Hemophagocytic histiocytosis: A Clinicopathological correlation

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ABSTRACT

Objectives: Histiocytic hyperplasia with hemophagocytosis (HP) is relatively uncommon condition that has often been mistaken in the past for neoplastic disorders. This study was conducted to investigate the possible etiology of HP, its intensity in the bone marrow (BM), and also its effect on hematological parameters with the extent of disease activity.

Methods: Blood samples were collected and BM examination was performed in 250 patients with varied etiology showing HP. Complete blood counts, reticulocyte count, and red blood cell morphology were determined. HP was examined in the BM smears by Leishman staining. The severity of HP was determined by grading of its intensity in the BM smears.

Results: Our data showed variable degree of HP (mild, moderate, and severe) in the BM smears of patients having different underlying disorders. HP syndrome (HPS) with clinical and biochemical derangements was found in 24 (9.6%) patients. HPS was mostly associated with infection. The etiological distribution in different group of disorders was nonmalignant hematological conditions (56.80%), infections (24.80%), storage disorders (4.40%), malignant hematological conditions (4.40%), autoimmune disorders (1.20%), and miscellaneous group (8.40%). Distribution of patients in different grades of intensity of HP was Grade I (35.50%; mild), Grade II (45.50%; moderate), and Grade III (19.60%; severe).

Conclusion: We conclude that severe degree of HP has profound effect on hematological parameters particularly hemoglobin and platelet counts. This phenomenon may present as HPS with fatal outcome. We also conclude that there was no effect of age on either intensity of HP or on blood counts.

Keywords: Hemophagocytic syndrome, hemophagocytosis, histiocytosis, mononuclear phagocyte system

Introduction

Histiocytic hyperplasia with hemophagocytosis (HP) is relatively uncommon condition that has often been mistaken in the past for neoplastic disorder.[1] The histiocytosis comprises a group of disorders of multiple etiologies involving cells of monocyte/macrophage system.[1,2] The disorders showing histiocytosis can be broadly classified into two categories, reactive (benign) and nonreactive (malignant). Although these disorders may have similar clinical features which include pyrexia, wasting, generalized lymphadenopathy hepatosplenomegaly with or without pancytopenia, the histopathologic findings vary from the proliferation of apparently benign histiocytes to cells that are obviously malignant.[3,4] In disorders with histiocytic proliferation, very often an element of HP is observed. A syndrome of exaggerated histiocytic proliferation and activation with HP has

been defined as HP syndrome (HPS). The syndrome is usually associated with systemic viral infections[4,5] but it can also occur with bacterial, parasitic, and fungal infections.[5] In histiocytic disorders particularly showing HP, there is an intricate interplay between macrophages and T-lymphocytes.[6] T-lymphocytes produce cytokines that influence macrophage migration, cytotoxicity, and secretary function as well as complement system. Macrophages, on the other hand, synthesize substances which promote proliferation of T-cells and their activation. The ultimate result is exaggerated phagocytosis of blood cells.[6,7]

HP is phagocytosis of all blood cells, mature and developing by activated histiocytes of mononuclear phagocyte system.[8,9] Histiocytic proliferation frequently shows an element of HP. It is reported that under various diagnostic terms, such as histiocytic medullary reticulosis, malignant histiocytosis, and infection

associated HP.[8,9] HP with or without histiocytic proliferation has been reported in several clinical situations with fatal outcome as well as in transitory benign disorders.[9] It may result from immunologic activation of the mononuclear phagocyte system (reactive) or may be due to a neoplastic proliferation of histiocytes (malignant), and sometimes this may occur as a result of genetic or chromosomal derangements.[8-10] HPS is a clinic-pathologic entity characterized by increased proliferation and activation of macrophages and T lymphocytes with HP.[10] In our previous report, we determined the etiology and grading of HP associated with infection and its effect on hematological parameters, and to correlate it with the clinicopathological effects of HPS.[11] Our previous data pointed out that those patients with increased intensity of HP in the bone marrow (BM) had profound effect on hematological parameters; particularly hemoglobin (Hb) and platelet count, resulting in the depression of these formed elements.[11] We and others have concluded that viral, bacterial, and parasitic infections play an important role in the causation of histiocytic hyperplasia with HP.[1,11] Severe intensity of HP has a profound effect on hematological parameters of patients particularly on Hb level, total leukocyte count and platelet count with a drastic decrease in all of the parameters during the severe disease course.[11] Now it is known that HPS may also be caused by a massive release of cytokines from activated T cells and macrophages.[11-13] In spite of numerous reports of the occurrence of HP and HPS particularly in various benign disorders, the literature is scanty on its correlation with BM function and effect of its intensity on hematological parameters. This study was undertaken to find out the etiological agents of HP and to correlate the effect of this phenomenon on hematological parameters and BM function.

