Evaluation of Post Chemotherapy Bone Marrow Changes in Acute Leukaemia

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***Abstract*- Introduction:** The incidence of Acute leukaemia both AML and ALL has been increasing over the years. Chemotherapeutic agents used in the treatment of acute leukemia cause damage to the hematopoietic environment of the bone marrow initially, followed by stages of hematopoietic recovery. The study of post chemotherapy bone marrow in acute leukemia is essential to analyse the response to chemotherapy and also to study the effects of chemotherapeutic drugs on the bone marrow micro environment.

Aims: 1) To study the changes in the marrow due to chemotherapeutic agents in acute leukemia patients.
2) To study the response of leukemia to these drugs.
3) To study and compare bone marrow findings in the post induction phase and maintenance phase.

Materials and methods: Total 65 cases of acute leukemia (50 cases of ALL and 15 cases of AML) in the post induction phase were enrolled for the study, out of these 44 cases (31 from ALL and 13 from AML) were followed up in the maintenance phase. Bone marrow aspirate and biopsy slides were studied for these cases.

Results: In the post induction phase bone marrow aspirates were predominantly hypocellular (43%) with normal to increased erythroid regeneration (64.6%) but decreased myelopoiesis and megakaryopoiesis. The erythroid maturation was mainly in the form of normoblastic or normoblastic with megaloblastoid changes (83.5%). Dyspoiesis was present in all three lineages (Erythroid series -40%, Myeloid series - 26%, Megakaryocytes-26.1%). In the maintenance phase bone marrow was normocellular or hypercellular (68.18%) with normal to increased erythropoiesis (72.7%) and predominantly normoblastic maturation (81%). Regeneration of myeloid series (63.6%) and megakaryocytes (90%) was also improved than in post induction phase. In the maintenance phase erythroid and myeloid dyspoiesis were decreased whereas megakaryocytic dyspoiesis was increased than in post induction phase.

Conclusion: Hence the study shows that though the bone marrow is hypocellular in the post induction phase , the cellularity improves during the maintenance phase. In the post induction phase erythroid regeneration appear first followed by myeloid and megakaryocytic regeneration.

***Index Terms*-** Post chemotherapy, Bone marrow, Acute leukaemia

I. INTRODUCTION

Acute leukemias (ALs) are heterogenous group of neoplasms due to uncontrolled proliferation of clonal neoplastic hematopoietic stem cells, leading to neutropenia, anaemia, and thrombocytopenia [1,2,3,4]. Acute leukemia is of two types : Acute Myeloid Leukemia (AML) and Acute Lymphoblastic Leukemia (ALL).

Acute leukemia can be diagnosed on the basis of peripheral blood smear examination alone but bone marrow aspiration and bone marrow biopsy further support the diagnosis. Peripheral blood smear and bone marrow aspirate help to study percentage of blasts and morphologic evaluation of the blasts and other cells. In addition to this, bone marrow biopsy helps to evaluate the spatial organization of hematopoietic cells and specific histological features associated with the process (e.g., fibrosis, necrosis)[5,6,7]. Also after chemotherapy bone marrow examination helps to study the hematopoietic regeneration in detail [2,8].

For the treatment of AML, in the induction phase Cytarabine, Daunorubicin or Idarubicin with or without Etoposide are used. For the treatment of ALL Vincristine, Asparginase, Prednisolone, Daunorubicin with or without Intrathecal Methotrexate are used.All of these chemotherapeutic agents supress normal haematopoiesis for variable period of time.

Haematopietic regeneration after chemotherapy is usually monitored by repeated peripheral blood smear examinations. Bone marrow examination after chemotherapy help to study the pattern of haematopoietic regeneration in more detail, abnormalities in the bone marrow cytology, localization of regenerating haematopoietic cells, the attainment of complete remission, any evidence of relapse or resistant disease and also the side effects of chemotherapeutic agents[5,6,9].

