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A Case Series Report of Iranian Children; Hemophagocytic Lymphohistiocytosis Syndrome

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ABSTRACT

Hemophagocytic lymphohistiocytosis (HLH) is an aggressive and potentially life-threatening disease and has to be considered in the differential diagnosis of many conditions. HLH comprises two different conditions that are difficult to differentiate; Familial hemophagocytic lymphohistiocytosis (FHLH) or familial erythrophagocytic lymphohistiocytosis (FEL), and Secondary hemophagocytic syndromes (secondary HLH, sHLH). Herein, we report a case series of Iranian children with HLH and describe the symptoms and outcome of this disease in Iran.

Implication for health policy/practice/research/medical education:
This article is useful for researchers who are seeking to advance knowledge in areas of HLH.

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1. Introduction

The term hemophagocytosis describes the pathologic finding of activated macrophages, engulfing erythrocytes, leukocytes, platelets, and their precursor cells. This phenomenon is an important finding in patients with hemophagocytic syndrome, more properly referred to as hemophagocytic lymphohistiocytosis (HLH) (1). HLH comprises two different conditions that are difficult to differentiate. The first condition is familial hemophagocytic lymphohistiocytosis (FHLH) or familial erythrophagocytic lymphohistiocytosis (FEL), which is an autosomal recessive disease, FHLH may be associated with decreased apoptosis triggering. 20-40% of all affected patients have mutations in the perforin gene and defect in NK and T cell cytotoxicity. Mutations in the gene hMunc 13-4 which is essential for cytolytic granules fusion (2) and Syntaxin gene (3) may be responsible too. Onset of FHLH may be triggered by infections. The second is Secondary hemophagocytic syndromes (secondary HLH, sHLH). The condition has been associated with viruses (virus-associated hemophagocytic syndrome, VAHS), bacteria and parasites (infection- associated hemo-

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phagocytic syndrome), malignancies (malignancy-associated hemophagocytic syndrome), rheumatoid disorders, metabolic disorders and prolonged intravenous nutrition. Remarkably, although sHLH may subside spontaneously, it may also be associated with mortality. Importantly, proving the infection at onset may not have major therapeutic importance, since both sHLH and FHLH sometimes feature a triggering infectious agent. Herein, we present 7 cases of HLH, in a children's hospital and describe their signs, treatment and outcomes. We also present three cases of HLH associated with disseminated Bacillus Calmette Guerin (BCG) Infection.

2. Cases Report

2.1. Case 1

A 5.5-month old girl was admitted to the hospital because of persistent fever, irritability, poor feeding in the previous days and axillary draining lymphadenitis at the same site of BCG vaccine injection. Abdominal ultrasound examination showed hepatomegaly with 3 hypoechoic focuses, heteroechoic splenomegaly and extensive paraaortic lymphadenopathy. Laboratory studies were done for HIV, disseminated BCG, other infections and also HLH. Echocardiography was normal and laboratory examinations showed an elevated erythrocyte sedimentation rate (ESR), anemia, thrombocytopenia, elevated ferritin and hypertriglyceridemia. Bone marrow aspiration (BMA) showed a few acid fast bacilli, and treatment with isoniazid, ethambutol, clarithromycin, ciprofloxacin, B6, gamma interferon with an impression of disseminated BCG was begun and cyclosporine A (CSA) and methylprednisone pulses were added later. She became afebrile and heteroechoic focuses in liver disappeared gradually. The organomegaly persisted and several blood transfusions were needed. Unfortunately the drug was discontinued by parents and the infant died after being discharged from the hospital.

2.2. Case 2

A 13-month old boy with silver-tinted hair was presented to another hospital with fever and lethargy. Symptoms began 20 days before admission with respiration difficulty and flu-like symptoms. On examination he had fever, exudative pharyngitis and heptosplenomegaly. Corticosteroid was begun with an impression of Infectious mononucleosis, but no improvement. Therefore, he was referred to our hospital with fever, respiratory distress due to tonsillar hypertrophy, cervical lymphadenopathy and huge hepatosplenomegaly. His Laboratory tests showed severe anemia, neutropenia and thrombocytopenia. EBVCA (IgM) was positive. Liver and spleen sonography showed low attenuated areas. On BMA few acid-fast bacilli without hemophagocytic cells were seen. Treatment for disseminated BCG was begun with ethambutol, ciprofloxacin, clarithromycin, isoniazid, rifampin and γ-INF with improvement in general condition but no change in spleen size or upper airway obstruction.