Methods

Human subjects

With the Institutional Ethical Committee approval, this study was conducted and was carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) for human samples.

Complete blood counts, absolute values, reticulocyte count, and red blood cell morphology

Cell counts were performed by hematology analyzer (Sysmex KX-21). Slides for the peripheral film were stained by Leishman stain (Sigma, St-Louis, USA). In the light of history, physical findings and findings in blood and BM one or more of the following investigations were carried out to determine the etiology: Viral screening, Widal test, liver function tests, coagulation profile, antinuclear factor, and BM culture for microbes.

BM aspiration and trephine biopsy

In the study, variable degree of HP was observed in the BM smears of 250 patients having different underlying disorders.

BM aspiration slides (Leishman stained) were systematically examined, under low power as well as under oil immersion lens, for following parameters: Cellularity of BM, cellularity and maturation of erythropoiesis, cellularity and maturation of myelopoiesis, megakaryocytes: Number and maturity, lymphocytes, plasma cells, abnormal cells, histiocytes/macrophages and HP, and parasites.

Grading of HP intensity

Grading of intensity of HP was performed on BM aspiration smears. The slides were mounted and scanned under low power ($\times 10$) in at least 10 random fields for presence or absence of HP. Slides were then scanned and examined under high power (high dry $\times 40$) to confirm the presence of HP. If HP was seen, it was graded in the following manner. The number of HP cells was counted in 10 random fields and average of such cells per high power field (HPF) was calculated. Following criteria were used to grade the intensity.

Grading	Number of hemophagocytic cell/HPF
Grade I (mild)	1-3
Grade II (moderate)	4-6
Grade III (severe)	7-10
Grade IV (very severe)	>10

Statistical analysis

Mean values and standard error were determined. *t*-test was applied to determine statistically significant difference between mean of cell counts in different grades of HP. Chi-square analysis was used to determine the significant difference in the number of male and female patients.

Results

Age and sex distribution in patients of HP

In this study, a total number of patients showing variable degree of hemophogocytosis in the BM smears was 250. Age of the patients ranged between 3 months and 76 years. Their mean age was 30.8 ± 1.20 years. The difference between number of male and female patients was not significant ($P > 0.05$) according to $\chi^2 = 2.304$ (Table 1).

Clinical features

Symptoms
Fever was the most common symptom at the time of presentation in patients of HP. It was documented in 163 (65.20%) patients, with variable degree. Generalized weakness was the next common symptom occurring in 139 (55.60%) patients, followed by diarrhea, 78 (31.20%) cough, 62 (24.80%) and bleeding in 57 (22.80%) patients in the form of epistaxis, purpuric spots, bruises, and bleeding per rectum.

Physical findings

Pallor was found in the majority of patients, 219 (87.60%). Splenomegaly was present in 93 (37.20%), hepatomegaly in 79 (31.60%), and lymphadenopathy in 54 (21.62%) patients. Jaundice was found in 31 (12.40%), bruises and purpuric spots in 29 (11.60%), and ascites 15 (6%) patient. Table 1 shows hematological parameters in patients of HP. Table 2 shows etiological breakup and intensity of HP in the BM. Figures 1-3 show phenomena of hemophagocytocysis in various disorders.

HPS

Out of 250 cases of HP in this study, 24 (9.6%) patients presented as HPS having varied underlying disorders. Most of these patients had severe (Grade III) degree of HP, that is, 19 patients, followed by patients with moderate intensity (Grade II). None of the patient had mild (Grade I) intensity (Table 3). All of these patients of HPS had pancytopenia/bicytopenia, organomegaly, and most of the clinic-pathological and biochemical derangements attributed to HPS, such as liver dysfunction, hyperferritinemia, coagulopathy, with BM histiocytic hyperplasia, and HP.

