Hemophagocytic Syndrome among Children in Basrah City / Iraq - A Retrospective Analysis of 36 Patients

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***Abstract* :** Hemophagocytic lymphohistiocytosis (HLH) is an aggressive and life-threatening syndrome of excessive immune activation. It most frequently affects infants from birth to 18 months of age, but the disease is also observed in children and adults of all ages. This study aims to evaluate patients with HLH with the clinical features, laboratory data, treatment modalities, and the prognostic significance of these factors on survival of patients after eight weeks of different therapies used. Thirty-six patients who were hospitalised in Basrah Children Teaching Specialty Hospital were evaluated for suspected HLH from January 2015 until October 2021. Retrospective analysis using descriptive statistics was done, applying demographic, clinical, and different laboratory indices as well as bone marrow analysis in an attempt to identify possible risk factors that might be related to prognosis and survival of patients. Of the total patients , 66.7% were males and 33.3% were females. The most common age group was infants less than 12 months old (47%), with a lesser percentage for other age groups. All patients had fever (100%), 94.4% of them had pallor, and hepatosplenomegaly was found in 77.8%. Furthermore, anaemia was detected in 94% of the cases, while neutropenia was in 80% of them and thrombocytopenia in more than 85% of the total cases. All patients present with elevated ferritin (100%) and an elevated triglyceride level (94.4%). Moreover, high bilirubin in more than 60% of patients with elevated liver enzymes in more than 50% of patients, low albumin less than 2.5 gm/dL in 61% of the cases, and LDH elevated in almost all of them (100%). Hemophagocytosis was found in 97.2% of the bone marrow, and coagulopathy with significant lengthening of the PT and PTT was found in 38.9% and 41.7%, respectively. It was discovered that age less than 12 months was associated with early death and poor outcome, as well as jaundice, decreased urine output, and generalised body oedema. SGOT, SGPT levels greater than 200 U/L, albumin levels less than 2.5 m/d, prolonged PT, and PTT all had a negative impact on survival, with patients with risk prognostic parameters having a higher mortality rate. The study concludes that HLH has a high mortality if undiagnosed early. Therefore, early identification of clinical symptoms and signs with adequate laboratory analysis and early initiation of therapy according to well-known therapies might improve the survival among affected patients.

***Index Terms* :** HLH, Basrah, Iraq, clinical feature, prognosis, outcome

I. INTRODUCTION

1.1. Background : Hemophagocytic lymphohistiocytosis (HLH) is a pathological immune activation syndrome that is characterised by clinical signs and symptoms of severe inflammation that result in life-threatening disorders requiring admission and treatment in an intensive care setting [1]. (HLH) is a multi-organ syndrome arising from deficient inflammatory control in which excessive activation of macrophages and CD8 + lymphocytes results in a hyperinflammatory state induced by elevated circulating tumour necrosis factor-α (TNF-α), interferon-α (IFN-α), interleukins (IL-6, IL-8, IL-10, IL-12, and IL-18) which will cause end -organ damage from severe protracted inflammation leading to marked elevation in morbidity and mortality [2]. The typical presentation can be mistaken for severe sepsis with multiorgan failure [3-5].

There are two main classification systems by Histocyte Society guidelines for diagnosis and management of HLH: HLH-1994 and the most recently updated one is HLH-2004. In 1986, Fischer et al. performed the first successful bone marrow transplant from a person who did not have HLH [6].

There are two subtypes of HLH: primary when there are underlying genetic defects in lymphocyte function or secondary when there are underlying triggers for HLH without genetic defects. These triggers may include infection (viruses, bacteria

,protozoa, fungi), malignancies, and autoimmune diseases. HLH may take place during the course of some metabolic diseases (e.g., Gaucher disease, lysinuric protein intolerance), immunosuppressive therapy, as well as after organ and stem cell transplantation [7-8].

