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REVIEW ARTICLE

Visceral leishmaniasis; literature review and Iranian experience

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INTRODUCTION

Visceral leishmaniasis (VL) or Kala-azar, a life-threatening parasitic infection, is often caused by various strains of Leishmania donovani, L. infantum, and L. chagasi and is endemic in Middle East and Mediterranean regions. Northwest and southern Iran are the primary foci for VL, which mainly affect children, therefore, L. infantum is expected to be dominant Leishmania strain in Iran (1-3). L. tropica which is typically more dermatotropic, has been confirmed as a causative agent for VL in Iran (2) and an etiological agent of viscerotropic leishmaniasis among US servicemen in Persian Gulf war in 1990 (4). It seems that L. tropica is the second etiological agent of VL in Iran, particularly in Southern regions. VL which was caused by L. major has been recently reported in a 30-year-old man living in Bushehr Province in Southern Iran (5).

While a minority of infected individuals develops full blown VL, characterized by fever, hepatosplenomegaly, anemia, neutropenia and hypergammaglobulinemia, most of them remain asymptomatic with the disease self-limiting (6).

Major investigations into VL in Iran have been carried out in Shiraz (in south of Iran) and Meshkin-Shahr (in northwest of Iran). Herein we review major topics and reports on our experiences on VL.

RESERVOIR, VECTOR, HUMAN INFECTION

The parasitological and serological examinations of animal reservoirs showed that dogs, jackals and foxes were infected in endemic foci (7, 8). L. infantum and L. tropica were isolated from viscera of both a wolf and a domestic dog (9). Gray and golden hamsters are other possible animal reservoirs (1, 10).

It is proved that Phlebotomus alexandri harbor L. infantum in Iran (11). Ph. major, Ph. kandelakii and Ph. Perfiliewi have been proposed as suspected vectors of VL in the endemic foci in Iran (12-14).

It has been revealed that L. donovani can be transmitted to the fetus of the affected but untreated mothers. Report of a case of confirmed VL due to L. infantum in Germany revealed that the asymptomatic mother must have had a subclinical infection with leishmania that was reactivated by pregnancy, and then congenitally transmitted to the child (15).

In a recent study in an endemic area of Fars province in Southern Iran, L. infantum K-DNA was detected by PCR-ELISA in 6 (6.8%) of 88 pregnant women peripheral blood samples. All 88 samples of cord blood had negative results. This

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study demonstrated that asymptomatic mother could not be accounted for as a major cause on transmitting L. infantum to child in the surveyed district. Should the disease originate from vertical transmission, it has to be a rare case (16).

The first case of human VL in Iran was reported by Pouya in 1949 and the first case series was reported by Tahernia and Jalayer in 1968 (17, 18). After that several case series reported from endemic and no endemic areas in Iran, but a precise number of patients is not available.

In the endemic foci, the disease is the most frequent among the rural population and nomads. Around 95% of patients are under 5 years and about 30% are under one year (1,19). Male to female ratio in endemic foci is 1.27-1.6 (1,20).

In a recent study, the seropositivity rate (defined as IFAT>1/20) in two endemic areas of Fars province was 54.6% and L. infantum K-DNA was detected by PCR-ELISA in 95 (24.5%) of 388 samples. All PCR positive individuals had IFA titer in their own serums but IFAT was not positive in all PCR positive individuals. It seems that appearance of the parasite in the body stimulates humeral immune system resulting in antibody production which in turn causes positive IFA. Meanwhile, stimulating of cellular immune system by the parasite causes the disappearance of the parasite in the blood and negative PCR, accordingly (Unpublished data).

New cases of VL are diagnosed throughout the year, but mostly from January till April (21) because incubation period varies widely from 6 weeks to 6 months and sand flies are active from mid-June through mid-October (14).

In a 1 year prospective survey in north-west Iran, the average incidence rate of infection was 2.8% per year with all ages equally at risk. One in 13 infections in children led to VL, and this ratio decreased significantly with age (22).