There are many studies where evaluation of bone marrow aspirate and biopsy have been done for the changes in the hematopoietic environment during the poat induction phase, but there are very few studies where study of bone marrow during the maintenance phase have been done. Hence in our study we have evaluated the changes in the bone marrow aspirate and biopsy in the post induction phase and in the maintenance phase for the cases of acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML). We have compared the findings of bone marrow during these two phases

II. MATERIALS AND METHODS

This is a retrospective and prospective cross sectional study done in the Department of Pathology, Kasturba Medical College, Manipal. We studied bone marrow aspirate and biopsy in patients with acute leukemia (both acute myeloid leukemia and acute lymphoblastic leukemia) who presented to our hospital from January 2006 to December 2010.

Inclusion criteria :

Cases of acute leukemia (ALL or AML) for whom bone marrow aspirate alone or bone marrow aspirate and biopsy both were done for the diagnosis and in the post induction phase.

Exclusion criteria :

1) Cases of acute leukemia (ALL or AML) which were diagnosed only by the study of peripheral blood smear alone and study of bone marrow was not done for the diagnosis.

2) Cases for whom bone marrow study was not done in the post induction phase.

3) Cases of acute leukemia (ALL or AML) diagnosed in some other centre but who received chemotherapy in our hospital.

4) Cases of acute leukemia (ALL or AML) diagnosed in our centre but referred to other centres for treatment.

Our patients received the following chemotherapy regimen in the Induction phase :

A) Patients with ALL were assigned to Protocol MCP-841 :

Induction therapy :

Daunorubicin (DNR) IV on days 8, 15, and 29;

Vincristine (VCR) IV on days 1, 8, 15, 22, and 29;

L-Asparaginase (ASP) intramuscularly (IM) every other day on days 2-20;

Oral prednisone (PRED) on days 1-28;

Methotrexate (MTX) intrathecally (IT) on days 1,8,15,and 22.

B) Patients with AML were assigned to Cytarabine + Idarubicin (7+3 regimen)

Induction therapy :

Cytarabine (Ara-C) 100 mg/m2/d iv day 1 -7 Idarubicin 12 mg/m2/d iv day 1- 3

We enrolled 65 cases of acute leukemia (50-ALL, 15AML) for the study. Bone marrow aspiration was done for all the cases, bone marrow biopsy was done for 38 cases (32 from ALL and 6 from AML). Bone marrow study was done in the fourth week of induction chemotherapy for ALL cases and in the second week of induction chemotherapy for AML cases.

We studied repeat bone marrow aspiration during maintenance phase for 45 cases (31 from ALL and 13) from AML. Both ALL and AML were found to be more common in males (64% in ALL and 66.7% in AML) which may be just a coincidence. The ALL cases were between the age of 1-39 years (mean 20years) and the AML cases were between the ages of 10-57 years (mean 33.5 years).

All the bone marrow aspirate slides were stained with Leishmain stain after necessary procedures. All the bone marrow biopsy slides were processed and stained with Haematoxyllin and Eosin.

III. RESULTS

NUMBER OF CASES ENROLLED IN THE STUDY

PHASE	STUDY	ALL	AML
POST INDUCTION (65 cases)	BONE MARROW ASPIRATE	50	15
	BONE MARROW BIOPSY	32 OUT OF 50 cases	6 OUT OF 15 cases
MAINTENANCE (44 cases)	BONE MARROW ASPIRATE	31	13
	BONE MARROW BIOPSY	11 OUT OF 31 cases	2 OUT OF 13 cases

BONE MARROW CELLULARITY IN POST INDUCTION PHASE AND MAINTENANCE PHASE

PHASE	TYPE OF LEUKEMIA	CELLULARITY			
		NORMAL	HYPOCELLULAR	HYPERCELLULAR	DRY TAP
POST INDUCTION	ALL (50 cases)	15(30%)	23(46%)	5(10%)	7(14%)
	AML (15 cases)	2 (13.3%)	5(33.3%)	3 (20%)	5(33.3%)
	TOTAL (65 cases)	**17(26%)**	**28(43%)**	**8(12.3%)**	**12(18.46%)**
MAINTENANCE	ALL (31 cases)	18(58.1%)	9(29%)	3(9.7%)	1(3.2%)
	AML (13 cases)	3(23.1%)	2(15.4%)	6(46.2%)	2(15.4%)
	TOTAL (44 cases)	**21(47.7%)**	**11(25%)**	**9(20.4%)**	**3(6.8%)**