The cut-off to distinguish primary from secondary HLH is somewhat hard to achieve without family history and the diagnostic genetic tests [9] Many of the clinical features seen in HLH are attributable to the underlying causative illness. HLH is often initially overlooked as a diagnosis, which may lead to delayed diagnosis of HLH. Early diagnosis and identification of HLH might improve survival and outcome. In Europe and Japan, an incidence of 1-2 per million has been reported, but this may be underestimated due to diagnostic difficulties and delays. The incidence of familial hemophagocytic lymphohistiocytosis (FHLH) in Sweden was estimated to be 0.12/100,000 children under age 15 per year [9–11], but in other study by Jordan et al. 2011 expected that1 case of HLH per 3000 admission the average duration of survival without HLH-2004 therapy had been estimated to be only two months [1]. There were 7.5 cases for every 10,000 hospitalised people in Turkey. This may be because many Turkish people are related to each other [12].

Certain criteria were required for the diagnosis of HLH and, over the last two decades, updates in the criteria for diagnosis have been established to facilitate early diagnosis, keeping in mind that bone marrow hemophagocytosis is an important diagnostic criterion but not essential for fulfilling the five criteria [13]. Hemophagocytosis of the bone marrow, lymph nodes, spleen, and/or liver is seen in 60% of HLH patients [14]. It can also happen without HLH after a blood transfusion, infection, or autoimmune disease [15, 16].

1.2. Objectives : To evaluate patients with hemophagocytic lymphohisteocytosis regarding the clinical features, laboratory data, treatment modalities, and the prognostic significance of these factors on survival after eight weeks of different therapies used.

II. METHODS

2.1 Data Collection : A retrospective analysis was conducted in October 2021 for patients with HLH who were admitted to Basrah Children Teaching Speciality Hospital/Iraq between January 2015 and October 2021. A total of 36 patients were registered. The collected information included the patient's demographic characteristics, clinical and laboratory findings, treatment methods, and disease outcome.

Data from patients' files such as patient age, gender, duration of hospitalization (intensive care unit, ward) were collected, other information such as the age of onset of HLH, presence of family history of HLH and number of affected siblings if there is family history of HLH. Symptoms of patients during hospitalizations were assessed like fever, jaundice, loss of appetite, cough, shortness of breath, body oedema, decreased urine output, lymphadenopathy, abdominal distension, hepatomegaly, splenomegaly, hepatosplenomegaly, bleeding, rash, hypotension, disseminated intravascular coagulation (DIC) signs, central nervous (CNS) manifestations like headache, fit, loss of consciousness, personality changes all had been evaluated.

The Laboratory data including white blood cell count (WBC), absolute neutrophil count (ANC), hemoglobin (Hb), platelet, fibrinogen, Prothrombin time (PT), activated partial thromboplastime (aPTT), serum glutamic oxaloacetic transaminase(SGOT), Total serum bilirubin (TSB), Serum glutamic pyruvic transaminase (SGPT), lactic dehydrogenase (LDH), ferritin, triglyceride (TG), albumin, potassium, sodium, and calcium.

Blood culture was not assessed for all patient to exclude presence or absence of infection like bacterial, fungal, or protozoal like leishmania donovani which is assessed by specific serological test or bone marrow examination. Viral infection had been searched for like hepatitis A, cytomegalovirus (CMV), Epstein Barr virus (EBV), herpes simplex virus (HSV), and COVID -19 virus.

Bone marrow examination for presence of hemophagocytosis , leishmania donovani bodies, storage diseases, and evidence of malignancy. Cerebral spinal fluid analysis was done searching for pleocytosis and or high protein.

The HLH diagnosis was made by applying the HLH-2004 diagnostic criteria. Cases were classified into primary and secondary types. Primary type is identified by presence of positive family history of affected sibling(s) with HLH called familial type (FHLH)/or age less than 12month old or presence of primary immune deficiency syndrome like Chediak-Higashi syndrome or Griscelli syndrome. A genetic mutation study is not available. The secondary type is classified by the presence of an underlying aetiology like infection, autoimmune disease such as systemic lupus erythematosus (SLE) and systemic onset juvenile rheumatoid arthritis (SJRA) or malignancy.

