SQU JOURNAL FOR SCIENTIFIC RESEARCH: MEDICAL SCIENCES
2002, VOL. 4, NO. 1–2, 45–48

Visceral leishmaniasis and haemophagocytic syndrome in an Omani child

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الليشمانيا الحشائية ومتلازمة الهيموفاجوستيوزيس في طفل عماني

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المستخلص: تناقش هذه الورقة حالة نادرة لطفلة عمانية عمرها ٤ سنوات وقد ظهر عليها بصورة مفاجئة شحوب في الوجه مع حمى وتضخم في الكبد والطحال. وبعمل الفحوصات المعملية وجد نقص شديد في كل مكونات الدم وارتفاع في نسبة الدهون ومخزون الحديد بالجسم. ولم يوجد في البداية أي دليل على وجود الليشمانيا وذلك لغياب الصورة السريرية الواضحة وأيضا لسلبية الفحوصات المعملية. وقد أثبتت صورة بذل نخاع العظم وجود متلازمة الهيموفاجوستيوزيس وبدأ العلاج باستخدام بروتوكول HLH٩٤ .ولعدم وجود إستجابة واضحة فقد أعيد فحص بذل النخاع عدة مرات وقد أظهرت إحداها وجود أجسام الليشمانيا في النخاع مما أدى إلى التشخيص الدقيق للمرض. وبعد اسبوع كامل في العلاج بإستخدام عقار الاميزوم شفيت الطفلة تماما.

ABSTRACT. The paper reports the case of a previously healthy 4-year-old-girl who presented with pallor, fever and hepatosplenomegaly. Laboratory findings included pancytopenia, hypertriglyceridemia and hyperferritinemia. Initial diagnosis of kala-azar could not be confirmed because of the absence of clinical evidence, negativity of bone marrow aspiration or specific serology for visceral leishmaniasis. Repeated marrow aspiration, performed due lack of clinical response, revealed histiocytes showing haemophagocytosis consistent with haemophagocytic lymphohistocytosis (HLH) and appropriate treatment was started. She continued to have high-grade fever, and a third bone marrow aspiration ultimately revealed presence of *Leishmania* amastigotes with evidence of active haemophagocytosis. The girl was treated with liposomal amphotericin (AmBisome) for 5 days, following which she recovered rapidly with definitive remission.

Key Words: haemophagocytic syndrome, visceral leishmaniasis, Oman.

A 4-YEAR-OLD OMANI GIRL, NOT KNOWN TO have any previous medical illness, presented with a one-month history of high grade fever associated with rigors 2–3 times a day, as well as body ache. She was admitted for 8 days in one of the peripheral hospitals where she was found to have hepatosplenomegaly and pancytopenia.

On physical examination she was pale and febrile (40°C). Her spleen was 3 cm and liver 3 cm below the costal margin. Laboratory tests showed: Hb 7.2 g/dl, absolute neutrophil count (ANC) 1.46×10^9/l, total leukocyte count (TLC) 4.0×10^9/l, platelets 117×10^9/l and reticulocytes 2.6%. Peripheral blood smear showed no blasts and also was negative for malarial parasite. Liver function tests showed aspartate amino transferase (ASAT) of 230 U/l, alanine amino transferase (ALAT) of 136 U/l and albumin of 26 g/l. Coagulation profile showed prothrombin time (PT) of 12.5 sec (normal range 11–14), activated partial thromboplastin time (APTT) of 35.1 sec (26–37), fibrinogen of 5.1 g/l (1.5–4.5) and D-dimers of >2.0<4.0 mg/l (<0.5). ESR was 91 in 2 hours and C-r-eactive protein (CRP) was 84 mg/l. Rheumatoid factor, ANA, anti DNA antibody and direct Coombs test all yielded negative results. Her serum triglycerides were 3.2 mmol/l (0.5–1.7) and immunoglobulin G (IgG) was 32 g/l. Chest x ray was normal and ultrasound abdomen showed hepatosplenomegaly. A provisional diagnosis of visceral leishmaniasis was made with the possibility of malignancy to be ruled out. Bone marrow aspiration (BMA) was negative for any malignant cells and despite extensive search no *Leishmania* amastigotes (LD bodies) were found. The child underwent a battery of investigations for pyrexia of unknown origin (PUO), and several blood, urine and stool cultures were negative. Hepatitis profile, *Brucella*, HIV 1 and 2, EBV, and Widal screening also yielded negative results. Serologic test results for leishmaniasis were also negative. The child continued to have high-grade fever (>40°C) despite antibiotic coverage with ceftriaxone and azithromycin. BMA was repeated afer one week, which showed hypercellular