Mean Hb, total leukocyte count (TLC), platelet count according to grades of intensity HP

Mean Hb (g/dl) in Grade I, II and III was 8.613 ± 0.31, 7.34 ± 0.19 and 6.37 ± 0.31, respectively. Mean TLC ($\times 10^9$/L) was 7.99 ± 0.92, 5.74 ± 0.31 and 5.09 ± 0.51 respectively, according to grades of intensity. Mean platelet ($\times 10^3$/µl)

count was 168.07 ± 14.32, 115.64 ± 7.74 and 81.55 ± 5.39, respectively. As a result of increased intensity of HP in Grade III group mean Hb (6.37 g/dl) and platelet count (81.55×10^3/µl) were low as compared to other groups. The mean Hb of Grade II was significantly decreased as compared to mean of Grade I ($P < 0.01$), the mean Hb of Grade III was significantly decreased as compared to Grade I ($P < 0.001$), and Grade II ($P < 0.01$). In case of TLC, the mean of Grade II

Table 1: Hematological parameters in patients of hemophagocytosis

Hemoglobin: g/dl		
Mean	7.60±0.16	
Range	2.7-17.6	
	Hb	**Number of patients (%)**
Severe anemia	<6	74 (29.60)
Mild to moderate	7-12	165 (66.00)
Normal	13-18	11 (4.40)
Leukocyte count: ×10⁹/L		
Mean	6.42±0.37	
Range	1.1-78	
	TLC	
Leukopenia	<4	85 (34.00)
Leukocytosis	>12	26 (10.40)
Normal	4-12	139 (55.60)
Platelet count: ×10³/ul		
Mean	127.42±6.54	
Range	3.0-619	
	Platelets	
Severe	<10.0	06 (2.40)
Mild to moderate	10-150	175 (70.00)
Normal	150-450	64 (25.60)

Hb: Hemoglobin, TLC: Total leukocyte count

Table 2: Etiological distribution with grades of intensity of hemophagocytosis

Etiology	*n* (%)		
	Grade I	**Grade II**	**Grade III**
NMHC (*n*=142)	51 (20.4)	64 (25.6)	27 (10.8)
Aplastic anemia	06 (2.4)	3 (1.2)	-
IDA	10 (4.0)	4 (1.6)	-
IDA, MBA	6 (2.4)	8 (3.2)	2 (0.8)
EMHypo	2 (0.8)	2 (0.8)	-
Hemolytic anemia	8 (3.2)	4 (1.6)	-
ITP	6 (2.4)	5 (2.0)	3 (1.2)
MBA	9 (3.6)	31 (12.4)	21 (8.4)
MDS	1 (0.4)	3 (1.2)	1 (0.4)
Mega hypo	1 (0.4)	-	-
Polycythemia	2 (0.8)	-	-
SA	-	1 (0.4)	-
TTP	-	1 (0.4)	-
Leo	-	1 (0.4)	-
CDA	-	1 (0.4)	-
MHC (*n*=11)	7 (2.8)	3 (1.2)	1 (0.4)
ALL	1 (0.4)	-	-
AML	(0.4)	-	-
CML	1 (0.4)	1 (0.4)	-
HD	1 (0.4)	1 (0.4)	-
MM	1 (0.4)	-	-
NHL	2 (0.8)	1 (0.4)	1 (0.4)
Infections (*n*=62)	10 (4.0)	31 (12.40)	21 (8.40)
Storage disorders (*n*=11)	8 (3.2)	3 (1.2)	-
Gaucher's disease	6 (2.4)	2 (0.8)	-
Niemann pick disease	2 (0.8)	1 (0.4)	-
Miscellaneous (*n*=21)	12 (4.8)	9 (3.6)	-
Carcinoma stomach	1 (0.4)	-	-
CHS	-	1 (0.4)	-
CUD	6 (2.4)	4 (1.6)	-
Hypersplenism	4 (1.6)	3 (1.2)	-
Lead poisoning	1 (0.4)	-	-
Sarcoidosis	1 (0.4)	-	-
SLE (*n*=3)	-	3 (1.2)	-