Data about treatment for all patients has been documented, including length of stay in ICUs and wards. Treatment details include the number of patients who received antibiotics only for confirmed secondary bacterial infection with other supportive therapies like packed red blood cell transfusion, platelets ,patients who received steroids only or received steroids and cyclosporine because of family refusal to start chemotherapy, other patients who received steroids and immunoglobulin, and patients who started chemotherapy using etoposide, steroids, and cyclosporine-A according to the HLH-2004 protocol. Remission state at 8 weeks following different treatment modalities has been documented for all patients. The outcome of all patients at end of eight weeks of different treatment modalities had been assessed whether Alfie or dead. Three patients were excluded from outcome analysis because of unavailability of their fate (family discharged the patients on their responsibility).

2.2 Updated hemophagocytic lymphohiteocytosis (HLH) diagnostic guidelines : The diagnosis of HLH can be established if one of either 1 or 2 below is fulfilled.

1. A molecular diagnosis consistent with HLH.
2. Diagnostic criteria for HLH were fulfilled (five out of the eight criteria below).

(A) Initial diagnostic criteria (to be evaluated in all patients with HLH)

- Fever.
- Splenomegaly.
- Cytopenia (affecting ≥ 2 of 3 lineages in the peripheral blood).
  - Haemoglobin < 9 g/dL (in infants < 4 weeks: haemoglobin < 10 g/dL).
  - Platelets < 100×10^9/L.
  - Neutrophils < 1.0×10^9/L.
- Hypertriglyceridemia and/or hypofibrinogenemia:
  - Fasting triglycerides ≥3.0 mmol/L (i.e., ≥265 mg/dL).
  - Fibrinogen ≤1.5 g/L.
- Haemophagocytosis in the bone marrow, spleen, or lymph nodes with No evidence of malignancy.

(B) New diagnostic criteria

- Low or absent NK-cell activity (according to local laboratory reference).
- Ferritin ≥ 500 μg/L.
- Soluble CD25 (i.e., soluble IL-2 receptor) ≥ 2400 U/mL.

2.3 Definitions

1. **Survival :** Patients who survived 8 weeks after disease onset
2. **Remission state at 8 weeks** : Patients who have the following criteria were considered to be in a complete remission state: no fever, no splenomegaly, no cytopenia, no

hypertriglyceridemia, ferritin less than 500 μg/L and normal cerebral spinal fluid (CSF) analysis.

3. **Central nervous system (CNS) involvement** was defined as when neurological symptoms were present, pleocytosis and/or proteinosis were found in the CSF, or abnormalities on magnetic resonance imaging were documented [17].

2.4 Statistical analysis : The data was classified into groups with percentages. The Chi-squared test was used to assess the difference between several risk factors, like clinical and laboratory findings, and the outcome of patients. Statistical analysis was performed using SPSS Software Version 26. A difference was considered significant if the P value < 0.05.

III. RESULTS

There was a total of 36 patients who were enrolled in this study. Of the 36 patients, 24 (66.7%) were males and 33.3% were females. The male to female ratio was 2:1. The most common age group at presentation was less than 12 months old (47.3%). Most of the patients were from Basra 22 (61.1%), followed by other governorates. Table 3.1 demonstrates the demographic characteristics of patients.

Table 3.1 : Demographic characteristic of patients with HLH

Age	No. (%)	Sex	No.(%)	Address	No. (%)
0-12month old	17(47.3)	Male	24(66.7)	Basra	22(61.1)
1-2 years old	3(8.3)	Female	12(33.3)	Missan	9(25)
2-5 years old	6(16.7)			Thi-Qar	4(11.1)
5-10 years old	4(11.1)				
10-15 years old	6(16.7)			Muthana	1(2.8)
Total	36	Total	36	Total	36

The most common presenting clinical features were: fever (100%), pallor (94.4%), splenomegaly (94.4%), followed by other symptoms. Table 3.2 illustrates the clinical features of patients with HLH.

Table 3.2 : Clinical manifestations of patients with HLH

Clinical manifestation	NO.* (%)
Fever	36 (100)
Pallor	34 (94.4)
Jaundice	20 (55.6)
Splenomegaly	34 (94.4)
Hepatomegaly	28 (77.8)
Hepatosplenomegaly	27 (77.8)
Lymphadenopathy	7 (19.4)
Bleeding	17 (47.2)
Bone pain	6 (16.7)
DIC	9 (25)
Oedema	24 (66.7)
Skin rash	9 (25)
Shortness of breath	11 (30.6)
CNS manifestations	12 (33.3)
* A respondent may have more than one clinical manifestation.	