INDIVIDUAL CELL LINEAGE IN POST INDUCTION PHASE AND MAINTENANCE PHASE

PHASE	TYPE OF LEUKEMIA	SERIES	CELL COUNT			DYSPOIESIS PRESENT
			NORMAL	DECREASED	INCREASED	
POST INDUCTION	**ALL (50 cases)**	ERYTHROID	18 (36%)	12 (24%)	19 (38%)	23 (46%)
		MYELOID	25(50%)	25 (50%)	0	14 (28%)

		MEGAKARYOCYTES	24 (48%)	23 (46%)	3 (6%)	14 (28%)
	AML (15 cases)	ERYTHROID	3 (20%)	7 (46.7%)	2 (13.3%)	3 (20%)
		MYELOID	1 (6.7%)	9 (60%)	3 (20%)	3 (20%)
		MEGAKARYOCYTES	3 (20%)	10 (66.7%)	0	3 (20%)
	TOTAL	ERYTHROID	21(32.3%)	19(29.2%)	21(32.3%)	26(40%)
		MYELOID	26(40%)	34(52.3%)	3(4.6%)	17(26.1%)
		MEGAKARYOCYTES	27(41.5%)	33(50.7%)	3(4.6%)	17(26.15%)
MAINTENANCE	**ALL (31cases)**	ERYTHROID	15(48.4%)	6 (19.4%)	10(32.3%)	5(16.1%)
		MYELOID	15(48.4%)	12(38.7%)	4(12.9%)	3(9.7%)
		MEGAKARYOCYTES	25(80.6%)	1(3.2%)	5(16.1%)	10(32.3%)
	AML (13 cases)	ERYTHROID	4(30.8%)	5(38.5%)	3(23.1%)	4(30.8%)
		MYELOID	5(38.5%)	4(30.8%)	4(30.8%)	6(46.2%)
		MEGAKARYOCYTES	8(61.5%)	2(15.4%)	2(15.4%)	5(38.5%)
	TOTAL	ERYTHROID	19(43.18%)	11(25%)	13(29.5%)	9(20.4%)
		MYELOID	20(45.45%)	16(36.36%)	8(18.18%)	9(20.4%)
		MEGAKARYOCYTES	33(75%)	3(6.8%)	7(15.9%)	15(34%)

Dyspoiesis in all three lineages

Erythroid dypoiesis was noticed in the form of nuclear budding, binucleate forms, multinucleate forms, increased mitoses and karyorrhexis

Myeloid dyspoiesis was seen in the form of abnormal nuclear lobation and giant bands (22%). In post induction phase increased myeloid precursors were seen in 26 cases (52%) of ALL and 6 cases (40%) of AML. Whereas in the maintenance phase increased myeloid precursors were seen in 13 cases (41.9%) of ALL and 6 cases (46.2%).

Megakaryocytic dyspoiesis in our patients was present in the form of hypolobated forms, immature forms, and micromegakaryocytes.

TYPE OF MATURATION OF ERYTHROID SERIES IN POST INDUCTION PHASE AND MAINTENANCE PHASE

TYPE OF MATURATION OF ERYTHROID SERIES	**POST INDUCTION PHASE**			**MAINTENANCE PHASE**		
	ALL	**AML**	**TOTAL**	**ALL**	**AML**	**TOTAL**
PREDOMINANT NORMOBLASTIC MATURATION	42 (84%)	6 (40%)	**48(73.8%)**	26(83.9%)	10(76.9%)	**36(81.8%)**
PREDOMINANT MEGALOBLASTIC MATURATION	3 (6%)	0	**3(4.5%)**	1(3.2%)	0	**1(2.7%)**
NORMOBLASTIC MATURATION WITH MEGALOBLASTOID CHANGES	20 (40%)	1 (6.7%)	**21(32.3%)**	10(32.3%)	4(30.8%)	**14(31.8%)**
MICRONORMOBLAST PRESENT	9 (18%)	0	**9(13.8%)**	9(19.4%)	3(23.07%)	**12(27.27%)**
TYPE OF MATURATION COULD NOT BE ASSESSED	5 (10%)	9 (60%)	**14(21.5)**	4(12.9%)	3(23.07%)	**7(15.9%)**