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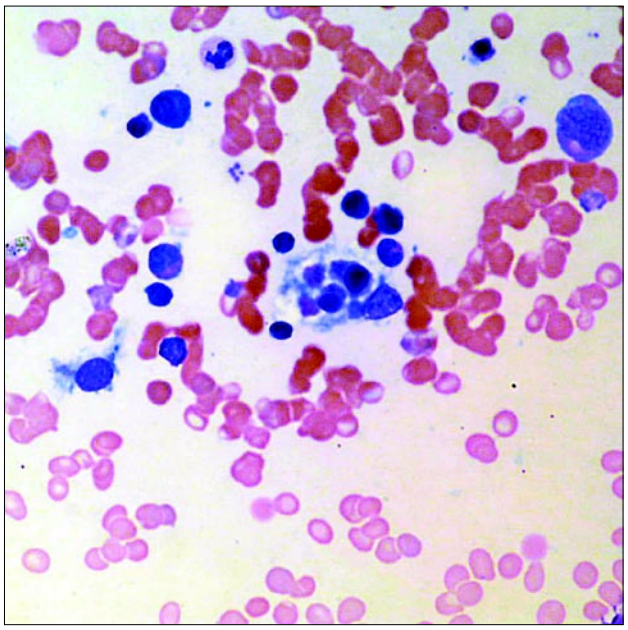

Figure 1. ***Photomicrograph of bone marrow aspiration, showing hypercellular marrow with erthroid hyperplasia showing histiocytes***

marrow with erythroid hyperplasia showing histiocytes [Figure 1] with haemophagocytosis consistent with haemophagocytic lymphohistocytosis syndrome (HLH). The hepatosplenomegaly, together with signs of haemophagocytosis on the marrow smear, and seronegativity for *Leishmania*, led to tentative diagnosis of familial lymphohistocytosis.

The child was put on chemotherapy as per HLH 94 protocol. She received etoposide intravenously (150 mg/m^2 twice weekly) and oral dexamethasone daily (10 mg/m^2) for 2 weeks. During this period the child improved clinically as the fever settled. Genetic study and HLA typing of the family was done preparing for bone marrow transplantation. As per the protocol, a third bone marrow examination revealed presence of LD bodies together with haemophagocytosis [Figure 2]. Etoposide and dexamethasone were stopped and the child was put on sodium stilbogluconate (20 mg/kg/day) but she continued to have high spikes of fever and pancytopenia, also she started to have very severe myalgia. Taking all the above into consideration, sodium stilbogluconate was stopped after receiving five doses and she was started on liposomal amphotericin intravenously (AmBisome), in a dose of 3 mg/kg for 5 days.

The child's general condition dramatically improved and she became afebrile. Liver size reduced to 1 cm and spleen was not palpable. Repeat BMA two weeks after starting AmBisome showed haemophagocytosis but no LD bodies. Her full blood count on discharge was as follows: TLC of 7.84×10^9/l, Hb of 11.2 g/dl and platelets of 188×10^9/l. Two weeks after the discharge, on her follow-up visit, she was doing well and her CBC showed: TLC 6.69×10^9/l, platelets 270×10^9/l, Hb 11.8 g/dl and ANC of 1.4×10^9/l.The fourth BMA was done at this time and it showed no LD bodies nor haemophagocytes. She was seen in the outpatient department after 4 month of the second discharge and was in good normal health.

DISCUSSION

It is important for any physician to be able to identify early potentially life-threatening disorders in diseases that may lead to severe permanent disabilities, in particular if they are treatable. Haemophagocytic lymphohistocytosis (HLH) is such a disorder. It usually affects infants who develop the disease very early in life with around 70% being less than one year of age at onset. A crude annual incidence of 1.2 cases of familial haemophagocytic lymphohistocytosis (FHL) per million children has been reported in Sweden.[2] Large series of HLH cases have been reported in Hong Kong[3,4] and Taiwan.[5] A seasonal pattern has been suggested; cases may occur more often in summer.[6] The disorder occurs in primary and secondary forms. The more common primary form, FHL, typically seen during infancy and early childhood, is inherited as an autosomal recessive trait. Possible loci for

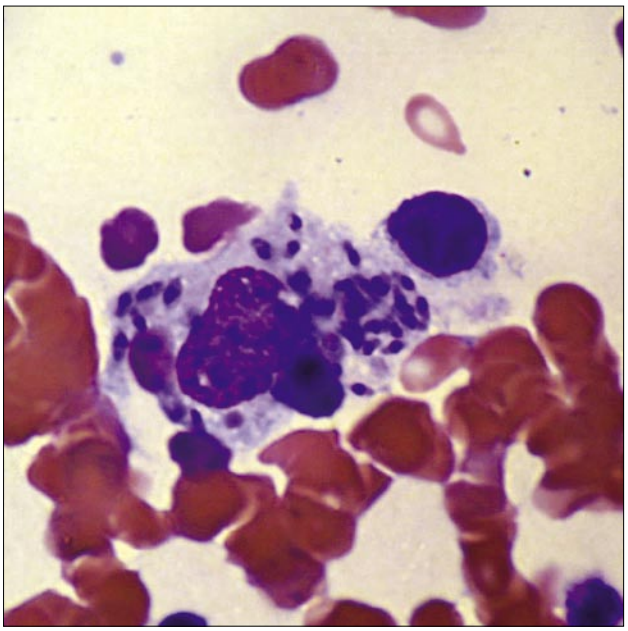

Figure 2. *Photomicrograph of aspirated bone marrow, showing macrophage with intracellular LD bodies with haemophagocytosis.*

a responsible gene or genes have recently been mapped to the long arms of chromosomes 9 and 10, andis invariably fatal with a median survival without therapy of two months after onset.[7,8] Secondary HLH can affect all ages and is also associated with high mortality. It has been linked to viral, bacterial, fungal and parasitic infections (the so called infection-associated HS) and to a broad spectrum of malignant and genetic disorders, such as Chediak-Higashi disease, Griscelli disease and XLP syndrome.[9,10]