The laboratory results for all patients with HLH revealed that anaemia had been found in 34 patients (94.4%), neutropenia (neutrophil count $< 1.5*10^9$) was observed in 29 cases (80.55%), and thrombocytopenia (platelet count $< 100*10^9$) was observed in 31 cases. All patients in this study presented with elevated ferritin levels, hypertriglyceridemia, and elevated levels of LDH. Bone marrow hemophagocytosis was detected in all patients except one. The highest ferritin, LDH observed among our patient was 5612ng/ml,3774 U/L respectively. Increased total serum bilirubin levels were observed in 18 cases (50%) among the patients, while elevated liver enzymes (SGOT and SGPT) were observed in about 40% of the patients. Prolonged prothrombin time (PT) and partial thromboplastin time (PTT) were observed in 14,15 patients (about 39% and 41.7%) respectively. Table 3.3 illustrated laboratory features of patient with HLH

Table 3.3 : Haematological and biochemical parameters of all patients

Parameters	No. (%)	Parameters	No. (%)
Hb (gm/dL)		**Triglyceride (mmole/L)**	
<5gm/dL	5(13.9)	<2.6	2(5.6)
5-8.9 gm/dL	29(80.6)	>2.6	34(94.4)
>=9 gm/dL	2(5.6)		
TWBC ($*10^9$)		**Ferritin (ng/ml)**	
$<2000*10^9$	12(33.3)	<1000	1(2.8)
$>2000*10^9$	24(66.7)	>1000	35(97.2)
ANC ($*10^9$)		**PT / seconds**	
<500	17(47.2%)	<20	22(61.1)
500-1000	4(11.1)	>20	14(38.9)
1000-1500	8 (22.2)		
>1500	7(19.4)		
Platelet ($*10^9$)		**PTT / seconds**	
$<20*10^9$	22(61.1)	<45	21(58.3)
$>20*10^9$	14(38.9)	>45	15(41.7)
TSB (µmole/L)		**CSF cytology**	
<100	20(55.6)	CSF pleocytosis	3(8.3)
>100	16(44.4)	Not evaluated	33(91.7)
SGOT (U/L)		**BM Hemophagocytosis**	
<200	17 (47.2)	Yes	35(97.2)
>200	19 (52.8)	Not evaluated	1(2.8)
SGPT (U/L)		**LDH (U/L)**	
<200	14(38.9)	<1000	29(80.6)
>200	22(61.1)	>1000	7 (19.4)
Albumin (gm/L)			
<2.5	22(61.1)		
>2.5	14(38.9)		

Searching for the causative underlying disease/conditions like primary or familial type with a positive family history of HLH has been identified in 9 cases (25%). One case has been diagnosed with primary immune deficiency disease(Griscelli syndrome). Secondary causes of HLH include infection, viral, and bacterial infections, but no Kalazar. Rheumatic disease was documented in one patient who presented with macrophage activation

syndrome(MAS). There is no malignancy associated with HLH in this study. Table 3.4 illustrated types of HLH and a family history of HLH, while Table 3.5 illustrated secondary causes of HLH.

Table 3.4 : Types of HLH and family history of HLH

Type of HLH	
Primary	19(52.8)
Secondary	16(44.4)
Undetermined	1(2.8)
Family history of HLH	
Yes	9 (25)
No	27(75)
Total	36(100)

Table 3.5 : Secondary causes of HLH

Infections				Autoimmune	
Viral	NO (%)	Bacteria	NO (%)	RA	NO (%)
HSV	3 (8.3)	Positive growth	5 (13.9)	MAS	1 (2.8)
Hepatitis A	2 (5.6)	Negative growth	6 (16.7)		
CMV	1 (2.8)	Not evaluated	25 (69.4)		
Covid 19	2 (5.6)				
Not evaluated/-ve culture	28 (77.8)				

Tables (3.6) summarise treatment modalities and remission state details at 8 weeks. This table reveals that most patients can attain complete remission with steroids or steroids and cyclosporine-A. Regarding HLH-2004 protocol treated patients only 4/21 of patients attained complete remission.