Later, his serum levels of triglyceride (TG), cholesterol and ferritin increased and fibrinogen diminished. Lymph node biopsy showed hemophagocytic cells. Cyclosporine (CSA), dexamethasone and colchicine treatment were begun. Because of unusual color of hair, it was sent for biopsy considering Griscelli syndrome, which was normal but his skull skin biopsy showed hyperpigmented basal melanocytes and sparse pigmentation of adjacent keratinocytes compatible with Griscelli syndrome.

Mutations in RAB27A were detected and he was considered as a candidate for hematopoietic stem cell transplant (HSCT), which was obtained from his matched sibling and performed after 9 months. His general condition has been good afterwards till now (6 months).

2.3. Case 3

A 2-month-old boy developed fever and irritability 6 days after first DPT (Diphteria, whole- cellular pertussis, tetanus) vaccination. Liver and spleen enlarged gradually and then gastrointestinal bleeding developed. He had anemia, thrombocytopenia, elevated transaminases and C-reactive protein and also hyperferritinemia. He had Hypertriglyceridemia, hypercholesterolemia, leukocyturia and elevated lactate dehydrogenase (LDH) and *Klebsiella* was grown in the urine culture.

Cerebrospinal fluid analysis and serum immunoglobulins were normal. BMA showed normal cellularity without increased histiocyte and phagocytic activity. His condition improved after beginning antibiotics, CSA and methyl prednisone pulses. Three months later he was admitted for pneumonia, which responded to antibiotics while he was on maintenance therapy for HLH. He was readmitted to the hospital, when was 11 months old with an impression of otitis media but he was staring and blinking too. Mastoid computed tomography (CT) and magnetic resonance imaging (MRI) revealed mastoiditis. The spleen and liver size increased gradually in spite of antibiotic therapy. The amount of TG, cholesterol, ferrittin and transaminases were increased and fibrinogen, hemoglobin, platelet, neutrophils and ESR levels decreased. BMA revealed increased histiocyte activity so methyl prednisone pulse was begun and CSA dosage increased again. Cerebrospinal fluid (CSF) analysis showed increased cells and elevated protein, but intratechal treatment for HLH was not begun. The CSA was discontinued by parents and he had 4 more admissions for HLH relapses. He was a candidate for HSCT but unfortunately no compatible donor was found. The central nervous system (CNS) symptoms increased. His last admission was accompanied by signs of infection and HLH flare up and ended in death. Brain MRI showed brain atrophy with ventriculomegaly, decreased white matter density, leukoencephalopathy and basal ganglia enhancement.

2.4. Case 4

A 5.5-month old girl came with fever from 14 days before admission. She had persistent symptoms respiratory infection since the last 1 month and developed diarrhea and vomiting. She had hepatosplenomegaly and left preauricular lymphadenopathy. The lab tests showed severe neutropenia and thrombocytopenia, increased TG, ferritin and liver transaminase and decreased fibrinogen. BMA showed few hemophagocytic cells. Plural effusion and ascites developed and respiratory distress worsened. CSA, Dexamethasone and VP16 were begun in addition to antibiotics and acyclovir but unfortunately she died 9 days after admission because of respiratory failure.

2.5.Case 5

A 9-month old girl was admitted for prolonged fever, mild vomiting, diarrhea since 2 days ago and splenomegaly. She had bandemia, anemia, mild neutropenia and thrombocytopenia. Liver enzymes increased significantly. Urine analysis showed leukocyturia and *Klebsiella* was grown in urine culture. Antibiotics were prescribed but hepatosplenomegaly increased. Laboratory tests revealed increased serum ferritin and TG; on the BMA some hemophagocytes were found. Pulse methylprednisone, and CSA were begun. The fever continued and seizure and pulmonary infiltration occurred and unfortunately she died because of respiratory failure.

2.6. Case 6

A 52-day old boy came with fever from 4 days ago. He had hepatosplenomegaly and had received BCG, the first dose of hepatitis B vaccine (HBV) and oral poliomyelitis vaccine (OPV). Laboratory tests showed anemia, thrombocytopenia, severe neutropenia, increased liver enzymes, TG, ferritin and hypofibrinogenemia, but no evidence for EBV, CMV, HBV, HIV1,2, HSV1,2, or Toxoplasma infections were detected. Treatment for *Mycobacterium bovis* and bacterial infection, pulse methylprednisone, colchicine and CSA were begun with improvement in signs and laboratory test results. He had two more admissions, one for fever and organomegaly and the other for respiratory symptoms with lung infiltration and hilar lymphadenopathy. HSCT was done in May 2009. His general condition has been well thereafter.