IDA: Iron deficiency anemia, MBA: Megaloblastic anemia, EM Hypo: Erythroid megakaryocytic hypoplasia, ITP: Idiopathic thrombocytopenic purpura, MDS: Myelodysplastic syndrome, SA: Sideroblastic anemia, TTP: Thrombotic thrombocytopenic purpura, Leo: Lofflers eosinophilia, CDA: Congenital dyserythropoietic anemia, ALL: Acute lymphoid leukemia, AML: Acute myeloid leukemia, CML: Chronic myeloid leukemia, CHS: Chidiakhigashi syndrome, HD: Hodgkins disease, MM: Multiple myeloma, NHL: Non-hodgkins lymphoma, CUD: Chronic underlying disorders, SLE: Systemic lupus erythematosus
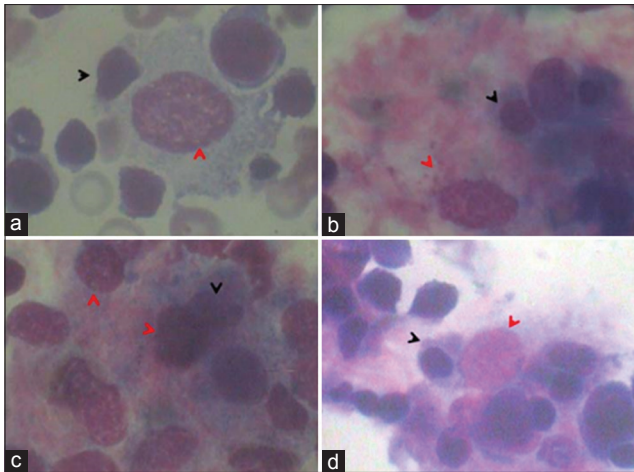
Figure 1: Micrographs showing phenomenon of hemophagocytosis in viral infection, enteric fever, tuberculosis and hemolytic anemia. (a) Viral infection: Micrograph of a bone marrow (BM) smear showing HP in a case of EBV infection. A large macrophage (red arrow) ingested blood cells (black arrow), (b) enteric fever: Hemophagocytosis in a case of enteric fever. Activated macrophage (red arrow) ingesting myeloid and erythroid cells (black arrow), (c) tuberculosis: BM blood cells (black arrow) phagocytosed by a large macrophage (red arrow) in a case of pulmonary tuberculosis, (d) hemolytic anemia: A case of hemolytic anemia showing mainly erythrophagocytosis (black arrow) by a macrophage (red arrow). Leishman stained BM smear high power field oil immersion
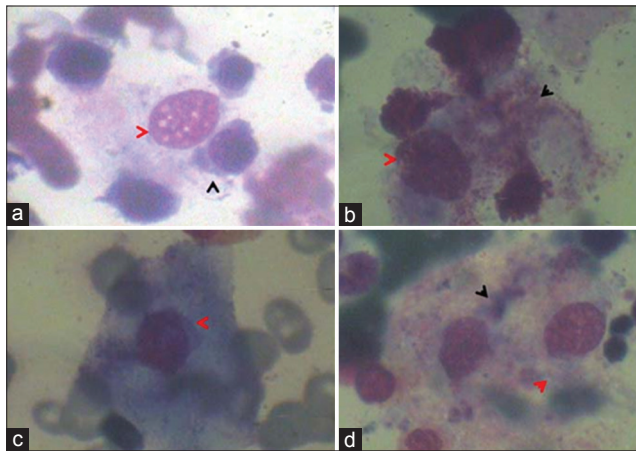

