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# Hemophagocytosis Mechanism in Life- Threatening Hemocytopenia in Several Infectious Diseases

**Hussaien Naji Abdullah** **Mohammed A.K.Al-Sa'adi**
*Medical College , Babylon University*

## Abstract

The study aimed to evaluate the epidimiologic and clinicopathological features of infectious associated hemophagocytosis (IAHS) and associated etiological pathogens of such disease process in Hilla and neighbored discrit-central of Iraq. This comprise 19 case of young children mean age ± SD (46.95±36.06), male: female ratio (11:8). All case-patiens had fever,(89.4%)had splenomegaly,(42.1%) hepatomegaly,(36.8%) skin rash,(89.4%)Severe anemia, (94.7%)Neutropenia, (68.4%) thrombocytopenia, (84.2%) Hypofibrinogenemia and (89.4%) hypetriglyceridemia. Bone marrow smear positive for hemophagocytosis in all cases. Regarding etiological pathogens, visceral lishmaniasis 10 cases, infectious mononucleosis 4 cases, brucellosis 2 cases and one case for each of typhoid fever, pulmonary tuberculosis, chronic ostiomylites.

The IAHS among children is an important illness should be considered in patient with unexplained fever and cytopenia in the setting of disseminated infection .

## الخلاصة

هذه دراسة عرض وتقييم العلامات السريرية والمتغيرات المرضية المختبرية وبيان اسباب متلازمة الهيموفاكوستيوزيس. في محافظة بابل والمقاطعات المجاورة في وسط العراق حيث الامراض المعدية مشكلة صحية كبيرة . تمت الدراسة على 19 حالة تم تشخيصها وجد ان المرض يحدث في كلا الجنسين و معظم الحالات بعمر مبكر في الاطفال وقد ظهر جميع المرضى لديهم حمى، 89.4% تضخم الطحال، 42.1% تضخم الكبد ، 36.8% لديهم نزف تحت الجلد ،وبعمل الفحوصات المختبرية وجد نقص شديد في مكونات الدم الخلوية (89.4% فقر الدم الشديد ، 94.7% نقص الخلايا البيض العدلة ، 68.4% نقص الصفيحات الدموية )، 84.62% نقص فايبرينوجين الدم، 89.4% نقص دهون الدم الثلاثي. وقد أثبتت صورة نخاع العظم وجود متلازمة الهيموفاكوستيوزيس في جميع الحالات. توزعت الاصابات الميكروبية بواقع 10 باللشمانيا و4 بفيروس وحيد الخلية المعدي و2 بالبروسيلا وحالة واحدة لكل من بكتريا التيفوئيد والتدرن والتهاب العظام المزمن.

ان متلازمة الهيموفاكوستيوزيس المصاحب للالتهابات مرض من المهم اعتباره في المرضى الذين لديهم حمى مزمنة ونقص خلايا الدم العام في حالات الالتهابات الواسعة.

## Introduction

Infection associated hemophagocytic syndrome (IAHS) is belong to a subtype of unusuall disorder the hemophagocytic lymphohistiocytosis (HLH)(Favara *etal*,1997) characterized by persistent fever, splenomegaly, hepatomegaly, and laboratory findings of blood cytopenia, hepatic dysfunction(Ravelli, 2002;Favara 1992) with pathognomic pathological picture of hemophagocytosis (phagocytosis by macrophages of erythrocytes, leukocytes, platelets and their precursors cells) in the bone marrow and other tissues(Ost *et al* 1998; Ravi and Morice ,2002).

The HLH disorder occurs in two forms ; primary (Inherited HLH) and secondary (Reactive) HLH which composed of macrophage activation syndrome (MAS), infections associated hemophagocytic syndrome (IAHS) and malignancy associated syndrome(Favarata *etal*,1997) The syndrome which has been referred to as histiocytes medullary reticulosis was first describe in 1939(Scott and Robben ,1939).

IAHS may be diagnosed in association with diverse infections diseases; viral (Chan *et al*,1969), bacterial (Zuazu *et al*,1979;Al-Ani *et al*,2006), fungal, and protozoa pathogens (Gagnaire and Stephan,2000;Kallfan *et al*,2002).

The pathogenesis of IAHS involves the excessive activation and proliferation of monocytes-macrophages system through immune dysregulation of cytotoxic T cells and natural killer (NK) cells with elaboration of high level activating cytokines (Fujiwara and Fibis ,1993;Osuigi *et al*,1997).

This study aims to determine the clinicopathological features and associated microbial causes of related hemophagocytosis with life-threatening hemocytopenia .

