA as a gene target can help discover asymptomatic instances of VL. By combining this methodology with epidemiological data analysis, it will be possible to improve the detection and treatment of asymptomatic cases. Priority should be given to stepping up efforts to better characterize asymptomatic illness in endemic areas and to develop a uniform case definition for leishmanial infection. Self-clearing infections vs. illness development must have all of their factors examined thoroughly. The problems in parasite and sand fly management methods, as well as changes in the epidemiology of VL in disease-endemic countries, are important threats to its eradication. In addition, the movement of infected but asymptomatic people from endemic areas has resulted in additional infections in non-endemic areas [86]. The xenodiagnosis approach validates whether asymptomatic infected people can be infectious to sand flies and might be a crucial step in determining whether or not to modify the existing VL management strategy. One study used xenodiagnosis to identify VL infection in HIV-positive people and found even asymptomatic patients in the early stages of the infection were able to infect others [87]. Although Xenodiagnosis findings from an Indian investigation indicate that neither asymptomatic nor treated patients were infected by vector sandflies [88]. Asymptomatic infected persons are not now the focus of drug research efforts. This is because the asymptomatic state is not clearly defined, but largely because an intervention is less of a priority as long as the role of asymptomatically infected people in transmission is not clarified. There have been significant gains in reducing infection rates thanks to the eradication programs, but there are still some obstacles to overcome along the way. Considering that humans are the sole reservoir for *Leishmania donovani*, the effectiveness of a control programme hinges heavily on treating both symptomatic and potentially asymptomatic persons, if such individuals are found to also function as a reservoir. The lack of techniques to identify live parasites in silent infections and their relationship to disease transmission, sterile cure characteristics, and PKDL development remains a serious danger to the disease's eradication. There has been tremendous progress in the control or elimination of tropical illnesses, with a large drop in the incidence of these diseases. Although it is critical to comprehend these underlying causes for each illness, asymptomatic

carriers are a common component that can contribute to resurgence; their effect in terms of population percentage and function in transmission must be established. More study is needed to completely understand the determinants.

Conflict of interest

None.

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