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Case Report: Recurrences of Visceral Leishmaniasis Caused by *Leishmania siamensis* after Treatment with Amphotericin B in a Seronegative Child

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Abstract. We report the first case of visceral leishmaniasis caused by *Leishmania siamensis* in a seronegative child. She was treated with amphotericin B at 1 mg/kg/day for 3 weeks; however, recurrences occurred twice. The patient was cured after the administration of amphotericin B for 5 weeks and monthly prophylaxis for 6 months.

CASE REPORT

In 2008, a 5-year-old girl living in a rural area of Satun Province, southern Thailand came to a provincial hospital because of abdominal enlargement for 2 months. Her condition was investigated; thalassemia and human immunodeficiency virus (HIV) infection were ruled out. After failure of diagnosis for 2 years with increased abdominal enlargement, she was referred to Songklanagarind Hospital, Prince of Songkla University, for further investigation in 2010. She was living with her parents and had no history of traveling to other areas. Her house was an untidy one-story house surrounded by trees and the rubber plantation where her parents worked as rubber tappers. Her parents had three cows and raised them nearby the house. On examination, she was an undernourished girl having good consciousness, normal vital signs, but pale, underweight by 13 kg, and had a height of 94 cm. She had abdominal distension, the liver was 2 cm below the right costal margin with soft consistency, and the spleen was 9 cm below the left costal margin. Laboratory findings showed anemia and thrombocytopenia (Hb 8.3 g/dL and Hct 27.3%, white blood cell [WBC] 6,000/μL; PMN 53%, Lymph 41%, Mono 4%, Eo 2%, and platelets 98,000/μL). Liver function test showed total bilirubin 0.6 mg/dL (normal range: 0.2–1.2 mg/dL), direct bilirubin 0.06 mg/dL (normal range: 0–0.2 mg/dL), aspartate aminotransferase (AST) 50 U/L (normal range: 0–32 U/L), alanine aminotransferase (ALT) 29 U/L (normal range: 0–33 U/L), Alkaline phosphatase 130 U/L (normal range: 167–577 U/L), Albumin 3.4 g% (normal range: 3.9–5.1 g%), globulin 7.2 g% (normal range: 2.6–3.0 g%). Coagulogram was normal. Abdominal ultrasonography revealed hepatomegaly without focal lesion and marked splenomegaly (12 × 4 cm.) without focal lesion. Liver biopsy showed portal inflammation with much mononuclear cell infiltration. Accumulation of inflammatory cells was seen in some areas of intrahepatic lobules. Amastigotes were seen in liver histiocytes. Bone marrow aspirates were positive for amastigotes of *Leishmania*. Serological and molecular diagnoses were performed at the Department of Parasitology, Phramongkutklao College of Medicine. Using the Direct Agglutination Test (DAT) (Royal Tropical Institute, Amsterdam, the Netherlands), antibody titer of 1:3200 was detected, although immunochromatographic rk39 strip test (InSure; InBios International, Seattle, WA) showed a negative result. Polymerase chain reaction (PCR) amplifications of the internal transcribed spacer 1 (ITS1) region as described by El Tai and others (2001) identified the infection of *Leishmania siamensis*.[1] Sequence analysis of 380 nucleotides of the ITS1 region showed 100% identity to those of *L. siamensis,* (GenBank accession no. JX195637). The patient was diagnosed with visceral leishmaniasis (VL) and the treatment was started with amphotericin B at 1 mg/kg/day for a period of 3 weeks.[2] After completion of the treatment, her spleen size decreased to 3 cm below the left costal margin, hemoglobin increased to 10.5 g%, and platelet count was normal (144,000/μL). She was then discharged.

During the follow-up period, recurrence occurred twice at 12 and 24 weeks, respectively, after completing the treatment. Clinical presentations of both recurrences were hepatosplenomegaly and thrombocytopenia. The first recurrence was in May 2010 and the second in August 2010. The positive results of bone marrow aspiration and PCR amplification also confirmed the first and second recurrence of VL. Sequence analysis of the ITS1 region showed 100% identity to *L. siamensis* collected from the first episode. The second recurrence occurred when doses of amphotericin B were given the same as at the beginning of treatment. Thus, to achieve a definite cure, amphotericin B was given at 1 mg/kg/day for 5 weeks after the second recurrence. Laboratory investigation of bone marrow smears showed no amastigotes of *Leishmania* after completion of the previous treatment. To prevent recurrence, amphotericin B at 1 mg/kg/day for 5 consecutive days every month for 6 months was administered. The patient was in good condition with increased weight of 16.7 kg, height 103 cm, no palpable spleen, normal hematocrit (37.5%), and a platelet count of 215,000/μL. The girl remained in good health for a follow-up period of over 2 years.