## Materials and Methods

The study was carried out through the period May1999-December 2007 in Hilla province and neighbored discrete-Southcentral of Iraq.

A total of 19 pediatric (11 males and 8 females) case-patients are very sick with pallor, high fever, organomegaly, blood cytopenia.They were referred for complete blood picture ,bone marrow study, microbial and related biochemical tests.

All relevant demographic clinical and laboratory data upon intial diagnosis with bone marrow aspirate, were collected and analyzed.

The diagnosis of IAHS based on criteria proposed by the Histiocytes Sociaty (Henter *et al*,1991 a) Clinical criteria ; fever and splenomegaly are the most clinical signs ,but hepatomegaly,lymphadinopathy,jaundice,and petechaiel skin rash are also seen; b) Laboratory criteria: cytopenias(affecting 2 of 3 lineage in peripheral blood with hemoglobin (<7 gm/dl), platelet (<100X$10^9$/L),and neutropenia(<1.0X$10^9$/L), hypertriglyceridemia(>2.0mmo/L), hypofibrinogenmia <1.5g/dl);
c) Histopathological criteria; hemophagocytosis in the bone marrow , or spleen , or lymph nodes , and no evidence of malignancy).

Venous blood was taken and used immediatly for blood cells count , blood smear, coagulation examinations, microbial and biochemical investigations.
Bone marrow aspirate from posterior superior iliac crest. Bone marrow smear and blood smear were stained by Leishman dye (Dacie and Lewis ,1995). Serum cholesterol, triglycride, total serum bilirubin, indirecthyperbilirubinaemia blood urea, serum fibrinogen assy, following manufacture instructions for pediatric age group. Bacterial cultures and serological diagnostic test for viruses and bacteria were performed (Talib,1996). The diagnosis of vsciral lieshmaniasis based on presence of L-D bodies in bone marrow smear confirmed in 4 cases with serodiagnosis. Monospot test used to diagnosed Epitein-Barr virus (E-B virus) infection. Brucellosis and typhoid fever based on positive agglutination titer test. Tubercullosis on sputm positive for AFB stain and chest x-Ray result. Chronic osteomylitis on history and clinical background. ( Hunter,2006 )

Statistic analysis results presented as number and percentage .Gender distribution tested by non –parametric Mann-Whitney test . The p value < 0.05 was considered statistically significant.

## Results

In the field of our study epidemiologic, clinical, laboratory features and etiological pathogens of 19 patients meeting the criteria of IHAS were analyzed.

Most of cases are young children (mean age 46.95±36.06 months) in both sex and there is no significant difference between male and female (p value> 0.05) .Also all cases were associated with disseminated infections were 10 cases visceral lishmaniasis, 4 cases E-B virus infection with history of infectious mononucleosis, 2 cases brucellosis , and 1 case for each of typhoid fever,chronic pulmonary tuberculosis, and chronic osteomylitis table (1).

**Table( 1) : Demographic features , sex , age, and diagnosis of infectious ssociated hemophagocytosis syndrome cases.**

| Case No. | Gender | Age / months | Time of Diagnosis* | | Type of infectious diseae |
|---|---|---|---|---|---|
| | | | Months | Years** | |
| 1 | female | 18 | May | 1999 | Infectious mononucleosis |
| 2 | female | 14 | July | 1999 | Visceral lishmaniasis |
| 3 | male | 9 | April | 2000 | Visceral lishmaniasis |
| 4 | male | 60 | August | 2000 | Visceral lishmaniasis |
| 5 | female | 11 | February | 2001 | Visceral lishmaniasis |
| 6 | male | 27 | October | 2001 | Infectious mononucleosis |
| 7 | male | 17 | June | 2002 | Visceral lishmaniasis |
| 8 | female | 52 | August | 2003 | Brucellosis |
| 9 | male | 18 | April | 2004 | Visceral lishmaniasis |
| 10 | female | 74 | September | 2004 | Typhoid fever |
| 11 | male | 108 | November | 2004 | Chronic ostiomylitis |
| 12 | male | 9 | December | 2004 | Visceral lishmaniasis |
| 13 | female | 57 | May | 2005 | Visceral lishmaniasis |
| 14 | male | 51 | July | 2005 | Infectious mononucleosis |
| 15 | female | 92 | August | 2005 | Pulmonary tuberculosis |
| 16 | female | 39 | April | 2006 | Visceral lishmaniasis |
| 17 | male | 100 | November | 2006 | Infectious mononucleosis |
| 18 | male | 111 | June | 2007 | Brucellosis |
| 19 | male | 20 | August | 2007 | Visceral lishmaniasis |