2.7. Case 7

A 5 year-old girl admitted with fever, vomiting diarrhea which had begun 10 days ago. She had mild splenomegaly and the lab tests revealed anemia, mild neutropenia, elevated TG and LDH. BMA showed some hemophagocytic cells. Methyleprednisone and CSA were begun. The symptoms and signs resided and the patient was discharged with HLH prescription. The patient has never returned for follow up.

3. Discussion

HLH is an aggressive and potentially life-threatening disease and has to be considered in the differential diagnosis of many conditions. Initial signs and symptoms of HLH may mimic common infections, fever of unknown origin, hepatitis, multiple organ failure syndrome, encephalitis, and even child abuse (2). Clinical signs and laboratory abnormalities associated with hemophagocytic lymphohistiocytosis are reported as follows:

Fever 60-100%, splenomegaly 35-100%, hepatomegaly 39-97 %, lymphadenopathy 17-52%, rash 3-65%, neurologic signs 7-47%, anemia 89-100%, thrombocytopenia 82-100%, neutropenia 58-87% , hypertriglyceridemia 59-100 %5, hypofibrinogenemia 19-85%, hyperbilirubinemia 74 % (3). In a Swedish study, major early clinical signs included: Fever 91% Hepatomegaly 90%, Splenomegaly 84%, Neurological symptoms 47%, Rash 43%, Lymphadenopathy 42%. Neurological symptoms could entirely dominate the clinical

Table 1. Clinical Data of Patients With HLH

Cases	1	2	3	4	5	6	7
Age (mo, at admission)	5.5	13	2	5.5	9	1.5	60
Gender	F	M	M	F	F	M	F
Fever	+	+	+	+	+	+	+
Splenomegaly	Huge, heteroechofocus	Huge, hypoecho focus	Huge	+	Huge	Huge	Mild
Hepatomegaly	Huge, hypoecho focus	Huge, hypoecho focuses	Huge	+	Huge	Huge	Mild
Lymphadenopathy	Extensive Para aortic	Cervical, para aorta	-	Preauricular	-	Parahilar	-
CNS involvement	nr	nr	+	GCS dec finally	Seizure	nr	-
Other Organ involvement	Liver, rash	Liver	Liver, ascitis CNS, lung	Rash, liver, respiratory, pleural effusion, ascitis, CNS	Respiratory, CNS, liver	Mild liver, mild respiratory, pleural eff, ascitis	-

Abbreviations: CNS, central nervous system; F, female;GCS, glasgow coma scale; M, male; HLH, hemophagocytic lymphohistiocytosis; nr, not reported

Table 2. Laboratory Data of Patients With HLH

Cases	1	2	3	4	5	6	7
White blood cells/μL	5000	1500	560	160	900	450	2200
Platelet x10³/μL	46	81	67	7	26	83	248
Fibrinogen,gr/L	< 2	0.5	Dec	Dec	2	2.1	
Ferritin,ng/mL	1292	1440	688.6	> 10000	2810	1269	
Triglyceride	131	228	327	315	Increased	452	428
Cholesterol,mg/dL	44	217	200	107		274	172
CSF analysis	nr	nr	+	Not detected	Not detected	nr	Not detected
ESR	66	8	3			32	18
CRP	3+	2+	3+			3+	2+
BMA	Few acid-fast bacilli ,some myeloid arrest	Few acid-fast bacilli without hemophagocytosis	Some hemophagocytosis	Few hemophagocytic cell, severe hypocellularof myeloid, erythroid, lymphoid, megakaryocyte	Some hemophagocytic	No hemophagocytosis	Few erythrophagocyte
Hemophagocytes presence	-	Lymph node	BM	BM	BM	-	BM

Abbreviations: BMA, bone marrow aspiration; BM, bone marrow; CRP, C-reactive protein; CSF, cerebrospinal fluid; ESR, erythrocyte sedimentation rate; HLH, hemophagocytic lymphohistiocytosis; nr, not reported

