

Research article

Presence of hemophagocytes in bone marrow- A study in a tertiary care center in South IndiaGowsik Kanimozhi¹, Priyathersini Nagarajan², Sri Gayathri², Mohan Arthi³¹Assistant Professor, Department of Pathology, Aarupadai veedu medical college, Puducherry, India²Associate Professor, ³Assistant Professor, Department of Pathology, Sri Ramachandra Institute of Higher education and Research, Chennai, Tamil Nadu, India

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ABSTRACT

Introduction and Aim: Haemophagocytic Lymphohistiocytic Syndrome (HLH) is a fatal disease with characteristic proliferation of histiocytes in the organs including bone marrow, liver, spleen, kidney, csf, meninges, lymph nodes and subcutaneous tissue which phagocytes the blood cells. In all unexplained cases of cytopenias, organomegaly and pyrexia of unknown origin the HLH should be thought off. Though there are established criteria to diagnose, early diagnosis can reduce the mortality rate due to HLH. Documenting the haemophagocytes in the biopsy can sometimes be very helpful in arriving at the diagnosis.

Materials and Methods: We identified patients who were diagnosed with or suspected to have HLH by bone marrow aspiration or biopsy admitted to Sri Ramachandra Medical College Hospital and Research Institution from January 2010 to June 2017. We retrospectively reviewed patients' medical records that have haemophagocytes in bone marrow aspirations and collected complete clinical history and laboratory findings. Those history included fever, cytopenias, and organomegaly. Their triglyceride levels, ferritin, ESR or any other abnormal elevated lab values were also noted down. If they had any established diagnosis then that was also noted.

Results: This is a descriptive study and the details of the data like clinical history, clinical features, laboratory investigations and abnormal values are represented using tabular column, Pie chart and bar diagrams.

Conclusion: From pathological point of view, documenting each case presenting with Haemophagocytes and looking into the factors like age, significant laboratory values along with clinical features can be a valuable tool to come to a provisional diagnosis and might help the clinician to proceed with the treatment for a life-threatening disease without much delay.

Keywords: Haemophagocytes; histiocytes; bone marrow aspiration.

INTRODUCTION

Haemophagocytic Lymphohistiocytic Syndrome (HLH) is a fatal disease leading to end organ damage and death. There is characteristic proliferation of histiocytes in the organs including bone marrow, liver, spleen, kidney, meninges, lymph nodes and subcutaneous tissue which phagocytes the blood cells (1,2) Earlier HLH is classified as primary form and secondary form, the former is genetically mediated and they have abnormal cytotoxic function of NK cell and T cells and the latter form is associated with infections and malignancy. Later the designation was changed to genetic for the primary and acquired for the cases of secondary HLH. All the cases which manifest at childhood does not have any genetic defects and all the cases with genetic defects does not manifest at young age. The basic mechanism is hypercytokinemia which results in end organ damage which in turn leads to death (1,3). In all unexplained cases of cytopenias, organomegaly and pyrexia of unknown origin the HLH should be thought off. Early diagnosis can reduce the mortality rate due to HLH. Documenting the haemophagocytes in the

biopsy can sometimes be very helpful in arriving at the diagnosis though we have we established criteria to diagnose (2,3).

MATERIALS AND METHODS

From patients' medical records we identified patients who were diagnosed with or suspected to have HLH by bone marrow aspiration or biopsy admitted to Sri Ramachandra Medical College Hospital and Research Institution from January 2010 to June 2017. The local institutional review board approved the study. Because the study was based on a review of medical record with clinical details, the requirement for written informed consent was waived.

We retrospectively reviewed cases that have haemophagocytes in bone marrow aspirations and collected complete clinical history and laboratory findings. Those history included fever, cytopenias, and organomegaly. Their triglyceride levels, ferritin, ESR or any other abnormal elevated lab values were also noted down. If they had any established diagnosis then that was also noted. This is a descriptive study and the details of the data like clinical history, clinical features, laboratory

investigations and abnormal values are represented using tabular column, Pie chart and Bar diagrams.