Table 3.6 : Remission state at 8 weeks for patients with different treatment modalities

Outcome state at 8 weeks	Frequency	Percent
Complete remission at 8week steroid only	7	19.4
Complete remission steroid+ CSA	2	5.6
Complete remission- HLH protocol	4	11.1
Incomplete response to HLH-2004 induction4	2	5.6
HLH protocol induction death	4	11.1
Died before 8 weeks on steroid ±antibiotic due to family refused chemotherapy	11	30.6
Not evaluated because of discharge or abandonment	3	8.3
Response with antibiotics only	3	8.3
Total	36	100.0

Several initial presenting clinical and laboratory parameters have been correlated with survival/death or worst outcome, including age of patients, jaundice, severe body oedema, CNS involvement, ANC, Hb, platelet count, SGPT, SGOT, TSB, ferritin, LDH. Among these factors, age less than 12 months old, platelet count<50×^{3} TSB >100 mmol /L ,SGPT & SGOT >200 U/L, low albumin, PT >39 second, PTT > 45, presence of DIC, acute liver failure, all these clinical and laboratory factors are associated with worse prognosis and early death. The P-value was < 0.05 for the aforementioned factors. Different demographic, clinical, laboratory findings, and treatment modalities have been assessed in relation to the survival of patients. Three patients were excluded from analysis for survival because their fate was not evaluated. That means 33 patients out of 36 patients were evaluated for survival. Tables 3.7 to 3.10 illustrate the relation of different demographic, clinical, laboratory findings, and treatment modalities to the survival of patients.

Table 3.7 : Relation of demographics, types and family history of HLH to survival of patients

Risk factors		Survived	Dead	Total	P-Value
Age	< 12 months	4 (26.7)	11 (61.1)	15	0.048
	> 12 months	11(73.3)	7 (38.9)	18	
Sex	Male	11 (73.3)	11 (61.1)	22	0.458
	Female	4 (26.7)	7 (38.9)	11	
Address	Basra	11 (73.3)	9 (50)	20	0.154
	Missan	4 (26.7)	5 (27.8)	9	
	Thi-Qar	0	4 (22.2)	4	
Type	Undetermined	0	1 (5.6)	1	
	Primary	5 (33.3)	12 (66.7)	17	
	Secondary	10 (66.7)	5 (27.8)	15	
Family history of HLH	Yes	3 (20)	6 (33.3)	9	0.45
	No	12 (80)	12 (66.7)	24	

Table 3.8 : Relation of Clinical features to survival of patients

Risk factors		Survived	Dead	Total	P-Value
Pallor	Yes	13(86.7)	18(100)	31	0.199
	No	20(13.3)	0(0)	2	
Splenomegaly	Yes	13(86.7)	18(100)	31	0.199
	No	2(13.3)	0	2	
Hepatomegaly	Yes	10(66.7)	16(88.9)	26	0.203
	No	5(33.3)	2(11.1)	7	
Hepatosplenomegaly	Yes	10(66.7)	16(88.9)	26	0.203
	No	5(33.3)	2(11.1)	7	
Jaundice	Yes	4(26.7)	14(77.8)	28	0.003
	No	11(73.3)	4(22.2)	15	
LAP	Yes	5(33.3)	2(11.1)	7	0.203
	No	10(66.7)	16(88.9)	26	
Skin rash	Yes	3(20)	5(27.8)	7	0.699
	No	12(80)	13(73.2)	26	
SOB	Yes	3(20)	7(38.9)	10	0.283
	No	12(80)	11(61.1)	15	
Bleeding	Yes	5(33.3)	11(61.1)	18	0.112
	No	10(66.7)	7(38.9)	17	
DIC	Yes	0	8(44.4)	8	0.004
	No	15(100)	10(55.6)	25	
Body edema	Yes	4(26.7)	17(94.4)	21	0.000
	No	11(73.3)	1(5.6)	12	
Decreased UOP	Yes	0	5(27.8)	5	0.049
	No	15(100)	13(72.2)	28	
CNS manifestation	Yes	9(60)	12(66.7)	21	0.709
	No	6(40)	6(33.3)	12	