HLH is now diagnosed on the basis of Henter *et al*'s guidelines:[10] (a) *clinical criteria:* fever and splenomegaly (both to be present); (b) *laboratory criteria*: cytopenias (affecting 2 of 3 lineages in the peripheral blood), haemoglobin (<9 g/l), platelets ($<100\times10^9$/l), neutrophils ($<1.0\times10^9$), hypertriglyceridemia and/or hypofibrinogenemia (fasting triglycerides ≥2.0 mmol/l or ≥3 SD of the normal value for age, fibrinogen ≥1.5g/l or ≥3 SD) (c) *histopathological criteria*: haemophagocytosis in bone marrow or spleen or lymph nodes, no evidence of malignancy.

Neurological symptoms may complicate and sometimes dominate the clinical course. Hypertriglyceridemia is common in systemic disease with fever. Hepatic abnormalities including elevated serum transaminases or hyperbilirubinemia appear to be related to degree of liver involvement. Elevated ferritin and lactate coagulation derangement is also common during active disease.[12] Pathophysiology of HLH is believed to be due to uncontrolled non-malignant proliferation of T lymphocytes and macrophages, leading to increased cytokine production. The haemophagocytosis mostly affects erythrocytes but occasionally also platelets and leukocytes.[9,10] The organs most frequently involved are the spleen, liver, lymph nodes bone marrow and Central nervous system.[13]

Infection by the protozoan pathogen *Leishmania* is a public health problem in certain regions of Oman as North Batinah.[14] Although there are a number of different species, all of which are transmitted by phlebotomine sand flies,[15] there are only two primary types of clinical disease, cutaneous and visceral leishmania (VL).[16] Visceral leishmaniasis revealed by haemophagocytic syndrome (HS) is an extremely rare event that can cause considerable diagnostic difficulty. The first case of leishmaniasis revealed by a reactive HS was reported by Matzner *et al* and concerned a 22-year-old adult.[17]

Our patient had an abnormal coagulation profile, elevated liver enzyme activities, very high triglyceride level, low plasma fibrinogen levels and bone marrow haemophagocytosis, keeping with all the diagnostic criteria of HS as defined by the HLH Study Group of the Histiocyte Society in 1991.[10] No other cause of HS was found despite extensive microbiologic and serologic investigation. The other signs presented by her were common to HS and VL (hepatosplenomegaly, fever and pancytopenia).

Leishmania was considered in the differential diagnosis, but *Leishmania* amastigotes were not present on the first marrow smear. Neurological involvement is very common in FHL with histiocytes in CSF. This should distinguish FHL from the secondary HLH.[18] The recent description of mutation in the perforin gene in patients with FHL linked to 9 q22 may also facilitate the diagnosis.[6]

In our case, etoposide was wrongly prescribed. This drug, cytotoxic for the monocyte-macrophage lineage, is the effective treatment of FHL but can have catastrophic consequences by increasing the risk of aplasia and aggravating the VL. Bone marrow transplantation was planned in our case, but fortunately the correct diagnosis was made during the pre-transplant workup.

Another important issue is the lack of response to pentavalent antimonials and the immediate and definite response to AmBisome. AmBisome was recently shown to be optimal for the treatment of VL in immunocompetent children.[19] In this case of secondary HLH, it seems to be particularly suitable as lipid formulation amphotericin B is taken up by macrophages and targets the drug to the site of infection leading to very high concentration in the liver, spleen and bone marrow.[20] Whatever treatment regimen is used, long-term follow up is essential to ensure complete cure.

CONCLUSION

This is the first reported case of visceral leishmania associated with HLH in Arabian Gulf region. It is also among the very few such reported cases in the literature. This diagnosis of HLH matched the diagnostic criteria by Henter *et al* in 1991,[10] but later on, the BMA on second week of therapy as per HLH protocol 94 proved the case to be secondary to leishmaniasis. Previous reports of association between erythroid hyperplasia, histocytosis with haemophagocytic changes and leishmaniasis have been reported by Al-Jurayyah *et al*.[21] LD antibodies should be sought stubbornly on BM smears, with repeated sampling and use of modern diagnostic methods. Leishmania can now be identified in tissues by means of PCR with species-specific probes and this should simplify the diagnosis of these very rare cases. This case also shows that it is not always easy to know

which occurs first, visceral leishmaniasis or haemophagocytosis.

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