Figure 2: Micrographs showing phenomenon of hemophagocytosis in brucellosis, V. leishmaniasis, gauchenteric fever and hemolytic anemia, (a) Brucellosis: Bone marow macrophage (red arrow) ingesting blood cells (black arrow) in a case of brucellosis, (b) V. leishmaniasis: Bone marrow (BM) smear of visceral leishmaniasis. Macrophage (red arrow) ingesting cells and cellular debris (black arrow) and some amastigotes of parasite, (c) gauchenteric fever: Sea blue histiocyte (red arrow) in a case of gauchers disease, (d) hemolytic anemia: Two activated macrophages (red arrow) ingesting cellular debris and malarial pigment (black arrow) in a case of Falciparum malaria. Leishman stained BM smear high power field oil immersion

was significantly decreased as compared to Grade I ($P < 0.05$). Mean TLC of Grade III was significantly decreased as compared to Grade I ($P < 0.01$), while the difference of mean was not significant between Grade II and III ($P > 0.05$). The mean platelet count of Grade II was significantly decreased

as compared to Grade I ($P < 0.01$), the mean platelet count of Grade III was significantly reduced as compared to Grade I ($P < 0.001$) and Grade II ($P < 0.001$). This study thus reveals that the main effect as a result of increasing intensity of HP affected the levels of Hb and platelet count which has been proved statistically. Table 4 shows mean values of blood counts in patients showing HP with different grades of intensity.

Discussion

In this study, we analyzed the variable degree of HP in 250 patients subjected to BM examination having diverse underlying etiologies. Despite many case reports on existence of hemophogocytosis in various benign and malignant disorders, the literature is, however, scanty on correlation between intensity of HP and its effect on hematological parameters.[14,15] This study deals with etiology of HP and the effect of intensity of this phenomenon on hematological parameters. Here, we showed variable degree of reactive HP that is, mild, 88 (35.20%), moderate, 113 (45.20%), and severe, 49 (19.60%). In other words, the majority of our patients had moderate or Grade II intensity of HP. The study done by Strauss *et al.*[14] Shows that those patients with mild HP were more as compared to moderate intensity patients. Most of the patients belonged to nonmalignant hematological conditions (NMHC) 142 (56.6%) followed by infections, 62 (24.8%); storage disorders, 11 (4.4%); malignant hematological conditions (MHC), 11 (4%); autoimmune disorders, 3 (1.2%); chronic underlying disorders, 10 (4.0%); hypersplenism, 7 (2.80%). These patients showed variable degree of HP in the BM, that is, mild, moderate, and severe, as a reactive phenomenon. None of these patients had malignant histiocytosis. As mentioned earlier NMHC ranked on the top in the list of etiology showing HP in the BM. Megaloblastic anemia (MA) was the most common cause in this category 61 (24.4%) followed by patients of mixed deficiencies, 16 (6.4%); idiopathic thrombocytopenic purpura, 14 (5.6%); hemolytic anemia, 12 (4.8%); iron deficiency anemia, 14 (5.6%) aplastic anemia, 9 (3.6%); and myelodysplastic syndrome, 5 (2.0%). Other important conditions, in this group, were polycythemia, megakaryocytic hypoplasia, sideroblastic anemia, and thrombotic thrombocytopenic purpura. Most of the patients of NMHC group showed Grade II (moderate) intensity of HP 64 (25.6%). Rest of the patients showed either mild 51 (20.4%) or severe 27 (10.8%) intensity. MA deserves special mention. It was the most frequent underlying disorder in NMHC group. Patients with MA either showed moderate 31 (12.4%) or severe 21 (8.4%) degree of HP. Three patients presented as HPS. Only 9 (3.6%) patients had mild intensity of phagocytosis.