OTHER FINDINGS IN POST INDUCTION PHASE AND MAINTENANCE PHASE

OTHER FINDINGS IN BONE MARROW ASPIRATE	**POST INDUCTION PHASE**			**MAINTENANCE PHASE**		
	ALL	**AML**	**TOTAL**	**ALL**	**AML**	**TOTAL**
MULTIVACUOLATED FAT CELLS	6 (12%)	3(20%)	**9(13.8%)**	1(3.2%)	2(15.4%)	**3(6.8%)**
INCREASED MITOSES	7 (14%)	1(6.7%)	**8(12.3%)**	3(9.7%)	0	**3(6.8%)**
INCREASE MACROPHAGES WITH INGESTED DEBRIS	7 (14%)	3(20%)	**10(15.3%)**	10(32.3%)	5(38.5%)	**15(34.09%)**
HEMOSIDERIN LADEN MACROPHAGES	13 (26%)	7(46.7%)	**20(30.7%)**	5(16.1%)	3(23.1%)	**8(18.8%)**
HAEMOPHAGOCYTOSIS	4 (8%)	2(13.3%)		0		

			6(9.2%)		0	**0(0%)**
SEA BLUE HISTIOCYTES	1 (2%)	1(6.7%)	**2(3.07%)**	0	1(7.7%)	**1(2.2%)**

BONE MARROW BIOPSY FINDINGS IN POST INDUCTION PHASE OF ALL AND AML

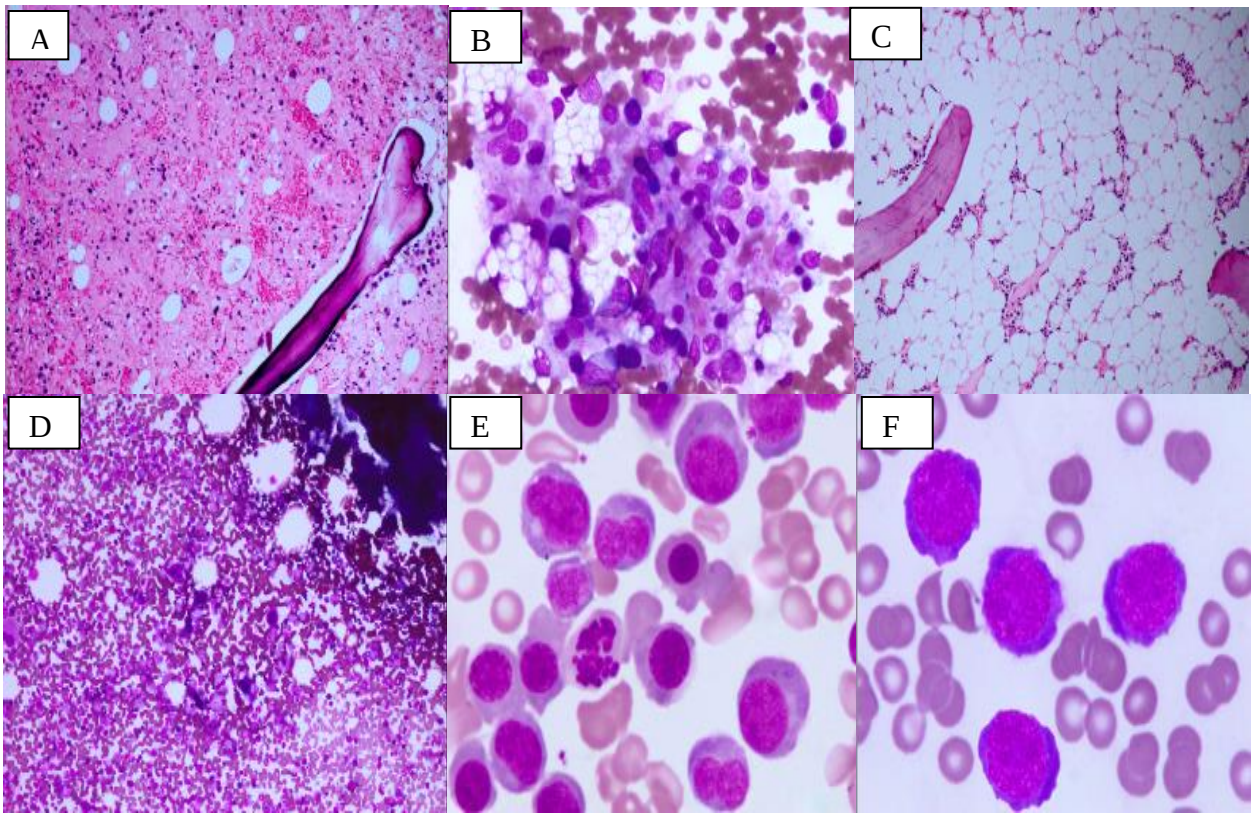
BIOPSY FINDINGS POST INDUCTION PHASE OF ACUTE LEUKEMIA 38 CASES	**NUMBER AND PERCENTAGE**
STROMAL EDEMA	6(15.8%)
PARATRABECULAR CLUSTERING	7(18.4%)
ALIP (abnormal localisation of immature precursors)	4(10.5%)
MEGAKARYOCYTE CLUSTERING	3(7.9%)
BONE MARROW FIBROSIS	1(2.6%)