RESULTS

Totally eighteen cases (18) out of which 7(39%) were female patients and remaining 11 (61%) patients were male patients. The age of distribution was more in paediatric age group 13 Patients that is 72% were less than 15 years, 28% of cases were adult. Among thirteen patients four (22%) patients were less than one year of age. Though seventeen patients (94.4%) had fever, the clinical diagnosis of pyrexia of unknown origin was the diagnosis for six cases. Three cases were known case of acute lymphoblastic leukaemia. Three cases were systemic disease including SLE with Evan's syndrome, Juvenile arthritis and septic shock. Two cases were childhood MDS. A rare case of Chediak Higashi syndrome (Fig.2) and a known case of Macrophage Activation Syndrome (MAS). One adult patient had prostatic carcinoma metastasis to the bone marrow and one

more was a case of malaria, gametocytes seen in bone marrow Fig. 1. The age, gender of the patient, clinical diagnosis, the criteria and other parameter are given in Table 1.

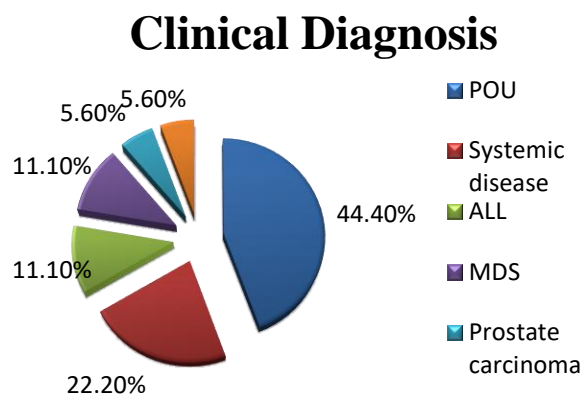


Fig. 1: Clinical diagnosis

Table 1. Patients' Age, gender, clinical diagnosis, clinical features and lab values

Pt ID	Age/Sex	Clinical Diagnosis	Fever	Cytopenia (Pan/ Bi)	SM	BMA	TG level (<150mg/dl)	Ferritin (6-159ng/ml)	LDH (<248)
1.	24/F	FFE	Y	Pan	Y	Hypo marrow HLH	242	>16,500	576
2.	15/M	FFE	Y	Bi (Hb & PLT)	Y	Hypo marrow HLH	257	7721.30	1359
3.	13/F	PUO	Y	Bi	Y	Hypo marrow HLH	187	2425.10	618
4.	21Days/M	FFE	Y	Bi (Hb, PLT)	Y	HLH	222	>16,500	530
5.	8/F	ALL relapse	Y	Nil	Nil	Hypo marrow HLH	UN	UN	UN
6.	3Months/F	CHS	Y	Bi (Hb, PLT)	Y	HLH	363	6149.90	735
7.	23/M	FFE	Y	Pan	Y	HLH	41	4.10	UN
8.	63/M	Prostatic ca mets to BM	N	Nil	Nil	Mets deposit wit HLH	Un	>16,500	898
9.	38/M	Malaria	Y	Nil	Nil	Malaria gametocyte HLH	123	Un	Un
10.	22/M	K/C/O MAS	Y	Bi	Y	HLH	73	1058.97	un
11.	15/M	SSS	Y	Bi (Hb, Plt)	N	HLH	79	Un	Un
12.	15/Fq	SLE with Evan's syn	Y	Pan	N	Hypo marrow dyEr, HLH	121	C3 139, c4 21.9	351
13.	1Month /Mq	FFE	Y	Pan	Y	HLH	92	Inc	Inc
14.	4/F	JR Ar	Y	Nil	Y	Er hy MAS	5	Un	un
15.	12/M	Fever, MDS	Y	BI	Y	MDS HLH	128	Un	1162
16.	1/F	POU	Y	Bi	Y	Dysery HLH	70	257.4	1506
17.	5/M	ALL	Y	Pan	Y	ALL, HLH	367	6253.80	69
18.	7/M	ITP, CHMDS	Y	Bi (HB, TC)	Y	HLH	385	7821.80	981

PUO- Pyrexia of unknown Origin, Pan- Pancytopenia, Bi- Bicytopenia, Hb- Haemoglobin, TC- Total count, Plt- Platelet, CHS- Chediak- Higashi syndrome, SSS - Septic Shock syndrome, SM- Splenomegaly, BMA- Bone marrow aspiration, TG

lvel- Triglyceride Level, FFE- Fever for Evaluation, PUO- Pyrexia Unknown origin , MAS- Macrophage activation syndrome, SSS- Septic Shock Syndrome, Hypo Marrow- Hypocellular marrow, SLE- Systemic Lupus erythematosus, JR Ar- Juvenile rheumatoid arthritis, Dy Er- Dyserythropoiesis, ALL- Acute Lymphoblastic Leukaemia