We have studied the morphological features of MA in the BM and emphasized the importance of HP along with dyserythropoiesis as an important cause of pancytopenia.[15] In another study, regarding the etiological break up of pancytopenia, we also highlighted the significance of HP in the BM in patients of MA[17] and suggested that MA with HP should
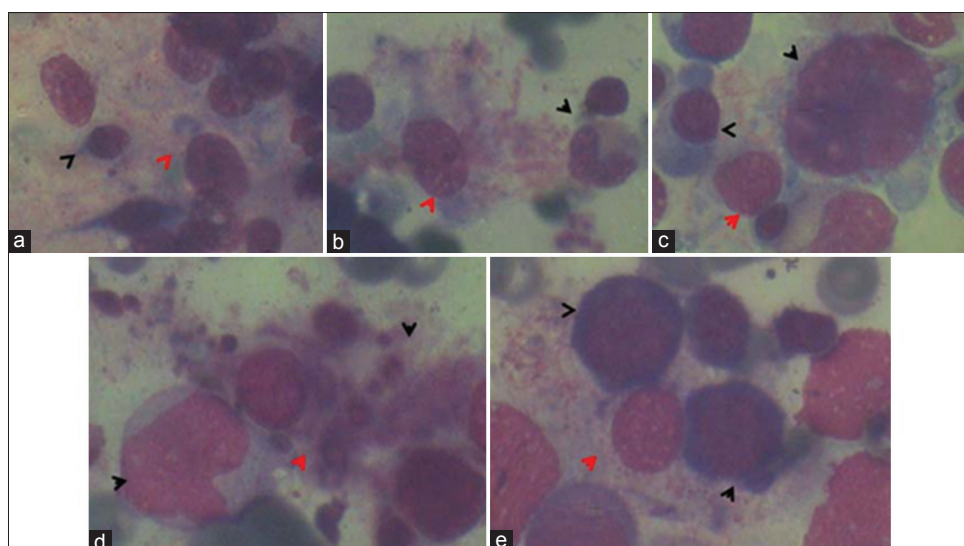
Figure 3: Micrographs showing phenomenon of hemophagocytosis in iron deficiency anemia, systemic lupus erythematosus (SLE), acute leukemia, megaloblastic anemia and multiple myeloma, (a) Iron deficiency anemia: Large activated macrophage (red arrow) engulfing blood cells (black arrow) in a case of iron deficiency anemia, (b) SLE: Macrophage (red arrow) in a process of engulfing a myeloid cell (black arrow), (c) acute leukemia: A large blast cell (black arrow) and cellular debris being engulfed by a macrophage (red arrow) in a case of acute leukemia, (d) megaloblastic anemia: Megalablasts (black arrows) phagocytosed by a large macrophage (red arrow), (e) multiple myeloma: An activated macrophage (red arrow) ingesting a plasma cells (black arrows) in a case of multiple myeloma. Leishman stained bone marrow smear high power field oil immersion

Table 3: Etiological break up of hemophagocytic syndrome *n*=24 (9.6%)

Etiology	Number (%)	Grade I	Grade II	Grade III
Infections	18 (7.2)			
Viral	9	-	2	7
Enteric fever	3	-	1	2
Tuberculosis	3	-	-	3
Malaria	2	-	-	2
V. leishmaniasis	1	-	1	-
NMHC	4 (1.6)	-		
MBA	3	-	1	2
MDS	1	-	-	1
NHL	1 (0.4)	-	-	1
Autoimmune (SLE)	1 (0.4)	-	-	1

N: Number of patients, NMHC: Nonmalignant hematological conditions, MBA: Megaloblastic anemia, MDS: Myelodysplastic syndrome, NHL: Non-Hodgkins lymphoma, SLE: Systemic lupus erythematosus

Table 4: Mean Hb, TLC, platelet count in different grades of HP

Grades	Hb, g/dl	TLC×10⁹/L	Platelet count×10³/μl
I	8.61±0.31	7.99±0.92	168.07±14.32
II	7.34±0.19a**	5.74±0.31a*	115.64±7.74a**
III	6.37±0.31b***c**	5.09±0.51b**	81.55±5.39b***c***

Grade I versus II (a), *P<0.05, Grade I versus III (b), **P<0.01, Grade II versus III (c), ***P<0.001, Hb: Hemoglobin, TLC: Total leukocyte count

be included in the list of causes of pancytopenia and HPS in the standard textbooks of hematology and other scientific literature which is so far quite scanty. What triggers this phenomenon, particularly in cases of MA, is still not known.

Perhaps dyserythropoiesis with exaggerated intramedullary destruction of BM precursor cells stimulates monocyte-macrophage system, along with T lymphocytes resulting in elaboration of cytokines. It is suggested that role of cytokines and BM microenvironment should be extensively studied which results in increased frequency of this phenomenon in the BM particularly in patients of MA.