In the maintenance phase out of 45 follow up cases bone marrow biopsy was done only in 13 cases (ALL:11, AML:2) and there was no significant finding except paratrabecular clustering of megakaryocytes which was seen in 1 case (7.7%)..

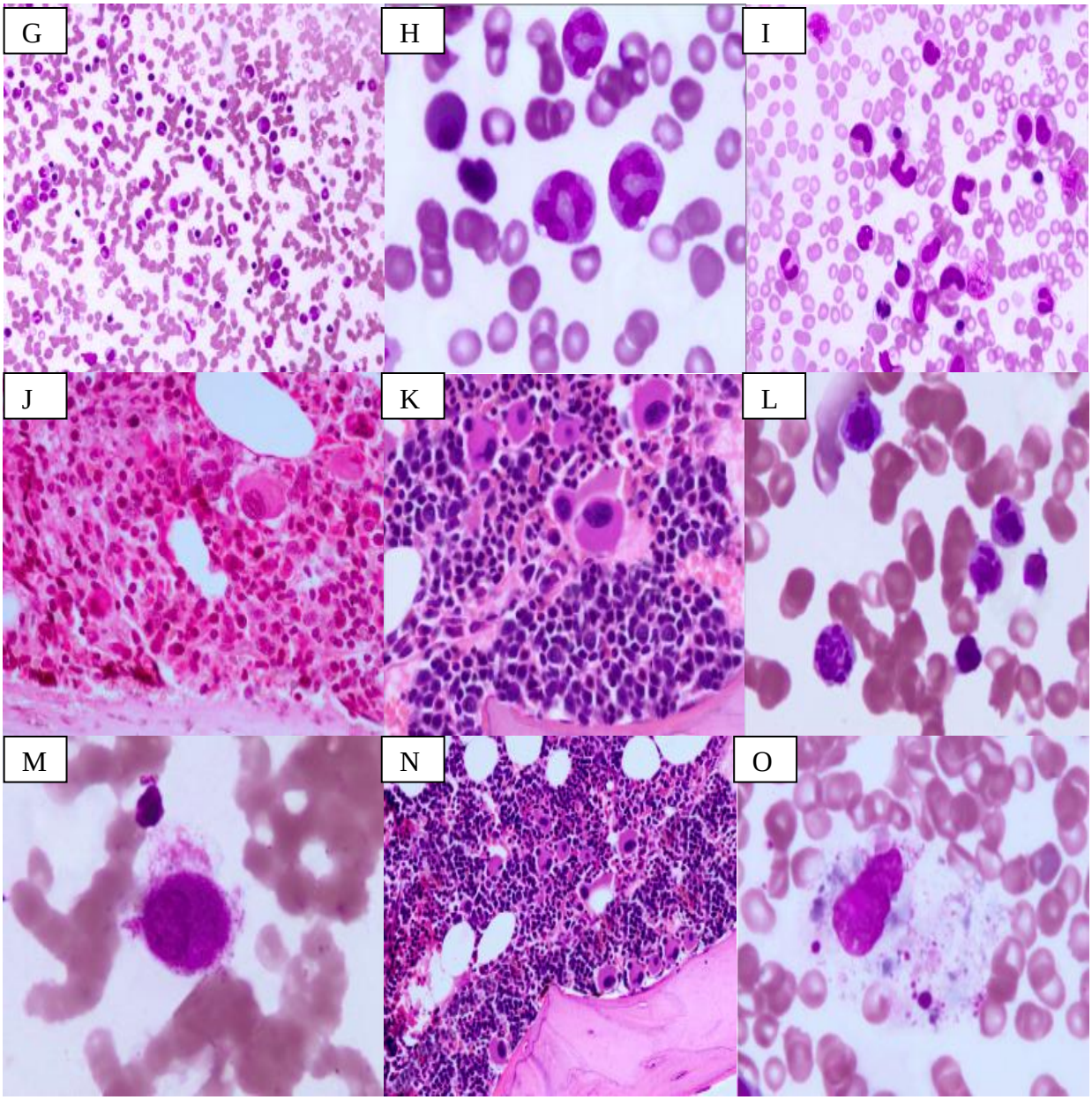
IV. RELAPSE CASES

In our study out of 50 cases 14 cases had relapsed during follow up, out of which 9 were ALL and 5 were AML. In ALL, relapse occurred between 82-834days (mean of 257 days) from the day of starting chemotherapy and in AML, relapse occurred between 51-242 days (mean of 154 days) from the day of treatment.

Bone marrow in Post induction phase



A.Marked ablation of haematopoietic elements replaced by proliferating sinusoids and fibroblast(10X). B.Multivacuolated fat cell(40X) C.Markedly hypocellular marrow(10X) D.Hypercellular marrow particle with features of regeneration(10X) E.Erythroid hyperplasia with megaloblastoid maturation and dyspoiesis(40X) F.Megaloblastoid changes(100X)