Male : female (8:11) P value > 0.05

Age : mean ± SD (46.95 ± 36.06 )

*to check the seasonal variation

**annual incidence is equal to 19/9(2.1)

**The seasonal distribution of casese was illustrated in figure (2)**

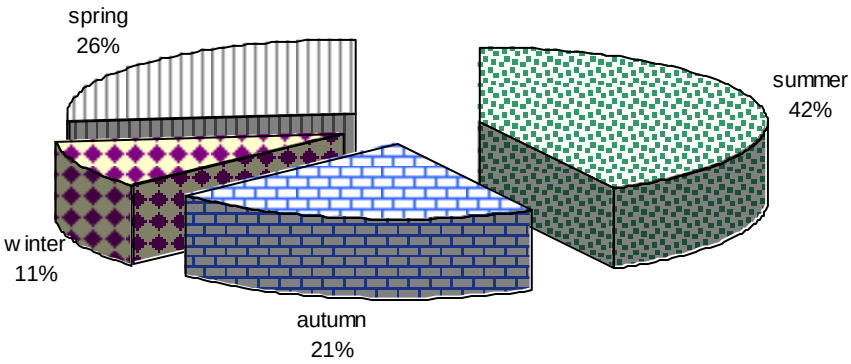


Figure 1 : Seasonal variation of cases w ith microbial infections.

The most important clinical characters ; fever reported in all cases followed by huge splenomegaly 89.4%,hepatomegaly 42.1% ,skin rash 36.8% as shown in (table2).

**Table 2 : characteristics of clinical features in case patients with infectious associated hemophagocytosis syndrome.**

| Clinical feature | No. of patients | percentage |
|---|---|---|
| Fever | 19 | 100 % |
| Splenomegaly | 17 | 89.4 % |
| hepatomegaly | 8 | 42.1 % |
| lymphadinopathy | 3 | 15.7 % |
| Skin petechiae | 7 | 36.8 % |
| Jaundice | 3 | 15.7 % |

Hematologic work up show cytopenia with anemia (Hb<10 g/dl) in most patients were severe anemia (Hb<7 g/dl) in 89.4% of them, absolute neutropenia in 94.7% and decreased platelate count with sever thrombocytopenia as showen in tables (3,4,5,6).

**Table 3 : Hemoglobin profile in 19 patients with infectious associated hemophagocytic syndrome.**

| Hemoglobin g/dl | No. Of Patients | Percentage |
|---|---|---|
| <4 | 3 | 15.7 % |
| 4 – 6.9 | 14 | 73.6 % |
| 7 – 9.9 | 1 | 5.2 % |
| 10 - 12 | 1 | 5.2 % |

The white blood cells count was less than 4.0 $X10^9$/ L in 73.6 % of cases as shown in (table 4)

**Table 4 : White blood cells count (WBC) in 19 patients with infectious associated hemophagocytic syndrome.**

| WBCC $x10^9$ / L | No. Of Patients | Percentage |
|---|---|---|
| <2000 | 9 | 47.3 % |
| 2000 – 3.900 | 5 | 26.3 % |
| 4000 – 5.900 | 4 | 21.0 % |
| 6000 – 7.900 | 0 | 0 % |
| 8000 – 9.900 | 1 | 5.2 % |
| > 10.000 | 0 | 0 % |

The diffrential leukocytes count was done for all patients through blood smear. There was decrease absolute neutrophile count, was illustrated in(table 5).

**Table 5 : White blood cells diffrential count in 19 patients with infectious associated hemophagocytic syndrome**

| diffrential count | Cells No. | No. Of Patients | Percentage |
|---|---|---|---|
| A.N.C. $x10^9$/L | 0.90 ± 1.05 | 18 | 94.7 % |
| A.L.C. $x10^9$/L | 4.40 ± 2.35 | 13 | 68.4 % |

A.N.C. : absolute neutrophile count .
A.L.C. : absolute lymphocyte count .

The platelets count was less than $100X10^9$ /l in 84.4% of cases with infectious associated hemophagocytic syndrome as shown in (table 6).