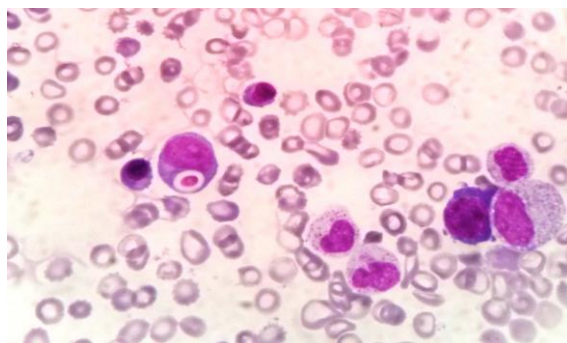


Fig. 2: The Myeloid series showing abnormal granules in their cytoplasm- Chediak Hegashi syndrome 400X magnification

Clinical features and laboratory values

Nearly most of the patients (seventeen) had fever as the presenting complaint except for one patient who had carcinoma prostate. Leucopenia is seen in 14 cases (83%) and out of those fourteen cases five cases had pancytopenia, nine patients had bicytopenia. Organomegaly was seen in 50% of cases, it was either hepatosplenomegaly or splenomegaly alone. Seven cases had elevated

triglyceride levels; ten cases had high LDL levels. Nine cases had elevated ferritin level. SGOT was elevated in ten cases, were as eleven cases had high SGPT. ESR was increased in thirteen cases fig.3. However, Bone marrow aspirates showed haemophagocytes in all the eighteen cases. In bone marrow biopsy some cases supported the finding in aspirate, (Fig. 4-6) but some of the biopsy was very difficult to spot.

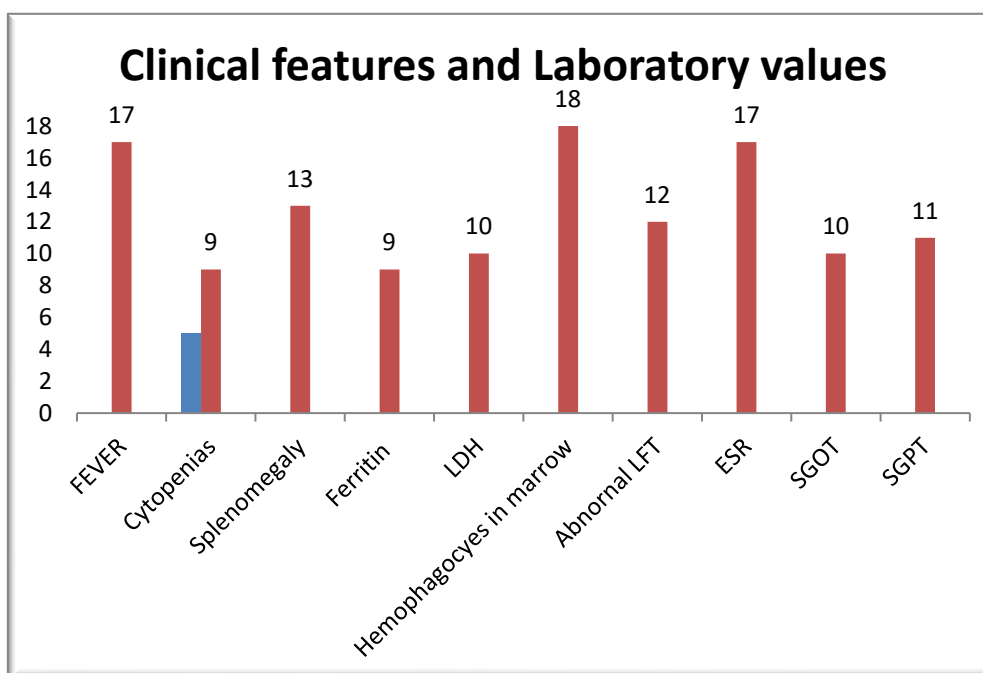


Fig. 3: Clinical features and laboratory values - Fever, Cytopenias, Splenomegaly, Ferritin, LDH-Lactose dehydrogenase, Haemophagocytes, LFT- Liver function test, ESR- Erythrocyte sedimentation Rate, SGOT- Serum glutamic oxaloacetic transaminase, SGPT- Serum Glutamic pyruvic transaminase.