G.Myeloid hyperplasia(20X) H.Myeloid dyspoiesis in the form of abnormal lobation(100X) I.Giant bands(40X) J.Trephine biopsy showing abnormal localaization of immature precursors(ALIP)(20X) K.Megakaryocytic dyspoiesis in the hypolobated form(20X) L.Atypical mitoses(100X) M.Micromegakayocyte(40X) N.Paratrabecular clustering of megakaryocytes(20X) O.Macrophage with ingested debris(100X)

V. DISCUSSION

Hematopoietic regeneration after chemotherapy for acute leukemia is usually monitored by repeated peripheral blood smear and bone marrow aspirate analysis. Bone marrow biopsy helps further by evaluation of spatial organization of the hematopoietic cells[5,6,7].

The main mechanism of action of most of the chemotherapeutic agents used for treatment of acute leukemia is by inhibiting DNA repair , inhibiting DNA and RNA synthesis and by causing fragmentation of DNA leading to cell cycle arrest, cell apoptosis and cell death. Hence the consequences of these chemotherapeutic agents are injurious to the hematopoietic microenvironment leading to bone marrow suppression, mitotic arrest in the blasts, megaloblastoid changes in erythroid precursors[10,11]. In our study we evaluated the changes in the hematopoietic environment after induction chemotherapy and in the maintenance phase of chemotherapy.

