INTRODUCTION

Visceral leishmaniasis (VL) or kala-azar is an endemic parasitic disease in some parts of Iran which is caused predominantly by *Leishmania infantum* [1-3]. Fever and splenomegaly are detected in more than 80% of patients as the most common clinical manifestations. Neutropenia, anemia, thrombocytopenia, elevated ESR, and hypergammaglobulinemia are the most common laboratory abnormalities of this disease [4].

Herein we report a rare case of kala-azar in a pediatric age group without fever.

CASE RECORD

An 11-month-old male was referred to Ali Asghar Children Hospital following pallor and illness for 5 months. Prior to admission in our hospital, he had received packed cell 4 times and underwent bone marrow aspiration 2 times in 2 previous admissions with no definite diagnosis. He had no history of fever, icterus, bleeding tendency, and gastrointestinal or respiratory symptoms; however, irritability, anorexia, and failure to gain weight were reported by his care givers during this time.

On physical examination his vital signs were within normal range. He had hepatosplenomegaly without lymphadenopathy and rash. The span of spleen and liver measured by ultrasound examination were 122 mm × 38 mm and 78 mm, respectively. His primary laboratory data was as follows: WBC 10.6 × 10³/mm³ (normal range: 6.0-13.5 × 10³/mm³), neutrophils 21%, lymphocytes 78%, eosinophils 1%, hemoglobin (Hb) 6.4 g/dl (normal range: 11-13 g/dl), platelet 138,000/mm³ (normal range: 140,000-440,000), ESR 82 mm/hr (normal: < 15 mm/hr), qualitative CRP 2+, reticulocyte count 2%, LDH 7,03I U/L (normal: up to 480). Serum albumin, total protein, and routine liver and kidney function test results were within normal limits. HIV test and direct coombs were also negative.

Examination of bone marrow aspirate revealed a large number (grade 5+) [5] of *Leishmania* sp. under a light microscope with high magnification (Fig. 1). The parasite load in the bone marrow smears before, in the middle, end of the treatment, and relapse time was graded based on World Health Organization guidance [5]. DNA from the slides was extracted and subjected to PCR-reverse fragment length polymorphism (RFLP) assay. The ribosomal internal transcribed spacer 1 (ITS1) was amplified with specific primers, and the PCR products were digested with a restriction enzyme (*Hae*III).

The species of parasite documented by PCR-RFLP assay was...
L. infantum (Fig. 2). Rapid kala-azar K39 immunochromato-
graphic dipstick serological test for L. infantum infection was
also positive. Specific anti-leishmanial antibodies detected by
indirect fluorescent antibody test (IFAT) and direct agglutina-
tion test (DAT) were at titers of 1:320 and 1:3,200, respectively.
Work-up for immunodeficiency disorders performed by flow
cytometry showed no abnormal findings. Two days after start-
ing treatment with meglumine antimoniate (Glucantime®), at
a dose of 20 mg/kg, the patient became febrile and fever per-
sisted despite continuing Glucantime® for 9 days.

We switched our treatment to amphotericin B deoxycolate
(1 mg/kg/day), and fever was stopped 48 hr after starting this
drug. He received amphotericin B for a period of 28 days. The
results of bone marrow aspiration in the middle of this time
showed decreasing load of Leishman bodies (grade 2+) [5] and
was negative at the end of the treatment.

Three months after discharge, he became pale and febrile
again, and his liver and spleen were found to become enlarged
on monthly examinations. He underwent bone marrow aspira-
tion and was admitted with positive bone marrow aspirate
for leishman bodies (grade 4+) [5]. He was leukopenic (WBC
2,500/mm³, neutrophils 37%, lymphocytes 61%, monocytes
1%, eosinophils 1%), anemic (Hb 7.6 mg/dl) and thrombo-
cytopenic (platelet 79,000/mm³). ESR was 108 mm/hr.

His treatment was started with liposomal amphotericin B
(Ambisome®) 4 mg/kg on days 1-5 and at the 14th and 21th
day in combination with daily subcutaneous human recombi-
nant interferon-γ at the dose of 100 µg/m² plus oral allopuri-
nol at the dose of 20 mg/kg/day in 3 divided doses. He was
discharged 24 hr after becoming afebrile on the 5th day of re-
cieving Ambisome®. He received the rest of the treatment in
outpatient manner. Six months follow-up showed no relapse
or complications.

DISCUSSION

Visceral leishmaniasis is known as an endemic disease in
some parts of the world including Iran [1-3]. The most com-
mon clinical findings are fever, splenomegaly, and anemia.
Hepatomegaly is less frequent than splenomegaly, and features
such as jaundice, edema, and ascites are less frequently report-
ed [3]. Signs and symptoms of VL in Iran are compatible with
the Mediterranean type except for the absence of significant
lymphadenopathy [3]. Fever with maximum rate of 100% is
the most common symptom but may be absent in immuno-
compromised patients, subclinical forms especially in endem-
ic areas, and rarely immunocompetent ones [4].

Our patient was afebrile at presentation despite no docu-
mented immunodeficiency, and his bone marrow aspirate
showed a large number of leishman bodies. It seems that high
parasite load might suppress the inflammatory response in
body and prevent fever, the phenomenon which could be not-
ed in other diseases such as tuberculosis and lepromatous lep-
rosy [4]. It might also address the normal leukocyte count and
serum albumin/total protein ratio in our case. The patient be-
came febrile 2 days after starting treatment, and this might be
due to massive release of antigens after starting therapy with a
subsequent triggering of an immune response. Subsiding fever
in kala-azar is usually considered as a clinical response to the
treatment. Our patient did not have fever at the beginning of
the treatment but fever was started after Glucantime® treatment. We had no rapid clinical marker for response to the treatment and were compelled to discontinue Glucantime® after 9th day of fever. It was according to some documented evidence of resistance to Glucantime® against L. infantum and L. tropica in our area [6-9].

We switched our treatment to amphotericin B deoxycolate and continued it for 30 days. Amphotericin B deoxycolate is usually used at a dose of 0.75-1.0 mg/kg/IV/daily for 15-20 doses for VL caused by L. donovani and up to 30 days for VL caused by L. infantum [10]. Three months after the end of treatment, the patient experienced an episode of relapse. Relapse could occur in kala-azar despite proper treatment and efficient immune state [11]. In general, diseases caused by intracellular microorganisms might recur secondary to residual organisms escaped pharmacologically and/or immunologically [11]. Heavy leishman body load at that first bone marrow aspiration might be a risk factor in our patient for relapse.

According to its high concentration in reticuloendothelial system, we decided to use Ambisome® and added human recombinant interferon-γ and allopurinol as adjuvant therapies to increase the eradication rate. The indication of using interferon-γ and allopurinol in combination with liposomal amphotericin B in patients with relapse but no documented immunosuppression state or resistance to antimonials is poorly defined. Different regimens of Ambisome® have been recommended for treatment of kala-azar [12-14]. The regimen which is usually administered in immunocompetent pediatric patients was scheduled for our patient [14,15].

In conclusion, medical practitioners especially in endemic areas should be aware of and consider VL among differential diagnosis of afebrile unexplained anemia.

CONFLICT OF INTEREST

We declare that we have no conflict of interest related to this study.

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