In this study, patients with different underlying infections were the second largest group resulting in reactive histiocytic hemophagocytosis, 62 (24.80%) with different grades of intensity. 17 patients with viral infection showed variable intensity of HP. These results have also been supported by other reports which showed HPS association with viral agents including herpes simplex, Epstein-Barr virus (EBV), cytomegalovirus (CMV), HIV, and parvovirus B19.[16,18,19] Our data showed that out of 17 patients of viral infection, 10 patients showed Grade II, and 4 had Grade III intensity of HP. Only three patients had mild HP. In other words, majority of the cases with viral infection had increased severity of HP. Viral screening was positive mainly for EBV, CMV, adenovirus, and rubella virus. HPS was found in 18 patients. All of these patients presented with bicytopenia or pancytopenia. Hepatosplenomegaly, liver dysfunction, hyperferritinemia, lipid derangement, and coagulation disturbance were other findings of HPS. The BM was mainly hypercellular or normocellular in these cases of HP. The macrophages in the BMs of all these patients were benign looking with low N/C ratio, inconspicuous nucleoli and abundant cytoplasm. Erythrophagocytosis was mainly present along with phagocytosis of leukocytes and platelets in some of the patients. 8 (3.2%) patients of malaria were diagnosed in this study. Three patients showed Grade II and

five patients had Grade III HP. Four patients had vivax malaria, two had falciparum infection, and two patients had mixed infection. Two patients of falciparum infection with Grade III intensity presented as HPS having peripheral cytopenia, hepatosplenomegaly, liver dysfunction, and hyperferritinemia. BM in both the patients was hypercellular with depression in erythroid series. HPS in association with malaria was first time reported in Pakistan by Anwar *et al.*[20] The presence of HP in malaria has been sparingly reported in the literature, perhaps because of its uncommon occurrence in the west.[21] In this study, two patients of falciparum malaria had HPS and pancytopenia. Whereas, 11 patients of MHC were diagnosed. Most of the patients had Grade I HP, 7 (2.8%). Only 3 (1.2%) had moderate and, 1 (0.4%) had severe degree of HP. The need for applying standard cytologic criteria of malignancy in the diagnosis of histiocytic proliferations was well reported.[22,23] Two patients of acute leukemia (acute lymphoid leukemia [ALL] and acute myeloid leukemia [AML]) were diagnosed in this study showing reactive HP of mild intensity in the BM. Four patients of non-Hodgkins lymphoma (NHL) with variable degree of HP were also diagnosed in the study. A case of NHL with Grade III intensity presented as HPS. Two patients of chronic myeloid leukemia and Hodgkins disease each were also present in this category. Liang *et al.*[24] reported three patients of ALL and two patients of NHL showing reactive hemophagocytic histiocytosis with HPS. Imashuku *et al.*[25] reported seven patients of AML showing HP by leukemia blast cells with translocation, t (p11, q22).[17,25] Most of the investigators whose findings have been cited above suggested that histiocytic medullary reticulosis like syndrome developing in patients with hematological malignancies was reactive to an underlying opportunistic infection (viral, bacterial, and fungal) in the presence of immunosuppressive state due either to disease process, itself and or chemotherapy. It is presumed that infiltration of malignant cells in the BM, hypersplenism, underlying infections with immunosuppression, hypercytokinemia with histiocytic proliferation, and prominent HP could be the cause of peripheral cytopenia in MHC. Relatively less number of MHC patients in this study could be due to the fact that not many patients with underlying malignancies are referred to the hospital where the study was conducted, for BM examination. It is an important to distinguish reactive histiocytosis with HP from malignant histiocytosis complicating a preexisting malignancy, because chemotherapy is deleterious in former patients, while specific antimicrobial, anticytokine therapy, plasmapheresis, and supportive treatment may save.[26]

This study reveals that the majority of the patients had increased severity of HP in the BM. Grade II and Grade III patients together were (64.80%) as compared to Grade I HP (35.50%) patients with mild intensity. Most of the patients of Grade II and III either belonged to NMHC or infection. According to the study of Strauss *et al.*[14] To identify the risk factors of HP, majority of the patients had mild (35.2%) degree of HP, followed by moderate (27.9%) intensity. In this study, however, patients with moderate intensity of HP were in majority (45.2%). HPS