Post induction bone marrow aspirates done at the end of 4th week in ALL cases, and at the end of 2nd week in AML cases were predominantly hypocellular (43% of cases), remaining 38% were normocellular to hypercellular where as in some cases bone marrow aspirate was dry tap. Our bone marrow findings were

similar to the findings in the studies done by Bruce D.Cheson et al[2] ,A study done by Islam et al[9] showed that bone marrow aspirate of AML cases in post induction patients were predominantly hypocellular. These findings are unlike the study done by Elizabeth L. Gerard [6] where the post induction bone marrow were predominantly normocellular. N. Hurwitz[5] in his study has stated that by fourth week of induction chemotherapy bone marrow aspirate should have normal age related cellularity. In other literatures hypocellularity is mainly reported in post induction phase of AML and not in ALL. But in our study hypocellularity was a feature of both AML and ALL in post induction phase. Though the bone marrow was predominantly hypocellular in post induction phase, the cellularity recovered to predominantly normocellular to hypercellular in the maintenance phase. Hence the hypocellularity observed during the post induction phase may be due to the antileukemic effect of the chemotherapeutic agents or could be due to their side effects as most of these drugs damage the hematopoietic environment.

Post induction bone marrow aspirate of our cases had predominantly normal to increased (64.6%) erythropoiesis, whereas myelopoiesis (52.3%) and megakaryopoiesis (50.7%) were predominantly supressed. Hence we concluded that in the post induction period regeneration of erythroid series precedes the regeneration of myeloid series and megakaryocytes. According to the article by N .hurwitz, the first sign of bone marrow regeneration is the appearance of unilinear islands of erythroid precursors followed by myeloid cells and megakaryocytes[5].

The type of erythroid maturation we observed in the post induction phases of our cases was predominantly normoblastic or normoblastic maturation with megaloblastoid change and few cases with predominantly megaloblastic maturation. Erythroid dyspoiesis was present in 40% of our cases, commonly in the form of nuclear budding, binucleate forms, multinucleate forms and karyorrhexis. Study done by B S Wilkins and N Hurwitz also showed erythroid cells with megaloblastic maturation[5,7]. These megaloblastoid changes could be the effect of chemotherapeutic drugs like Methotrexate, Danorubicin / Idarubicine, Cytarabine due to inhibition of DNA synthesis. We noticed increase in the stage of basophilic normoblasts in 10.7% of our cases. In a study done by B S Wilkins et al, erythroid cells arrested in the stage of basophilic normoblasts was seen in 45% of the cases in the post induction phase. N. Hurwitz et al, in his study had mentioned about the partial arrest of maturation of erythroid series in the basophilic normoblastic stage during the post induction phase[5]. All we can conclude from these findings is that the heterogenecity in the stages of erythroid maturation seen in a normal marrow will be lost after chemotherapy and there will be maturation arrest in the erythroid series.

In normal marrow erythropoietic foci are not found in the paratrabecular region. Bone marrow biopsy done among 38 of our cases in the post induction phase showed abnormal paratrabecular clustering of erythroid cells in 18.4%. B S Wilkins et al in their study has mentioned about abnormal paratrabecular clustering of erythroid cells in 60% of their cases and showed marked suppression of granulopoiesis at that time. But biopsies done later on in their cases showed returning of erythroid cells to their normal place with recovery of granulopoies[7].

Hence we assume that after chemotherapy the maturing erythroid cells may lose their normal heterogenecity and may have abnormal paratrabecular clustering. Both of these findings will recover later on.

When we evaluated the bone marrow in 44 cases during the maintenance phase, erythroid regeneration was normal to increased in 72.68%. The regenerating erythroid cells were predominantly in the normoblastic stage in 81% of the cases, whereas normoblastic maturation with megaloblastoid changes had decreased from 32.3% in the post induction phase to 22.7%. The erythroid dyspoiesis also reduced from 40% in the post induction phase to 20% in the maintenance phase. Also the findings seen in the post induction phase like erythroid precursors in the stage of basophilic normoblast and abnormal paratrabecular clustering of erythrocytes got resolved as the patients entered into maintenance phase.

These findings indicate that not only the number of erythroid series but also the type of erythroid maturation reverts back to normal (predominantly normoblastic maturation) once the patient progresses from post induction phase to maintenance phase. Also there will be reduction in dyspoiesis and megaloblastoid changes with regain of normal heterogenecity and normal topography.