Table 3. Treatment and Outcome of Patients With HLH

Cases	1	2	3	4	5	6	7
Treatment	INH, ETH, CLAR, CIPRO B6, γINF, CSA, pulse methyl, Colchicin	INH, ETH, CLAR, CIPRO B6, γINF, CSA, dexa, Colchicin	CSA, methyl prednisone pulse and colchicine	CSA, dexVP16	Pulse methylprednisone, colchicine and CSA	INH, CIPRO, CLAR, ETHA pulse methylprednisone, colchicin and CSA	Pulse methylprednisone, colchicine and CSA
Transplant	-	HSCT	Candidate , no donor	-	-	HSCT	-
Outcome	Death	Good	Death	Death	Death	Good	No f/u
Predisposed infections	Diss BCG	Diss BCG, EBV, *Griscelli*	DPT, vaccination, UTI with *Klebsiella*	Enteritis, res infection	UTI with *Klebsiella*	Diss BCG	Gastroenteritis

Abbreviations: CIPRO, ciproheptadin; CLAR, clarytromycin, CSA; cyclosporine A; Diss BCG, disseminated BCG; HLH, hemophagocytic lymphohistiocytosis, HSCT, hematopoetic stem cell transplant; EBV, Ebestein barr virus; ETH, ethanbutol; INH, isoniaside

picture and may develop prior to other symptoms and signs. Frequent laboratory findings were pancytopenia, hypertriglyceridemia, hypofibrinogenemia, elevated serum transaminases, hyperbilirubinemia, hyponatremia, hypoalbuminemia, and a moderate spinal fluid pleocytosis. Chest X-ray often revealed typically discrete pulmonary infiltrates (4).

In our study: All 7 patients presented with fever, hepatosplenomegaly, 4 out of 7 patients had lymphadenopathy and 3 had CNS signs. Anemia was present in all, thrombocytopenia in 6 out of 7, neutropenia in 4, hypertriglyceridemia in 6, hyperferritenemia in 7 and largely increased LDH in 6 patients (*Tables 1, Table 2*).

Fitzgerald *et al.* declared that many of these patients had clinical and radiologic findings similar to the acute respiratory distress syndrome, with alveolar-interstitial opacities and pleural effusions and Nahum *et al.* reported that patients with HLH were also found among those with the multiple organ failure syndromes. They studied a total of 11 children with HLH, in seven of them (63%) one or more organ failures were detected. Organ failure was noted most often in the respiratory system (n = 7) attributable to severe, acute respiratory distress syndrome and pleural effusion, Liver failure occurred in three and central nervous system involvement and coma in three (5, 6). In our study, respiratory failure that ended in death was present in 2 out of 7 patients and one more patient had symptoms of respiratory tract infection even after HSCT (*Table 3*).

Imashuku *et al.* declared that persistently low natural

killer cell (NK) activity and a high incidence of central nervous system (CNS) disease increase the probability of high risk FHL (3). In our case series CNS involvement was associated worse prognosis; all 3 patients with CNS involvement died. Only one of those without CNS involvement died. Her triggering factor was severe disseminated BCG infection which has a poor prognosis; additionally, her parents discontinued treatment.

In Aerico *et al's* survey, in nearly 20 percent of cases, more than one bone marrow specimen as required in order to demonstrate hemophagocytosis (7). In our study hemophagocytosis were found in 4 out of 7 early specimens from bone marrow aspiration. In one patient, evidence of hemophagocytosis was found in the lymph node biopsy but not in the bone marrow specimen.

In 3 of our patients clinical manifestations of HLH triggered after disseminated BCG infection. We did not find a similar report in the literature search. In two of these children HSCT was successful. One of these two had Griscelli syndrome and also EBV infection accompanied with disseminated BCG infection and HLH syndrome (*Table 3*).

HLH is often fatal, therefore one must have a high index of suspicion in patients presenting with the following clinical manifestations: high fevers, failure to thrive, central nervous system symptoms, hepatosplenomegaly, lymphadenopathy, cytopenias, abnormal liver function tests, high levels of serum ferritin and LDH. Early diagnosis, thorough investigation for the detection of CNS involvement, appropriate treatment and HSCT if needed are critical steps in patient survival. In countries where newborns are vaccinated with BCG, disseminated BCG may be the triggering factor for HLH, especially in patients with underlying disease. Early treatment of both conditions and considering HSCT may be lifesaving in these patients.

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Authors' Contribution

1. Conception and design: Karimi A, Ghanaie RM, and Shiari R.
2. Provision of study material or patients: Karimi A, Shiari R, Fahimzad AR, Armin Sh, and Arzanian MT.
3. Collection and/or assembly of data: Ghanaie RM.
4. Data analysis and interpretation: Karimi A, Ghanaie RM and Shiari R.
5. Manuscript writing: Ghanaie RM, Shiari R, and Shiva F.
6. Final approval of manuscript: Karimi A & Shiari R.

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