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SECONDARY HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS (HLH): EXPERIENCE FROM A TERTIARY CARE HOSPITAL.

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Abstract: Background: Haemophagocytic lymphohistiocytosis (HLH) is a rare clinical syndrome characterized by fever, hepatosplenomegaly, cytopenia, and progressive multiple-organ failure. Secondary HLH is often triggered by autoimmune diseases, malignancy, or infection.
Aim: To evaluate cases of hemophagocytosis and to identify the triggering diseases in secondary hemophagocytic lymphohistiocytosis(HLH).
Materials and Methods: We evaluated cases of hemophagocytic lymphohistiocytosis over a period of two years in which peripheral smear, bone marrow aspirate, bone marrow biopsy sections and other laboratory diagnostic parameters were studied.
Results: The majority of our patients (96.9%) had secondary HLH as opposed to 3.1% with primary HLH. We observed that infection was present in 68.8% cases of secondary HLH and viral infection was the most common aetiology in 20.3% of cases, followed by 12.5% with typhoid as aetiology, 9.4% with hepatitis C, 7.8% with sepsis as etiology and 7.8% with tuberculosis as an etiology. There were 3 deaths reported in secondary HLH, making the mortality rate 4.8% .
Conclusion: Haemophagocytic lymphohistiocytosis is are and left threatening disease if left untreated. Mortality is high, even among patients who are treated according to the HLH-2004 protocol. Thus, early recognition and treatment of this disorder is essential to decrease associated morbidity and mortality.

Keywords: Hemophagocytic lymphohistiocytosis, Secondary, Etiology.

Introduction:

Haemophagocytic lymphohistiocytosis (HLH) is a rare clinical syndrome characterized by fever, hepatosplenomegaly, cytopenia, and progressive multiple-organ failure (1). Secondary HLH is often triggered by autoimmune diseases, malignancy, or infection (1). The pathogenetic mechanisms behind HLH are not completely understood but involve defective granule-mediated cytotoxicity and uncontrolled T-cell activation, leading to an exaggerated inflammatory response (2), the consequence of which is tissue damage and progressive multiple-organ failure. The spleen, liver, and lungs are the most frequently affected organs (1), but HLH can involve virtually all tissues and organs in the body. In contrast to familial HLH, which often (but not always) presents in paediatric age (3), secondary HLH predominantly occurs in adults. Secondary HLH is arbitrarily divided into three groups depending on triggers and associated diseases. Autoimmune disease-associated HLH is denoted A-HLH, whereas HLH triggered by malignancy and infection is denoted M-HLH and I-HLH, respectively. M-HLH is often associated with haematologic malignancies, and the annual incidence of M-HLH in Sweden is less than 0.4 in 100,000 (4). I-HLH is predominantly triggered by Epstein–Barr virus (EBV) or cytomegalovirus (CMV) (1). Bacterial infections are reported less frequently than viruses as HLH triggers, and mycobacteria dominate among bacteria associated with I-HLH (5).
According to the HLH-2004 Protocol A diagnosis of HLH can be made if either criteria 1 or 2 is met:

1. Molecular diagnosis consistent with HLH
2. Clinical and laboratory criteria (at least 5/8 criteria should be fulfilled) –
Fever , Splenomegaly , Cytopenia ≥2–3 cell lines in peripheral cell lines, Hypertriglyceridemia and/or hypofibrinogenemia (fasting triglycerides ≥3.0 mmol/L, fibrinogen ≤1.5 g/L) , Hemophagocytosis in bone marrow, spleen, cerebrospinal fluid(CSF), or lymph nodes, No sign of malignancy , Decreased or absent NK-cell activity (according to local laboratory reference) , Ferritin ≥500 μg/L , sCD25 (soluble IL-2–receptor) ≥2,400 U/mL.
- Supportive evidence includes: Cerebral symptoms with moderate pleocytosis and/or elevated protein ,Elevated transaminases, Elevated bilirubin, Elevated LDH .
Newer laboratory data have recently shown the utility of monitoring levels of soluble hemoglobin scavenger receptor (sCD163) in patients with HLH. CD163, a receptor for hemoglobin-haptoglobin complexes, is a marker for the activation of alternative pathway scavenger macrophages. Levels of this molecule in HLH patients are markedly higher than those found in patients with malignancy, infections, and autoimmune conditions.(7) Thus, monitoring levels of sCD25 and sCD163 can be effective in tracking HLH disease activity.(8)
Left untreated, the prognosis of HLH is poor and generally fatal. Therefore, prompt recognition and timely treatment are critical. Mortality in secondary HLH has been reported to vary from 8–22% in rheumatologic HLH to 18–24% in EBV HLH.(6)

Material and methods: Complete blood count and peripheral blood smear examination was performed. Bone Marrow Aspiration and Bone Marrow Biopsy studies and other ancillary investigations such as USG, cytogenetics and biochemistry were performed.