In the post induction phase granulopoiesis was seen to regenerate only after erythropoiesis. In almost 52.3% of the cases myelopoiesis was decreased. There was paucity of mature granulocytes and increase in myeloid precursors(49.23% of the cases). Dyspoietic myeloid cells with abnormal lobations and giant bands were seen in 26.1% of the cases. Abnormal localization of immature precursors (ALIP) was seen in 10.4% out of 38 biopsies in the post induction phase. B S Wilkins in his study has mentioned about the reduction in the number of granulopoiesis, paucity of mature granulocytes, increase in precursors forms but not the myeloid dyspoiesis and abnormal localization in the post induction phase[7]. But most of these changes were seen to revert back to normal in the maintenance phase. Myelopoesis regeneration in the maintainance phase was seen in (63.6%) cases with increase in mature forms, decrease in dyspoiesis (20.45%) and normalization of ALIP .

In the post induction phase of our cases megakaryopoiesis was decreased in 50.7% of the cases and megakaryopoietic regeneration was seen to occur last. Megakaryocytic dyspoiesis seen in 26.15% of the cases were in the form of hypolobated forms, immature forms, micromegakaryocytes, mononuclear variants and megakaryocytes with bizarre angulated nuclei. Abnormal clustering of the megakaryocytes was seen in 7.9 % of the bone marrow biopsies. All of these findings were also seen in the study done by B S Wilkins[7]. Though megakaryopoietic regeneration occured last during the post induction phase, in the maintenance phase megakaryocytic regeneration (90%) was better than regeneration of the erythroid series and myeloid series. Despite of having good regeneration, megakaryocytic dyspoiesis persisted for long duration even in the maintenance phase in the form of hypolobated forms, immature forms and micromegakaryocytes. This is well supported by the study done by B S wilkins in which megakaryocytic dyspoiesis persisted for a long duration (for weeks to months) during follow up[7]. Hence our study showed that in the post induction phase, megakaryocytic regeneration occured last , with dyspoieis and

abnormal clustering , which will recover as the patient progresses to the maintenance phase however dyspoiesis may take long time for recovery.

We also noticed some other findings in the bone marrow like stromal oedema (15.8%), multivacuolated fat cells (13.8%), increased mitoses, macrophages with ingested debris (15.3%), hemosiderin laden macrophages (30.7%), hemophagocytosis(9.2%) and sea blue histiocytes (3.07%). These findings were also present in the studies done by N. Hurwitz[5] and Islam et al[9]. According to their literature the earliest bone marrow changes after chemotherapy were stromal oedema with granular eosinophillic exudates, marrow hypocellularity, multiloculated fat cells sinusoidal ectasis, macrophages with ingested debris, haemosiderin laden macrophages. All these findings either reduce or disappear in the later biopsies. They have also mentioned that precursor multilocular fat cells give rise to unilocular fat cells and the islands of regenerating hematopoietic cells are seen around the aggregates of fat cells hence fat cells play important role for restitution of hematopoiesis.

On retrospective analysis we found that those cases which had relapsed of had suppressed erythropoiesis during the maintenance phase as compared to those non relapsed cases which had good erythropoiesis in the post induction phase. Hence we can assume that erythroid regeneration may play a significant role in the course of the disease. Similarly hypocellularity in post induction phase, erythroid dyspoiesis and marked suppression of megakaryocyte in post induction phase may also play a significant role in the prognosis of the disease.

VI. CONCLUSION

We conclude that chemotherapeutic drugs can cause various effects on the bone marrow microenvironment. It is essential to study these post chemotherapy changes for proper evaluation of the response to therapy and the to analyse the regeneration of bone marrow following chemotherapy.Bone marrow studies should be done both in post induction and in the maitainance phase for better evaluation of the response and the regeneration of the marrow. Though there is suppression of the marrow with evidence of marrow damage in the post induction phase,most of these changes revert back to normal in the maintainance phase.Marrows which show poor regeneration in the maintainance phase can indicate poor prognosis for the patient.

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