was found to be associated with 24 (9.6%) patients, mostly with different underlying infections showing Grade III intensity in majority of patients. Such patients presented with peripheral cytopenia in addition to other clinicopathological and biochemical derangement. Pancytopenia was present in 78 while bictyopenia was found in 92 patients. Patients showing pancytopenia or bicytopenia mostly belonged to Grade II or Grade III groups of HP. As a result of increased intensity of HP in Grade III group mean Hb (6.37 g/dl) and platelet count (81.55×10^3/L) were low as compared to other groups. The mean Hb of Grade II was significantly decreased as compared to mean of Grade I ($P < 0.01$), the mean Hb of Grade III was significantly decreased as compared to Grade I ($P < 0.001$), and Grade II ($P < 0.01$). In case of TLC, the mean of Grade II was significantly decreased as compared to Grade I ($P < 0.05$). Mean TLC of Grade III was significantly decreased as compared to Grade I ($P < 0.01$), while the difference of mean was not significant between Grade II and III ($P > 0.05$). The mean platelet count of Grade II was significantly decreased as compared to Grade I ($P < 0.01$), the mean platelet count of Grade III was significantly reduced as compared to Grade I ($P < 0.001$) and Grade II ($P < 0.001$).

This study thus reveals that the main effect as a result of increasing intensity of HP was on Hb and platelet count which has been proved statistically. The mechanism of peripheral cytopenia has been studied by various workers in HP patients in relation to different underlying disease processes. Ohga *et al.*[27] highlighted the role of cytokines in EBV infection resulting in HP and pancytopenia. They found that interferon (INF-gamma, IL-10 along with CD_3+ HLA-DR cellsplay an important role in BM HP and suppression causing peripheral cytopenia.[27] Role of cytokines in the causation of HP resulting in peripheral cytopenia was extensively studied by Fujiwara and his coworkers. According to them pancytopenia is an essential sign in HPS and it is induced by a large number of cytokines with overlapping functions particularly interferon-gamma along with 1L-18 acting as potent suppressor of hematopoiesis.[7] Role of BM function in relation to intensity of HP causing peripheral cytopenia was studied by Strauss *et al.*[14] Most of the patients of HP in his study presented with pancytopenia. The cytological examination of the BM revealed that activated macrophages were diffusely distributed. The macrophages appeared mature with low nuclear/cytoplasmic ratio and inconspicuous nucleoli. Mainly erythrocytes and less frequently platelets and neutrophils and their precursors were phagocytized.[14] The causation of pancytopenia in patients of HP is thus multifactorial and largely depends on the underlying disease process. In viral infections the antigens may directly target the cells. Other possible mechanisms include hypersplenism, immune destruction of cells immunosuppression with underlying infections, drug therapy and above all exaggerated HP with hypercytokinemia, particularly INF-gamma and tumor necrosis factor alpha. In the light of the study and previous studies by other workers, it is believed that cellularity of BM

apparently does not correlate with the degree of intensity of HP and severity of peripheral cytopenia.

Conclusions

HP is seen in a wide variety of hematological and nonhematological conditions, mainly as a reactive phenomenon. It mainly results from immunological activation of mononuclear phagocyte system. Macrophages and T lymphocytes play a major role through elaboration of various cytokines. Reactive hemophagocytic histiocytosis is seen mostly in NMHC and infections. Among the nonhematological conditions, it is typically seen in MA with increasing intensity and usually results in the depression of blood cell counts. Large number of viral and nonviral infections can give rise to this phenomenon. It may present as HPS due to hypercytokinemia with varied etiology showing pancytopenia/bicytopenia, liver dysfunction, coagulopathy, hyperferritenemia. Mechanism of peripheral cytopenia is multifactorial but mainly occurs because of exaggerated HP with hypercytokinemia. The extent of intensity of HP and depression of cell counts is independent of BM cellularity. Age of the patient apparently has no effect on either intensity of HP or peripheral cell counts. Increase intensity of hemophagocytosis has a significant effect on hematological parameters, particularly Hb and platelet counts, resulting in depression of these hematological parameters.

Future Scope

Studies are required to clarify the possible role of BM microenvironment in the pathogenesis of hemophagocytosis and peripheral cytopenia, to characterize the activation status of the macrophages in HP and to investigate in detail the role of T lymphocytes and their associated cytokine profile (Th1/Th2 immune response).

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