STAINS USED: Leishman stain was used for peripheral blood film and Bone Marrow Aspiration staining while as hematoxylin and eosin method was used for staining bone Marrow Biopsies. • Edta-k2 vacutainer was used for complete blood counts. • Salah's needle 16G was used for aspiration. • Jamshidi's needle 11G was used for doing biopsies.
Written and informed consent was taken from patients prior to Bone Marrow Aspiration and Bone Marrow Biopsy procedures. All the questions were answered and patients were given opportunities to sign the written consent form. Clinical findings such as fever, organomegaly along with biochemical parameters like serum ferritin, serum triglycerides, and fibrinogen level was assessed. Slides were prepared and evaluated for proper bone marrow examination and reporting with special emphasis on demonstration of Hemophagocytosis of Marrow. Increase in Reticuloendothelial activity was established with demonstration of hemophagocytosis by the marrow Macrophages. Hemophagocytosis was also graded on microscopy.

STATISTICAL METHODS: The recorded data was compiled and entered in a spreadsheet (Microsoft Excel) and then exported to data editor of SPSS Version 20.0 (SPSS Inc., Chicago, Illinois, USA). Continuous variables were expressed as Mean±SD and categorical variables were summarized as frequencies and percentages. Graphically the data was presented by bar diagrams.

ETHICAL ISSUES: Ethical clearance was taken from institutional ethical committee.

Results: A total of 64 patients were seen over a period of two years. 62 cases out of 64 cases were of secondary hemophagocytsois(HLH).
In our study mean age was (41.5±19.74) years, in secondary HLH , there was a predominance of male patients, over females (65.6% vs. 34.4%). The most frequent clinical manifestation in the study subjects was fever (100%), which was followed by splenomegaly (87.5%), bicytopenia (73.4%), hypertriglyceridemia (60.9%), hyperferritinemia (48.4%), hypofibrinogenemia (32.8%), and pancytopenia (26.6%). Bone marrow examination showed (82.2%) cases had hemophagocytosis on

bone marrow aspirate only, followed by 17.2% with bone marrow aspirate and biopsy both showing haemophagocytosis (TABLE: 1)

TABLE 1: Clinical and laboratory findings of patients in hemophagocytic lymphohistiocytosis (HLH)

Patient characteristics	Hemophagocytic lymphohistiocytosis (HLH) -N = 64
Age(Mean)	(41.5±19.74) years.
Male/Female (%)	65.6% vs. 34.4%)
Fever	64/64 (100%)
Bicytopenia	56/64 (87.5)
splenomegaly	47/64 (73.4)
Hypertriglyceridemia	38/64 (59.3)
Hypofibrinogenemia	21/64 (32.8)
Hyperferritinemia	31/64 (48.4)
Pancytopenia.	17/64 (26.5)
Hemophagocytosis on bone marrow	64/64 (100%)
Infections	44 (68.7%)
autoimmune	7 (10.9%)
Malignancy	1 (1.5%)
Others(Primary HLH and deficiency anaemias)	12 18.7%)

When patients were assessed according to the aetiology, we found that viral infection was the most common aetiology in 20.3% of cases, followed by 12.5% with typhoid as aetiology, 9.4% with hepatitis C, 7.8% with sepsis as etiology and 7.8% with tuberculosis as an etiology. 3 patients out of 62 patients with se died, making the mortality rate 4.8% in secondary HLH.(TABLE 2).

TABLE 2: Showing aetiology of study cases.

Etiology	Number	Percentage
Viral infection	13	20.3
Typhoid	8	12.5
Hepatitis C	6	9.4
Sepsis	5	7.8
Tuberculosis	5	7.8
Dengue	2	3.1
Pneumonia	2	3.1
Hepatitis A	1	1.6
Infectious mononucleosis	1	1.6
Malaria	1	1.6
Dual deficiency anemia	4	6.3
Iron deficiency anemia	3	4.7
Rheumatoid arthritis	3	4.7
Vitamin B12 deficiency	2	3.1
Primary HLH	2	3.1
Colliod nodule	1	1.6
Idiopathic thrombocytic purpura	1	1.6
Non specific colitis	1	1.6
Multiple myeloma	1	1.6
Pustular psoriasis	1	1.6
Splenic haemangioma	1	1.6
Total	64	100

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Discussion:

In the present study, on evaluating the spectrum of presentations of haemophagocytic syndrome and correlating clinical and hematopathological features, we have comprehensively analysed patients' data on the basis of demographic aspects, clinical parameters, etiological consideration and hematopathological features. We observed that with an average age of (41.5±19.74) years, the age of studied patients was ranging from 10 months to 85 years. The majority of patients (39.1%) were belonging to the age group of 46-65 years, followed by 35.9% belonging to the age group of (19-45) years, 17.2% patients were ≤ 18 years, and 7.8% patients were above 65 years of age. The clinical presentation among the studied patients was assessed, we found that the most frequent clinical manifestation was fever (100%), which was followed by splenomegaly (87.5%), bicytopenia (73.4%), hypertriglyceridemia (60.9%), hyperferritenemia (48.4%), hypofibrinogenemia (32.8%), and pancytopenia (26.6%). The commonest manifestation fever has been document by a multitude of scholars (Melissa et al, Iqbal et al, Li et al, and Sundari et al).[9, 10] Melissa et al, and Li et al, in their studies reported that almost 100% of HLH cases presented with fever while as Iqbal et al reported that 65.2% of cases presented with fever. [11] These results essentially infer that fever is the predominant clinical manifestation among patients with HLH. Antibiotics frequently have no effect on protracted, persistent fevers.. Broadly, HLH can be classified according to the underlying etiology into either primary (genetic) or secondary (acquired) HLH, although in practice this distinction is often difficult because the symptomatic presentations of primary and secondary HLH overlap significantly. The main cause of primary HLH is genetic abnormalities that impair the normal function of NK cells. Any gene that contributes to the creation, movement, or export of perforin granules that are used by NK cells to lyse target cells could be the culprit. Secondary HLH can result from a wide range of conditions, including infections, autoimmune diseases, and cancer. In the present study, majority of our patients (96.9%) had secondary HLH as opposed to 3.1% with primary HLH, which is consistent with the study of Sundari et al, who reported primary HLH in 3% of the cases compared to 97% cases of secondary HLH.[12] .When patients were assessed according to the aetiology of HLH, we found that infection etiology was found in 68.8%, of them; viral infection was the most common aetiology in 20.3% of cases, followed by 12.5% with typhoid as aetiology, 9.4% with hepatitis C, 7.8% with sepsis as an aetiology, and 7.8% with tuberculosis as an aetiology. The other less common etiologies observed were colliod nodule, idiopathic thrombocytic purpura, non-specific colitis, multiple myeloma, pustular psoriasis, and splenic haemangioma. Melissa et al, in their study reported that 50% of their patients had infection associated HLH while as Sundari et documented 70% of their cases had infection associated HLH, which is very much similar to our observation.[13] as an aetiology, and 7.8% with tuberculosis as an aetiology. The other less common etiologies observed were colliod nodule, idiopathic thrombocytic purpura, non-specific colitis, multiple myeloma, pustular psoriasis, and splenic haemangioma. Melissa et al, in their study reported that 50% of their patients had infection associated HLH while as Sundari et documented 70% of their cases had infection associated HLH, which is very much similar to our observation.[13] While Melissa et al, found an increased frequency of viral infections contributing to HLH, Chandra et al found equal presentations of bacterial and viral illnesses.[14] Sundari et al, reported in their study that viral infection was the commonest etiology found in 28% of their patients with HLH and 15% of their patients had typhoid associated HLH cases, which is compatible with our results. IAHS, also known as infection-associated hemophagocytic syndrome (IAHS), has been found to account for nearly 50% of all identified cases of HLH.[15] Epstein-Barr virus (EBV) is most frequently linked to secondary HLH, but other significant tropical illnesses, including as tuberculosis, malaria, leishmaniasis, and typhoid, can also cause IAHS, particularly in the subcontinent.[15] Controlling and eradicating infections that are endemic to developing nations has always been difficult, especially in Asia. The issue seems to be exacerbated by poor sanitation, overcrowding, and tainted water. This is a cause for concern because many of these illnesses, like typhoid fever, can be made worse by secondary HLH, an uncommon but serious illness with a fatal outcome. In the present study; out of 64 patients with

HLH, five patients expired, making the overall mortality rate of 7.8%. Three patients were adults who died from sepsis, making the adult mortality rate 5.66%. Two of the patients had primary HLH and were under the age of 18. According to our data, the mortality rate for HLH in kids was 18.18%, which is in line with other studies that have found that the mortality rate for children varies from 13% to 28%. According to reports, sepsis, haemorrhage, or multiorgan failure are the leading causes of mortality in HLH, which is consistent with our findings.[16]

Declaration of conflicts of interest: Authors have no conflicts of interest.

Conclusion: Our study tries to raise the awareness of the clinicians for this underdiagnosed condition that leads to multiorgan failure and lethal complications and, therefore, it should be considered as a medical emergency with immediate treatment.

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