CLINICAL MANIFESTATIONS

The most common clinical features are anemia, fever, splenomegaly that present in >90% of cases in southern and northwest Iran (1,23). Hepatomegaly is less frequent than splenomegaly. Findings such as jaundice, edema and ascites reported less frequently (1). It seems that all of signs and symptoms are compatible with Mediterranean type except for the absence of significant lymphadenopathy.

Cutaneous manifestations of VL are encountered frequently. Post kala-azar dermal leishmaniasis (PKDL) is a complication of VL, characterized by a macular, maculopapular and nodular skin lesions, which usually starts periorally in patients recovered from VL and who are otherwise healthy. The main loci are in the Sudan and India, where PKDL follows treated VL in 50% and 5-10% of cases, respectively (24). To date, only two cases of PKDL have been reported from Iran (25, 26).

Recently K-DNA of L. tropica was detected by specific PCR on whole blood and skin lesions of a 15 year old girl referred from Kazeroun district of Fars province with hepatosplenomegaly, multiple intraabdominal lymphadenopathies, hyperglobulinemia and numerous papulonodular, painless, non-pruritic, pink lesions, appeared on extremities, back and face ,few of which became ulcerative. She was labeled as a case of VL with atypical disseminated cutaneous leishmaniasis (27). VL with disseminated dermal leishmaniasis was reported in five cases, but the type of Leishmania species was not identified (28).

The principal involved organs are in reticuloendothelial system, other organs such as kidney and the submucosa and mucosa of the digestive system may be filled with infected macrophages. Duodenal involvement has been reported in three immunocompetent cases with VL from southern Iran (29).

Bacterial superinfection such as pneumonia, septicemia, otitis media, urinary tract infections and skin infections is one of the major complications leading to death in patients with visceral leishmaniasis. Bacterial infections were found in 22 (41%) of 54 patients admitted to hospital with visceral leishmaniasis (30).

The most common laboratory findings are anemia, elevated ESR, thrombocytopenia and neutropenia (1,21,31). Bandemia (>10% of total WBC) were reported in 33% of patients (1). Hyperglobulinemia typically is found. Rheumatoid factor was positive in 34% (17 of 50) which indicates immune complex process in the infantile type of VL (32).

Erythrophagocytosis by histiocytes is seen commonly, and the anemia so typical of kala-azar may in part be the result of such sequestration of red cells. Benign hemophagocytic syndrome in VL was reported in southern Iran (33).

Relapse has been detected in 1 (1.3%) of 76 patients that followed for 6 months (34). Mortality rate is fortunately low and has been reported in 2.7% of patients (35).

PATHOGENESIS

Parasite factors and host mechanisms are inextricably linked in pathogenesis. To initially establish infection, promastigotes enter macrophages silently to evade triggering host responses (36).

Leishmania promastigotes bind to some of the surface molecules like complement receptor 1 and 3 (CR1&3) and C3b of macrophage before they are internalized. CR1 constitutes the major macrophage ligand for mature promastigotes, though additional parasite surface glycoprotein (e.g., gp63 membrane protease) and other macrophage receptors (e.g., CR3, mannose fucose receptor) have been implicated in various studies. Once internalized, promastigotes transform into intracellular amastigotes (37).

Amastigotes replicate by binary fission, eventually rupturing the macrophage and spreading to uninfected cells. Progressive intracellular (amastigotes) infection depends on the maintenance of macrophages in an inert, deactivated state. At the same time, however, the immunocompetent host is also equipped and responds with interdigitating non-specific (innate) and antigen-specific (acquired) mechanisms (cell-mediated immunity, delayed-type hypersensitivity). These inflammatory responses mediate disease expression and may (asymptomatic infection, self-healing disease) or may not (non-healing disease) produce the desired clinical end-results (figure 1) (36).

Leishmania do not behave as inert particles in their hosts. Rather, they actively secrete proteases and other factors that affect immune cells and cytokines of the host. Leishmania lipophosphoglycan (LPG) is a multifunctional molecule expressed only in the promastigote stage, which has been suggested to promote parasite survival through several mechanisms including retrograde progression through the gut of the sand fly vector, resistance to complement mediated lysis, inhibition of oxidative responses, evasion of phagosome-lysosome fusion, and inhibition of protein kinase C (38).