Table 3.9 : Relation of Laboratory features to survival of patients

Parameter		Survived	Dead	Total	P-Value
Hb gm/dL	<5	0	4(22.2)	4	0.074
	5-9	13(86.7)	14(77.8)	27	
	>9	2(13.3)	0	2	
ANC (10 ×^{9})	<1000	6(40)	12(66.7)	18	0.126
	>1000	9(60)	6(33.3)	15	
Platelet count (10 ×^{9})	<50	4(26.7)	15(83.3)	19	0.001
	>50	1(73.3)	6(16.7)	7	
PT/seconds	Normal	15(100)	7(38.9)	22	0.0001
	Up to 39	0	7(38.9)	7	
	>39	0	4(22.2	4	
PTT/seconds	<45	15(100)	6(33.3)	21	0.0001
	>45	0	12(66.7)	21	
TSB/mmole/L	<100	15(100)	5(27.8)	20	0.0001
	>100	0	13(72.2)	13	
SGOT U/L	<200	12(80)	5(27.8)	17	0.003
	>200	3(20)	13(72.2)	16	
SGPT U/L	<200	10(66.7)	4(22.2)	14	0.01
	>300	5(33.3)	14(77.8)	19	
Albumin gm/dL	<1.5	0	3(16.7)	3	0.0001
	1.5-2.4	3(20)	13(72.2)	16	
	2.5-4.5	12(80)	2(11.1)	14	
TG mmole/L	<2.65	2(13.3)	0	2	0.119
	>2.65	13(86.7)	18(100)	31	
LDH U/L	200-400	5(33.3)	0	5	0.022
	401-600	7(46.9)	5(27.8)	12	
	601-800	1(6.7)	3(16.7)	4	
	801-1000	2(13.3)	3(16.7)	5	
	1000-2000	0	6(33.3)	6	
	2000-4000	0	1(5.6)	1	
Ferritin ng/ml	500-1000	1(6.7)	0	1	0.633
	1000-1500	3(20)	2(11.1)	5	
	1501-2000	6(40)	7(38.9)	13	
	>2000	5(33.3)	9(50)	14	
Serum sodium (mmole/L)	Normal	13(86.7)	12(66.7)	25	
	Low	2(13.3)	6(33.3)	8	0.242
Serum Potassium (mmole/L)	Normal	12(80)	15(83.3)	27	0.577
	Low	3(20)	3(16.7)	6	
Serum calcium	Normal	12(80)	13(72.2)	25	0.699
	Low	3(20)	5(27.8)	8	

Table 3.10 : Relation of treatment to survival of patients

Treatment modalities	Survived	Dead	Total	P-Value
Antibiotics	4 (26.7)	4 (22.2)	8	0.877
Steroids	5 (33.3)	6 (33.3)	11	
Steroid + CSA	2 (13.3)	2 (11.1)	4	
HLH protocol induction	4 (26.7)	4 (22.2)	8	
Immunoglobulin therapy + steroid	0	2 (11.1)	2	

IV. DISCUSSION

In addition to the improvement of diagnostic criteria and the addition of new criteria for the HLH-2004 protocol and the application of hematopoietic stem cell transplantation as a curable step, all these factors played a fundamental role in the detection of suspected cases and the resultant improvement in treatment and survival.

The most common age group in this study is below 12 months old (17 patients), which accounts for 47.3% while in a cohort by Histeocyte Society it was 57% for patients less than 12 months old [8], while in Badheka et al., 2018 it was 19.6% for those less than 1 year old and a higher percentage was reported in those older than 6 years old [19], while in another study by Xue et al., 2017, age less than 12 months comprised 17% of the total number of patients [17] while it was just 15% in a study by Ishii et al., 2007 [11], while in another study by Chen et al., 2020 ,the median age was 7 years old [20]

The male gender had a higher number among the total number of patients, 24 (66.7%), with a male: female ratio of 2:1, which is nearly similar to the result in a study by Xu et al., (2017) with a predominance of male gender with a male: female ratio of nearly 1.7:1[17] In a study by Sasan et al., (2019), the male: female ratio was nearly 1.5:1, while there was female predominance in a study by Chen-T et al., 2020, with a male: female ratio of 0.59:1 [20], while in a study by Ishii et al., (2007), the male: female ratio was 0.94 [11].