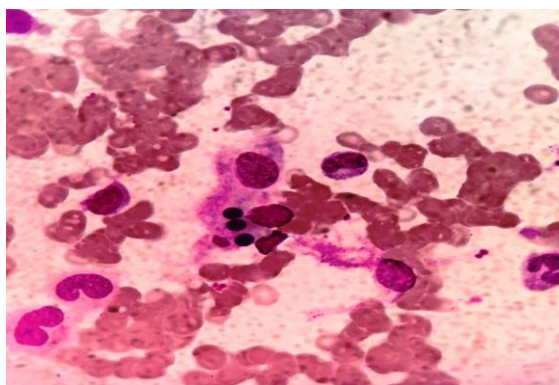


Fig. 4: Haemophagocytes in bone marrow aspiration 400X magnification

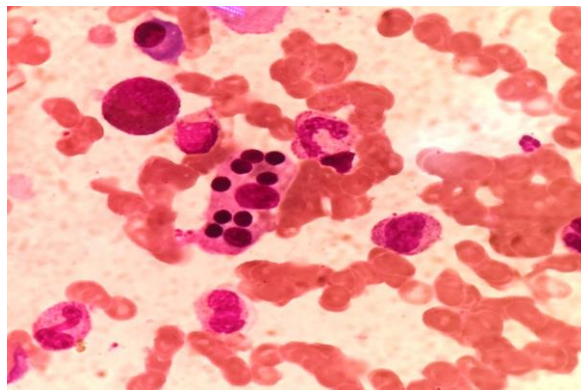


Fig. 5: Haemophagocytes in bone marrow aspiration 400X magnification

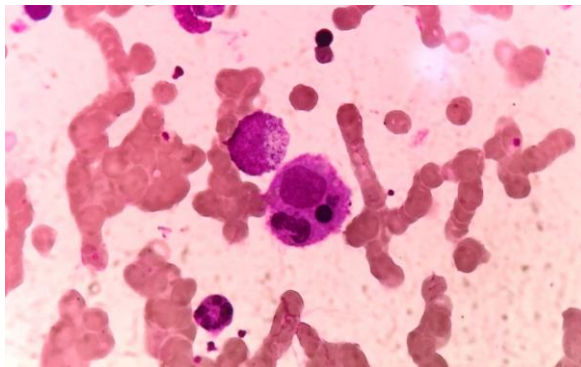


Fig. 6: Haemophagocytes in bone marrow aspiration 400X magnification

DISCUSSION

The HLH is usually diagnosed based on the HLH diagnostic criteria revised (1) by the Histiocyte Society in 2004 (HLH-2004). Five out of the following nine diagnostic criteria for HLH are to be considered for diagnosis: which includes fever, splenomegaly, cytopenia's (affecting two or more of three lineages in the peripheral blood), hypertriglyceridemia, hypofibrinogenemia, elevated ferritin, hemophagocytosis in bone marrow/spleen/lymph nodes, low or absent natural killer (NK)-cell activity, or elevated soluble CD25 (interleukin [IL]-2 receptor;) (3-6). But the patients with an established genetic abnormality (eg., FHL mutations), the diagnosis can be established without meeting the above-mentioned criteria. The 2004 diagnostic criteria for HLH does not apply to MAS (Macrophage activation syndrome) because of the overlap of clinical and laboratory findings between HLH and autoimmune diseases. Modified diagnostic criteria for MAS have been suggested by Ravelli et al., who suggested that a change in baseline laboratory findings as an indication of MAS along with the appropriate clinical symptoms (6,7). In the absence of arthritis, a very high C-reactive protein, high ESR, only moderate cytopenias, reduced erythropoiesis, increased granulopoiesis with a left shift, and a high level of interleukin 1 β might suggest MAS (6,7).

The tests like soluble CD25 levels and NK cell activity were not done in our institution, we considered the haemophagocytes presence in the

bone marrow aspiration and biopsy along with that other criterion like fever, splenomegaly, cytopenias, triglyceride levels elevated ferritin levels and other abnormal lab values including LDH, Abnormal LFT and elevated ESR levels. None of the patients were subjected to genetic testing.