The production of interleukin (IL)-12 by antigen-presenting cells (APCs) and interferon (IFN)- γ by T cells appear to be required for control of the parasites and development of acquired resistance. Although peripheral blood mononuclear cells (PBMCs) from humans with subclinical/asymptomatic infection (i.e. leishmanin skin-test-positive individuals, with no history of disease) respond typically to stimulation with leishmanial antigen with the production of IL-2, IFN- γ and IL-12, a key immunological feature of VL is the inability of PBMCs to proliferate or to produce IFN- γ in response to leishmanial antigens (39).

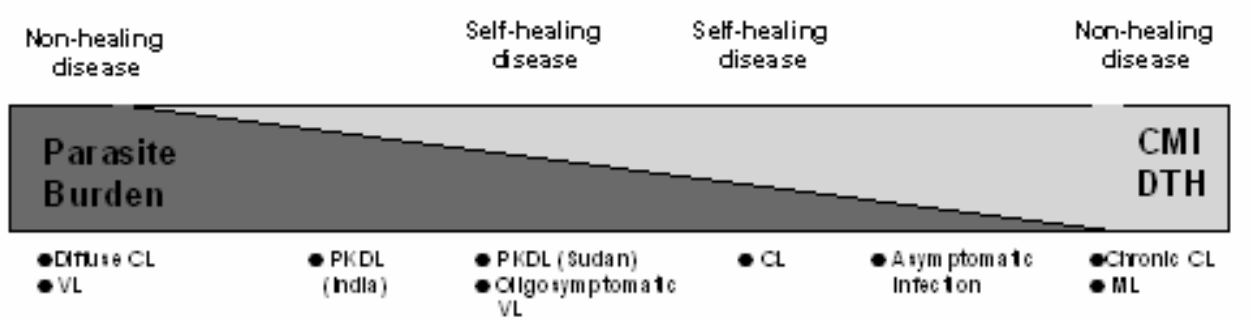

Figure 1. *The relation of clinical spectrum of human leishmaniasis between effective cell-mediated immunity (CMI), delayed-type hypersensitivity (DTH), and parasite burden.CMI/DTH and parasite burden are inversely related. Asymptomatic infection (vigorous but not pathologic CMI/DTH; low parasite burden). Nonhealing chronic disease is produced differently, by either: (a) uncontrolled infection because of absent [diffuse cutaneous leishmaniasis (CL)] or ineffective CMI/DTH [visceral leishmaniasis (VL), PKDL] or (b) unrestrained CMI/DTH and pathologic inflammation despite low parasite burden [mucosal leishmaniasis (ML), chronic cutaneous leishmaniasis (CL)]*(adapted from reference 36).

Recent reports have indicated that despite the presence of high levels of IFN-γ, infected host may fail to control Leishmania infection due to defective response of IFN-γ induced intracellular signaling mechanisms. Studies on IFN-γ receptor-1(IFN-γ R1) have highlighted that during the active VL there is low expression of IFN- γ R1, and after treatment with sodium antimony gluconate (SAG) there is up-regulation in the receptor level (40).

Both experimental and clinical data support a relevant pathogenetic role for IL-10, especially in visceral leishmaniasis and PKDL, and cytokine balance (e.g., interferon γ to IL- 10 ratio) can affect both clinical outcome and responses to treatment (39). It seems that IL-10 can inhibit type-1 immune response and cause a more severe form of the disease. After medical therapy, IL-10 serum level decreases significantly, which may be associated with recovery (41).

In a study about polymorphisms in IL-10 gene promoter, there were no significant differences in genotype and allele frequencies of investigated IL-10 gene polymorphisms between pediatric patients with Kala-azar, healthy individuals who were patients' siblings and healthy individuals who lived in an endemic area without any history of Kala-azar or cutaneous leishmaniasis and with positive leishmanin skin test. Since the results showed no significant differences in genotype and allele distributions between the groups, other cytokines or cytokine gene polymorphisms may be involved (42).