Leishmaniasis, caused by a novel *Leishmania* species, *L. siamensis* is an emerging vector-borne disease in Thailand. This is the first case report of leishmaniasis caused by *L. siamensis* in a seronegative child. Similar to most reported cases, this case was living in the South of Thailand with no history of traveling abroad.[3–5] All human VL cases caused by *L. siamensis* in the published literature were HIV/acquired immunodeficiency syndrome (AIDS) patients.[3–6] We report here the first VL case in a girl without HIV/AIDS. From the available data, the distribution of VL, caused by *L. siamensis* might be similar to that found in *Leishmania infantum*, which mostly affects immunocompromised hosts and children.

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In contrast, *Leishmania donovani*, another causative agent of VL affects all age groups. To confirm this observation, prevalence of leishmaniasis, caused by *L. siamensis* in both seronegative and seropositive populations should be properly determined. The other characteristic of leishmaniasis caused by *L. infantum* is zoonotic transmission. A little evidence might support that *L. siamensis* is zoonotic as well. Phylogenetic analysis showed that *L. siamensis* was closely related to *Leishmania enrietti*, the *Leishmania* species infecting guinea pigs.[4] In addition, recent reports from different geographical areas identified *L. siamensis* as the causative agent of cutaneous leishmaniasis in animals including cows and horses.[7–9] However, factors involving animal-to-human transmission of *L. siamensis* in Thailand such as reservoir hosts and potential vectors have to be explored.

Amphotericin B-deoxycholate, a sterol-complexing agent is the anti-leishmanial drug of choice in Thailand because other effective drugs such as pentavalent antimony and miltefosine are not commercially available for treatment of VL in Thailand. Membrane sterols of *Leishmania* are the primary target of amphotericin B. The effect of amphotericin B markedly inhibits binding of *Leishmania* promastigotes to host macrophages, and thus prevents internalization of the parasites inside the macrophages.[10] Amphotericin B for the treatment of VL was shown to be highly effective. A study in India showed the efficacy of amphotericin B was 99.3% for VL, which had a definite cure rate at 6 months.[2] Either relapses or resistance to amphotericin B treatment in immunocompetent patients did not occur.[11] However, patients with severe underlying diseases such as HIV/AIDS are generally more difficult to treat and have a high relapse rate.[12–14]

In this report, the seronegative Thai girl showed recurrence of VL twice after the completion of a 21-day amphotericin B regimen. The ITS1 sequences of the parasites isolated from these three episodes showed 100% similarity. Sequence polymorphisms of the ITS1, *hsp70* and *cytb* loci showed two different lineages, i.e., lineages PG and TR, in *L. siamensis* isolated from Thai patients.[15] The parasites isolated from all three episodes were *L. siamensis* lineage PG. Because most parasites isolated from these areas shared the same lineage, it could not be used as a genetic marker to determine the nature of parasite's recurrence, i.e., relapse or re-infection. Genetic markers for this purpose need to be developed.

Treatment failure in leishmaniasis could be caused by either host and/or parasite factors. Unfortunately *in vitro* sensitivity of parasite isolated from this patient was not determined. Until now, no evidence of decreased *in vitro* susceptibility to amphotericin B has been found among *Leishmania* strains isolated from VL patients.[14] Regarding the host, multiple factors could be involved, e.g., genetics, nutritional status, and immune status, etc. Definite proof of these host factors would require special investigations. It has been known that an intact immunity, mainly Th1, cytokines (interferon-gamma [IFN-γ], interleukin-2 [IL-2], IL-12) and activated macrophages play an important role in curative ability and to determine responses to chemotherapy.[16] This patient was an undernourished girl, and thus it could significantly affect her immune status and other host factors. A systematic review recently identified the risk factors of VL relapse in HIV-infected patients.[17] Some of these risk factors were associated with the CD4+ count, i.e., the absence of an increase in CD4+ counts at follow-up and CD4+ counts below 100 cells/mL at the time of primary VL diagnosis. Unfortunately, we had no information of CD4+ count in this patient.

In this case, a 5-week extended course of amphotericin B with continuous prophylaxis was beneficial when relapses had occurred twice during the two episodes of treatment. Thus, relapse of infection should also be a concern in seronegative patients who have underlying nutritional and other medical conditions. A few studies showed the usefulness of secondary prophylaxis with pulsatile lipid formulations of amphotericin B after the initial clinical cure of VL in HIV-infected patients.[18–21] Because only amphotericin B-deoxycholate was available in Songklanagarind Hospital, Prince of Songkla University, this drug was used for this purpose. Determination of the efficacy of amphotericin B-deoxycholate for secondary prophylaxis of VL is required.

CONCLUSION

This is the first report of an autochthonous VL caused by *L. siamensis* in a seronegative child. The distribution of *L. siamensis* might be similar to that found in *L. infantum*, which causes the disease mainly in immunocompromised hosts and children. Awareness of leishmaniasis caused by *L. siamensis* in seronegative children, apart from seropositive patients, is suggested in the endemic areas. Recurrences occurred twice in this patient after the standard 21-day amphotericin B administrations. Further study of genetic polymorphisms of *L. siamensis* should be done to identify the suitable markers for differentiation of relapse and reinfection. Monitoring of drug susceptibility should be performed to know the situation of drug resistance in *L. siamensis*.

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