HLH usually presents with variable clinical features, but most of the patients presented with high fever, organomegaly, cytopenias, hemophagocytosis in various organs including bone marrow, spleen, liver and lymphnodes, coagulation abnormalities and some time they present with multiple organ failure or shock. It is considered that the clinical features are due to hypercytokinemia that releases proinflammatory cytokines, such as tumour necrosis factor (TNF)- α , interferon (IFN)- γ , interleukin (IL)-10, IL-12, and IL-18 in large amounts by activated lymphocytes and macrophages. Though there are established diagnostic and therapeutic guidelines for hereditary HLH which have been widely accepted, the causes of secondary (also referred to as "reactive or acquired") HLH are yet to be understood (1-3, 8-13).

The eighteen 18 patients in the current study were thought to have HLH, includes primary and secondary form and they have varied clinical diagnosis. In our study 72 % of cases belonged to paediatric age group and in literature we found other authors reported to have 42% to 68 % of cases belonged to paediatric age group (4,5,14-17). The clinical presentation of the underlying diseases is usually very broad, and is consistent with the results

previously reported in the literature. In the current study, 22.2% patients had hematologic malignancy as compared to the study conducted by Tiong et al., almost half of their patients (47.6%) were having haematological malignancy (12). Whereas study conducted by Mesilla et al., had 27% of cases showed haematological malignancy and in there study ALL cases were predominant. In our study 44.4% patients had infectious diseases, and 22.2% patients had systemic disease like autoimmune disorders, septic shock syndrome and MAS (4-8,12).

The special form of HLH developed in association with autoimmune diseases, which is termed as macrophage-activation syndrome and that was supposed to be the underlying cause in 2- 5% of reactive HLH cases (6,7). In our study 5.6% had Macrophage-activation syndrome and the systemic diseases which include SLE with Evan's syndrome and Juvenile Rheumatoid Arthritis. Most of the authors from their study states that MAS is very frequently associated with systemic diseases or autoimmune diseases like systemic lupus erythematosus, systemic juvenile idiopathic arthritis and AOSD, but they can also be rarely reported to be associated with systemic vasculitis, Sjögren syndrome and systemic sclerosis (7,13-16). In our study one case of pyrexia of unknown origin turned out to be malaria infection in the bone marrow aspirate along with Haemophagocytes. According to the data only 20 patients having HLH secondary to malaria has been reported (5-7). In review of literature malaria and few other parasitic infections including toxoplasmosis, babesiosis, and strongyloidiasis have also been described to be a cause for HLH (9-11).

In our study all the cases showed haemophagocytes in the bonemarrow aspiration, the study conducted by Gupta et al., (14)58% of cases showed haemophagocytes in bone marrow aspirate however they conclude that absence does not exclude the diagnosis of HLH. The ferritin levels were high in nine patients ie., 50% of cases. In most of the studies high ferritin levels were considered as important factor for diagnosis and prognosis. The ferritin levels usually peak more than 11,000 ng/ml at three week and their decrease in levels immediately will have a good prognosis (4,17). There were cases which showed elevated ESR, Abnormal SGOT and SGPT where see in our study. The studies conducted by Parodi et al., and Gupta also shows that the ESR, C-reactive proteins and abnormal liver function test and they were mostly associated with acquired HLH cases (18,19).

CONCLUSION

In conclusion, here we have discussed a single centre experience with the patients presenting with haemophagocytes in bone marrow, along with supporting clinical features and laboratory values. Though a set of criteria are available for the HLH, it

has a broad spectrum of aetiology and varied clinical features. The investigations like CD 25 levels and NK cell activity which are included in the diagnostic criteria are not available in most of the institutions, so a bone marrow aspiration or biopsy revealing hemophagocytes could be helpful to arrive at the diagnosis. From Pathological point of view, documenting each case presenting with haemophagocytes and looking into the factors like age, laboratory values and clinical features will be a valuable tool to come to the diagnosis and might help the clinician to come to a provisional diagnosis and can proceed with the treatment without much delay.

CONFLICT OF INTEREST

Authors declare that there is no conflict of interest in this study.

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