Under optimum conditions, macrophages are eventually activated to a leishmanicidal state largely governed by an intact T-helper cell-type 1 (Th1) response. This complex response revolves around antigen-presenting dendritic cells, responding CD4+T cells, and secretion of pro-inflammatory cytokines, including interleukin 12, interferon γ and tumor necrosis factor. This same Th1 response also prevents recrudescence of latent, chronic infection. In subclinical infections, host responses are by definition effective and presumably tightly regulated since signs of inflammation are not noticeable. By contrast, inflammation is prominent and underlies pathogenesis in nearly all forms of clinically apparent infection (36).

Protective immunity against L. donovani is dependent on an IL-12-driven type 1 response and IFN- γ production, which results in the induction of parasite killing by macrophages primarily via the production of reactive nitrogen and oxygen intermediates (38). Since not all infected humans

develop disease, there are probably differences in the response between individuals. This could be due to differences in the NO-production (43).

DIAGNOSIS

Demonstration (microscopy) and isolation of parasite (culture): Direct visualization of amastigotes in clinical specimens is the diagnostic gold standard in regions where tissue aspiration is feasible and microscopy and technical skill are available. In three studies, carried out in Shiraz, bone marrow aspiration and staining were positive in 35-50% of symptomatic Kala-azar cases (44). The results of culture in Novy-McNeal-Nicole (NNN) and RPMI 1640 media have been disappointing (45). On the other hand, 89.5% of the splenic smears were found to be positive for Leishman bodies. Spleen puncture resulted in severe hemorrhage and hypotension in two patients which necessitated splenectomy in one. The report of the procedure in Kenya was of high safety, as compared to ours. It could be explained in part by the fact that children in Kenya were over 5 years while in ours they were under 2 years of age, and perhaps the trivial amount of blood leakage commonly occurring after this procedure which is due to lower weight might have caused hypotension. We, therefore, do not use this procedure except under certain circumstances (45).

Serological methods: Serological methods are highly sensitive and being non-invasive. They are comparatively more suited for diagnosing VL in endemic regions. These methods are either based on detection of antibodies (produced against parasite by polyclonal activation of B cells) or antigens. Many conventional methods for antibodies detection ,for instance, gel diffusion, complement fixation test, indirect haemagglutination test, indirect fluorescent antibody detection test (IFAT), and counter current electrophoresis have been evaluated with varying sensitivities and specificities. The sensitivity and specificity of the IFAT were 100 and 92.7 per cent in Shiraz, respectively (6).

Direct agglutination test (DAT) has been found to be 91-100% sensitive and 72-100% specific in various studies elsewhere in the world (47). Our experience in Shiraz revealed that this test is not suitable for the diagnosis of the acute illness because titer remains high after previous illness.

Detection of anti-K39 by immuno-chromatographic strip testing is a rapid and non-invasive method of diagnosing Kala-azar. It entails a good sensitivity and specificity and is well studied for use in field conditions. In symptomatic patients, anti-K39 strip-test sensitivity is high (90–100%), while specificity might vary by region (36).

In our study, the sensitivity and specificity of anti-K39 strip-test were 82.4 and 100 percent, respectively (6). The rK39 strip test can be readily used under field conditions and needs only one drop of peripheral blood. Clinicians can perform it independently without laboratory facilities and treat patients without any requirement to refer them to equipped hospitals or clinics. Our results confirm that the rK39 strip test is suitable for use in this region and other developing countries for the diagnosis of visceral leishmaniasis in infants (6). The rK39 ELISA has high predictive value for detecting VL in immunocompromised patients, like those with AIDS (46).

Two urinary antigens of 72-75 and 123 kDa have been reported to be very useful in diagnosis and prognosis of Kala-azar with sensitivity of 96% and specificity of 100% (47).

DNA detection methods: A variety of nucleic acid detection methods targeting both DNA and RNA have been developed. The most suitable target for the DNA based diagnosis is kinetoplast DNA minicircle (K-DNA). The leishmania polymerase chain reaction (PCR) assays using peripheral blood as clinical specimen showed to be a highly efficient non-invasive alternative with sensitivity varying from 80-100% (46).