HLH is characterised by multisystem inflammation and immune activation, which can be manifested by fever in most patients except in the neonatal period [21]. The most common clinical presentation was fever, which was presented in all patients, followed by splenomegaly , pallor, and hepatosplenomegaly with a percentage similar to that mentioned in other studies [22] CNS manifestation was present in 12 patients (33.3%), but CNS involvement was not evaluated by cerebral spinal fluid analysis in most of the patients because of family refusal and belief that a sick baby may not tolerate the procedure, so CNS involvement couldn't be assessed accurately in our patients. We have just three patients who have proven CNS involvement by CSF analysis.

The majority of patients had cytopenia, which is consistent with Wang et al., 2016 [22]. Cytopenia in HLH is thought to be caused by elevated levels of TNF-alpha and IFN-gamma in the bone

marrow microenvironment, which inhibit and/or cause apoptosis of blood cell precursors. Bone marrow suppression is also a common feature of many infectious, inflammatory, and malignant disorders [23].

The highest values were 5612 ng/ml for ferritin and 3774 U/L for LDH, respectively. In this study, hypertriglyceridemia occurs in HLH because increased TNF-alpha and IFN-gamma suppress the activity of lipoprotein lipase. Most studies revealed the presence of hypertriglyceridemia in 60–70% of patients, while in this study, hypofibrinogemia was documented in 94.4% of patients and hypofibrinogemia in about 40% of patients [24]. In this study, fibrinogen couldn't be assessed in most patients. Bone marrow hemophagocytosis has been reported in all patients except one, for whom bone marrow examination was not done for him because of the very critical situation of the infant at that time with severe bleeding diathesis. Hemophagocytosis in the bone marrow, spleen, lymph nodes, and/or liver caused by macrophage activation is neither sensitive nor specific for HLH. It is found in approximately 50% to 60% of the population [14], and it can be seen in other conditions such as blood transfusion, infection, and autoimmune diseases [15][16]. Activated macrophages produce plasminogen activator, which leads to hyperfibrinolysis and hypofibrinogenemia. The fibrinogen level had not been assessed in all of the patients because the test was not available, and the patients could not afford to do it in a private laboratory.

Elevated liver function tests with or without evidence of acute liver failure have been documented in more than 50% of patients, which may relate to uncontrolled inflammation and cytokine release with evidence of different degrees of hepatitis from mild-severe at any point. These abnormalities may have a prognostic significance for patient response to specific therapy related to the underlying causes of HLH or an immuno-chemotherapy protocol based on HLH.

HLH types are classified into primary and secondary subtypes. The presence of a family history of HLH and younger age, especially those less than 12 months old, among patients was considered to have primary HLH. Unfortunately, genetic studies were not available to detect the different gene mutations in HLH. For that reason, cases of primary HLH may be missed by using the family history of HLH because some cases of primary HLH may not have a positive family history of HLH and the case of HLH is primary but sporadic, which can be identified by genetic mutational analysis only. In this study, 52.8% of patients were considered to have primary HLH, including one patient with a primary immune deficiency syndrome called Griscelli syndrome. That meant primary HLH included patients with primary immune deficiency syndrome in addition to patients with familial HLH (FHLH), and a family history of HLH was detected in 9/36 patients (25%). In a study by Xue et al. (2017), genetic sequencing analysis was done in 86/323 patients. Mutations in HLH-related genes were found in 40 (27.9%) patients [17], while in another study by Sasan et al. (2019), 60% of cases were categorised as primary HLH ,and FHLH was observed in 45% [25], while in a study by Wang et al. (2016), there were no cases of primary HLH had been identified [22].