**Table 6 : Changes in platelates count in 19 patients with infectious associated hemophagocytic syndrome.**

| Platelate $x10^9$/L | No. osf Patients | Percentage |
|---|---|---|
| <10 | 3 | 15.7 % |
| 10 – 50 | 7 | 36.8 % |
| 50 - 100 | 3 | 15.7 % |
| 100 – 150 | 5 | 26.3 % |
| >150 | 1 | 5.2 % |

About 47.3% of patients had prolonged bleeding time,low serum fibrinogin was lower than normal value in 84.2% of patients.whereas prothrombine time and activated partial thromboplastin time showed normal values in most cases (table 7)

**Table 7 : Coagulation profile in 19 patients with infectious associated hemophagocytic syndrome.**

| test | time | No. Of patients | Percentage |
|---|---|---|---|
| Bleeding time (5-9 minute)* | >10 minute | 9 | 47.3 % |
| Prothrombine(12 second)* | 13 second | 18 | 94.7 % |
| A.P.T.T.(28-35 second)* | 28-35 second | 18 | 94.7 % |
| Fibrinogene (2-4 gm/dl)* | <2 gm | 16 | 84.2 % |

A.P.T.T.: activated partial thromboplastin time .
*normal value

Most of cases show abnormal liver function test; high serum triglycrides and increased total serum bilirubin and indirect hyperbilirubinia.

**Table 8 : Biochemical tests for19 patients with infectious associated hemophagocytic syndrome.**

| Biochemical tests | Mean ± SD | No. of patients | Percentage |
|---|---|---|---|
| Blood urea mg/dl | 27 ± 2.15 | 6 | 31.5 % |
| Serum albumin g/dl | 169 ± 11.5 | 13 | 68.4 % |
| Total bilirubin mg/dl | 5.8 ± 2.3 | 9 | 47.3 % |
| Cholesterol mg/dl | 137 ± 9.47 | 11 | 57.8 % |
| Triglycerides mg/dl | 548 ± 16.90 | 17 | 89.4 % |

Indirect serum bilirubin 4.4±1.17 in 8/9 with jundice.

## Discussion

It is an important to identify early potentially life-threating diseases, particularly if they are treatable. Hemophagocytic syndrome is a such disorder. Our patients had abnormal clinical and laboratory feature keeping in all the diagnostic criteria of IAHS which is supported by positive bone marrow pathognamic pathologic picture, phagocytosis of blood cells and their precursors by macrophages in all cases.

Most of the study cases in early childhood with median age at onset of diagnosis (46.95±36.06 months-table 1), no significant difference in sex factors (M:F 11:8) which is similar to other studies (Hoffibrand *et al*, 2005;Janka *et al*, 2004; Hsin-Hsu, 2007).

Annual incidence of 2.1 cases (table 1) with seasonal pattern of most cases in Summer which is considered as a favourable period for most microbial infectious diseases(table 1 and figure 1).Higher annual incidence of cases was reported in Hongkong and Taiwan (Fishman *et al*,2006) The most prodromal clinical signs of the disease were: Fever in all patients ,and splenomegaly (100%, 89.4 respectively),this in accordance with (Henter *et al*,1991) , while severe anemia,neutropenia and marked thrompocytopenia were obsereved in the hematologic work up . Prolonged bleeding time in 47.1% of patients and serum fibrinogen is typicaly low in 84.2% (table 6),and this may suggest disseminated intravascula coagulopathy (Kaito *et al*, 1997).Serum chemistry (table 8) where most of cases show hypertiglyceridemia, increased serum total bilirubin with inderect hyperbilirubinaemia which may suggest hemolysis mostly intramedullary (Fishman, 2006) .

Regarding associated etiologic factors, infection by the protozoa pathogens; visceral lieshmaniasis were noted in 10 cases.The first case of visceral leishmaniasis revealed with IAHS was reported by Matzner *et al* (1979) .The high incidence of visceral leishmanasis parrellel to other studies where visceral leishmaniasis endemic (Matzner *et al* ,1979)and it is endemic in Hilla proviance and south of Baghdad (Al-Marzoki, 2003).The E-B virus infection is the second causative pathogen. E-B virus associated hemophagocytosis has been described in 1979 as distinct clinical entitey with poor outcome (Waom and Cheng ,1994).

*Brucella* and *Salmonella typhi* are small Gram negative bacilli capable of surviving within macrophage of any organ ,thus the proliferation of which can produce organomegaly and hemophagocytosis with hemocytopenia (Colmenero JD et al 1996; Yaramis A et al 2001) .

The resolution of HLH following treatment of disseminated infection suggestions that HLH is a secondary to the underling infection (Janka and Schnieder 2004; Fishman, 2006 ).

Because so many infectious agents may associated with IHAS clinician should work closely with pathologist and microbiologist to clearly define precipitating or underling illness, and improve treatment of underling causes and survival in other wise fatal conditions.

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