In a cross-sectional study performed on 388 blood samples from healthy persons living in two endemic areas from August to September 2005 in Fars province, anti-leishmania antibody was detected in 212 cases (54.6%) using IFA method but we did not note anti-leishmania antibody in over 1/80 dilution of IFAT positive samples. L. infantum K-DNA was detected by PCR-ELISA in 95 (24.5%) out of 388 samples, thus, detection of K-DNA by PCR cannot serve as a sufficient diagnostic tool and it seems that a positive PCR test together with IFAT ≥1/80 can easily diagnose acute Kala-azar (unpublished data).

The leishmanin skin test (LST): LST is a measure of delayed hypersensitivity to leishmanial antigen. The test remains negative through the period of active disease. The change from negative to a positive LST is regarded as an important prognostic sign (48).

In our study, LST was positive in 132 (34%) healthy persons living in two endemic areas and, surprisingly, we showed K-DNA with PCR in blood samples of 55 LST negative individuals. In this study, K-DNA was detected in blood samples of 42 (30.3%) individuals with positive skin test which indicate that stimulation of CMI alone cannot cause the elimination of parasite in the blood (unpublished data). We can conclude that LST is not sufficient for surveying the prevalence of VL infection in endemic regions, and has to be verified by DNA detection PCR.

TREATMENT

There are nearly 25 compounds having antileishmanial effects, but only a few are classified as antileishmanial drugs used for humans and most of these are parenteral. However, the first effective drug was ureastibamine, which was discovered in 1912 by Prof. Brahmchari, a Nobel laureate in 1929 (49).

Pentavalent antimonials: Antimony remains the therapeutic cornerstone in all regions except regions with high resistance such as Bihar State, India (36). The most commonly used organic compounds of antimony (Sb) are sodium antimony gluconate (SAG) and meglumine antimoniate (Glucantime). Although the precise mechanism of action is not fully understood, the antimonials are known to inhibit glycolytic enzymes and fatty acid oxidation in leishmania amastigotes, and there is a dose-dependent inhibition in net formation of adenosine triphosphate (ATP) and guanosine triphosphate (GTP) (49).

The recommended dosage is 20mg of pentavalent antimony (SbV) per kg of body weight per day for 28 days. Subjects who relapse may respond to a second course and some experts recommend the second course for two months (50).

We recommend short course therapy for treatment of visceral leishmaniasis in southern Iran. In a randomized clinical trial, a single deep intramuscular injection of 20mg/kg/day Glucantime continue for seven days after defervescence was found adequate. The mean period for treatment was 11.7 days (34).

Common side effects with stibogluconate sodium and meglumine antimoniate include abdominal pain, anorexia, vomiting, nausea, myalgia, arthralgia, headache, and malaise, but these complications seldom prevent completion of therapy. Elevated amylase and lipase occur in most recipients, but only a minority manifests clinically apparent pancreatitis. Electrocardiographic changes are dose-dependent and include T-wave inversion and a prolonged QT interval (50). Hepatic and renal function may be impaired (51).

Amphotericin B: Amphotericin B is a polyene antifungal antibiotic agent which binds to cell wall ergosterol and causes holes in the membrane, leading to parasite death (49). Various doses and durations of therapy have been used; 1.0mg/kg/day for 15 days or 1.0 mg/kg/every other day for 30 days are two alternatives (50).

In a patient with jaundice, we suggest amphotericin B rather than Glucantime due to its liver toxicity effect.

Lipid formulations of amphotericin B: Lipid formulations of amphotericin B representing macrophage-targeted treatment induce side effects much less frequently than the free drug and are very active in 5–10 day regimens (36). Safety of liposomal amphotericin B permits administration of total dose requirement in a single infusion. Lipid formulations of amphotericin B, though expensive, represent a major advance in treating patients with VL resistant to conventional drugs (52).