Secondary HLH can be caused by a variety of infectious agents (viral and bacterial), protozoal parasites such as Leishmania donovani, autoimmune diseases such as SLE and RA, or cancer. In this study viral infection had been screened in only 8(22.3%) cases because of limited resources and unavailability of laboratory tests and a shortage of tests for viral load. Viruses that have been identified were herpes simplex virus, hepatitis A virus, CMV, and COVID-19 virus, while in a study by Wang et al. (2016) infectious agents were identified in 35/57 (61.9%). EBV was the most common viral infection(22) Bacterial infections have been evaluated in about 30% of patients, with a positive culture in 5(13.9%). While autoimmune causes were identified in one patient with systemic onset juvenile rheumatoid arthritis. In infants and adults with any form of HLH, therapy should rely on inhibiting the activated CD8+ T lymphocytes and macrophages and hyperactivated immune system by destroying active T cells and treating underlying triggers if any have been identified [26].

Some patients had positive infectious agents with the crucial rule of adding antibiotics, other supportive therapies like blood products, immunoglobulin, and aggressive supportive therapy

according to the system affected during treatment. However, very close monitoring of patients, even for those secondary agents like infection, is necessary because sometimes patients should be treated like primary HLH with HLH-1994/2004 . Dexamethasone monotherapy can be used alone, especially in patients who didn't fulfil the HLH criteria but are clinically and hemodynamically stable or have family refusal to start etoposide. Close monitoring of fever and ferritin levels after 24 hours of dexamethasone monotherapy can be continued if the patient's fever subsides, ferritin level improves, and their clinical condition improves. Etoposide can be added according to clinical response to dexamethasone if the patient remains febrile with an increasing level of serum ferritin after 24 hours of starting dexamethasone alone or if the patient demonstrates deterioration in his clinical condition. HLH-1994 or 2004 protocols can be started immediately in patients who are systemically unwell with CNS involvement or unstable hemodynamically [2]. In this study, remission state at 8 weeks following different modalities has been evaluated with attained complete remission with steroids alone (19.4%), antibiotics only (3.8%), and with the HLH-2004 protocol, 4 attained complete remission while a significant number of patients died before completing 8 weeks (some of whom died within the first few days of admission) because of very critical conditions (with the presence of other comorbid conditions like sepsis, multiorgan failure or coagulopathy, late presentation, and in addition to family declined initiation of etoposide).

Several clinical and laboratory risk factors have been assessed to assess their relation to the survival of patients and or early death. These factors are the age of patients less than 12 months old, associated with poor prognosis and early death from associated comorbidities like acute liver failure, high LDH, DIC, and coagulopathy with possibly associated CNC manifestations or immune deficiency diseases. Patients aged less than 12 months may harbour a genetic mutation for HLH. Another important factor is the late presentation of patients with this fatal immune dysregulation syndrome, which is associated with grave prognosis and mortality without early recognition and immediate intervention.

The presence of jaundice, severe body oedema, high levels of LDH, TSB, liver enzymes ,albumin and coagulopathy with moderate to severe thrombocytopenia has been associated with a high risk of morbidity and death in comparison to patients who did not have these abnormal liver function tests and coagulopathy. This agrees with Wang et al. (2016) [20][22].

The treatment modalities used in this study and their relationship to outcome analysis found no significant differences among patients who received different treatment modalities, which can be explained by several possible causes, including a small sample size, late presentation, and delayed diagnosis of HLH with the presence of comorbid conditions, which had an obvious effect on the response and survival of patients, particularly acute liver failure with coagulopathy or multiorgan failure. Another important factor is late referral of patient with suspected primary HLH to hematopoietic stem cell transplantation (HSCT) service firstly because of unavailability of HSCT center in Iraq and secondly sometimes unavailability of Human Leukocyte Antigen (HLA) matched donor for HSCT. Future studies with a higher number of patients and the availability of genetic studies may clarify the outcome of patients with FHLH and genetic base of HLH and clarify the outcome with HLH more efficiently.

V. FUNDING AND FINANCIAL SUPPORT

The study will be funded mainly by the researchers

VI. DATA CONFIDENTIALITY AND STORAGE

The data will be processed with a higher degree of confidentiality and privacy.

VII. CONFLICTS OF INTEREST

The researchers did not report any conflicts of interest at the current time.

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