Allopurinol: Allopurinol, a hypoxanthine analogue, was the first oral drug. Allopurinol is hydrolyzed to allopurinol riboside, an analogue of inosine that is incorporated instead of ATP into leishmanial RNA and interferes with the normal protein synthesis. Allopurinol has been used to treat leishmaniasis for decades. Allopurinol is not effective as monotherapy for VL (49). SbV plus allopurinol was found superior to SbV when used as monotherapy (52).

Miltefosine:Miltefosine, an alkyl phospholipids, developed as an anti-tumor agent, has excellent antileishmanial activity and is the first effective oral treatment for visceral leishmaniasis, as well as for antimony-resistant infection, opened the door to self-administered outpatient therapy; approved in India (2002), Germany (2004), and Colombia (2005) in a 28-day course. In all clinical studies, a cure rate >94 per cent has been found consistently with this drug. Gastrointestinal reactions are common but usually transient (36). Miltefosine has a median long terminal half-life of 154 hrs, which could encourage development of clinical resistance, and it should be administration in combination with other drugs. It is teratogenic and abortifacient, therefore, drug cannot be used in pregnancy, and females with child bearing potential must observe contraception for the duration of treatment and an additional 2 months (46).

We believe that the drug must be used by order and supervision of physician because early discontinuation of drug by patient due to the side effects my lead to developing drug resistance.

Pentamidine: Its efficacy gradually declined over the years. It now cures only 70% of patients. This drug is associated with serious adverse events like insulin dependent diabetes mellitus, shock, and hypoglycemia (46). We have no experience with this drug in Iran.

Paromomycin, Sitamaquine: Paromomycin is an aminoglycoside antibiotic and sitamaquine is a primaquine analogue (8-aminoquinolene) with limited antileishmanial activity (46).

Cytokines: Interferon-γ, in combination with Sb, could speed up the elimination of parasites in patients who were not responsive to treatment (46). Granulocyte-macrophage colony-stimulating factor also enhances macrophage antileishmanial activity, and readily mobilizes and delivers myelomonocytic cells to infected tissue foci. It was given by Brazilian investigators to ameliorate the leukopenia of Kala-azar, and in a single study, this effect appeared to reduce secondary, complicating infections (53).

Corticosteroid: Generally we do not use steroid for treatment of VL because it inhibits production of IFN-γ and may suppress cell-mediated immunity and can be harmful (54,55).

Bacterial superinfection: Bacterial superinfection is one of the major complications leading to death in patients with visceral leishmaniasis. In infants with visceral leishmaniasis, fatal bacterial infections can be accompanied by nonspecific signs and symptoms. Thus, we advise to initiate broad-spectrum antibiotic treatment in all patients under one year old (30). It is noteworthy that hypercaloric food, rich in protein and vitamins should be provided.

Response to treatment: Most patients respond with clinical improvement after 7–10 days. More than 90% of properly treated individuals show apparent cure response in two weeks and 5–10% do not respond to or die during treatment (far-advanced disease, intercurrent illness, or drug toxicity). In our experience, relapse has been detected in 1 (1.3%) of 76 patients that followed

for 6 months (34). Relapse warrant a new treatment regimen (36). Recently we monitor serum INF-γ, IL-12, and IL-10 as indicators of response to treatment in patients with VL. Normalization of plasma levels of INF-γ, IL-10 can serve as a reliable parameter for being "cured" (Unpublished data).

Hematological abnormalities and spleen size improve after the treatment. In our study, the spleen was palpable below the costal margin in 20% of the patients six months after the completion of therapy, so a palpable spleen after this period of therapy can not be considered as the sign of relapse. None of the patients showed a noticeable hepatomegaly after six months (34).

Recrudescence is frequent in patients with underlying immune deficiency especially HIV infection (50).

Splenectomy in Kala-azar: Splenectomy is a fatal issue in patients with visceral leishmaniasis (56). If there is not an alternative option and it is mandatory to perform splenectomy, we suggest treatment with Amphotericin B for minimum of 14 days until bone marrow aspiration is free from leishman bodies, then splenectomy could be performed followed by another course of Amphotericin B.

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