Role of Bone Marrow Trephine Biopsy in Diagnosing Hematological Disorders Which Shows Bone Marrow Aspiration Failure-Two Year Observational Study of 58 Cases

PoonamT.Dambhare[a],V. D. Tote[b], P. N. Wasnik[c], D. T. Kumbhalkar[d]
[a]Blood transfusion officer (Medical Officer), Model Blood Bank, Dept of Pathology Government Medical College Nagpur, Maharashtra, India
[b]Associate Professor, Dept of Pathology Government Medical College Nagpur, Maharashtra, India
[c]Assistant Professor, Dept of Medicine Government Medical College Nagpur, Maharashtra, India
[d]Professor & Head, Dept of Pathology Government Medical College Nagpur, Maharashtra, India
Corresponding Author: PoonamT.Dambhare

Abstract

Bone marrow examination is an important diagnostic tool for evaluation of many hematological diseases. The present study was conducted to compare the role of trephine biopsy with bone marrow aspiration for effectively diagnosing wide spectrum of hematological diseases. Few studies have compared the relative value of aspirate with trephine biopsy. In present study we particularly selected and studied the cases which showed aspiration failure or dry taps. This is a two year observational study of 58 cases, done from September 2009 to August 2011 in the Department of Pathology, Government medical college and hospital Nagpur in Maharashtra. Bone marrow aspiration, trephine biopsy and trephine imprints done simultaneously for correlation. All the smears and sections were studied for morphological details and compared to each other. Out of 58 trephine biopsies, 2 biopsies were inadequate for diagnosis. 56 cases were diagnosed on trephine biopsy with diagnostic accuracy of 96.55%, of imprint smears 91.37% and based on patients selection criteria, approximately 96 % inadequacy found on bone marrow aspirate smears. All three sets of smears complement each other for evaluating haematological disorder. Histopathological study of trephine biopsy gives well preserved marrow architecture with its all cellular and stromal components. Imprint smear also allows cytological evaluation of biopsy findings and rapid diagnosis, when aspirate smears are inadequate.

KEYWORDS: Bone marrow aspiration, trephine biopsy, trephine imprint, pancytopenia, leukemia

INTRODUCTION

Bone marrow examination is an important diagnostic tool for evaluation of many hematological diseases. Bone marrow aspiration is the safe invasive procedure done routinely for the diagnosis and management of hematological disorder. About trephine biopsy, it is an invaluable diagnostic procedure which can provide information about the structure of relatively large piece of marrow, pattern of bone marrow involvement, to evaluate cellularity in aplastic anemia and leukemia, topographical alterations and fibrosis of bone marrow. Also provides information of prognostic significance which are not always apparent in aspiration smears. By

making imprint smears from the core obtained, morphological features of individual cells can be studied.

The present study was conducted to compare the role of trephine biopsy with bone marrow aspiration for effectively diagnosing wide spectrum of hematological diseases. Bone marrow aspiration, trephine biopsy and trephine imprints done simultaneously for correlation. All the smears and sections were studied for morphological details and compared to each other. .

MATERIAL AND METHOD

This was a two year observational study done from September 2009 to August 2011 in the Department of Pathology, Government medical college and hospital Nagpur, Maharashtra. Total 58 cases of haematological disorder were studied. Bone marrow aspiration and trephine biopsy performed simultaneously in each case. Imprints were also prepared from the core obtained. All the smears and sections were studied for morphological details and compared to each other.

Only those cases which showed bone marrow aspiration failure or dry tap, cases with peripheral pancytopenia were included in the study. Also cases were included to confirm their diagnosis established on peripheral blood smears and/or bone marrow aspirate, to detect early fibrosis in myeloproliferative disorders. Whole procedure was explained and written consent of patient was taken in each case from patient or relative before starting the procedure. Both BMA (by Klima needle) and BMB (using Jamshidi needle) were taken from posterior superior iliac spine. 0.2 to 0.5 ml of fluid was aspirated, smears prepared, dried and stained with Leishman stain. Bone marrow biopsy core obtained with average length of 1.5cm to enable the evaluation of at least 10 partially preserved intertrabecular areas. It was gently rolled across a glass slide and imprint smears were prepared before the specimen was transferred into fixative. Imprints were allowed to dry and stained with Leishman or MGG stain. Core fixed in 10% formalin for 6-12 hours, decalcified by 10% EDTA for 24-48 hours and embedded in paraffin blocks in histopathology laboratory. Stepwise four to five micron thick sections were taken from paraffin block and were stained with Haematoxylin and Eosin stain in all cases. Reticulin staining done in all cases of leukemia and pancytopenia. As and when indicated Masson trichrome, Prussian blue, PAS, Reticulin and peroxidase stains were used. Aspiration smears, trephine imprints and trephine sections were compared for the relative efficacy in yielding a definite diagnosis.

RESULTS

A total of 58 cases were done. Out of which 56 were adequate for interpretation. Aplastic anaemia was found as the commonest diagnosis. Acute myeloid leukemia and megaloblastic anaemia were diagnosed as the second common diagnosis. Distributions of total cases diagnosed by bone marrow biopsy are shown in table 1.

Out of 58 trephine biopsies, 2 biopsies were inadequate for diagnosis. 56 cases were diagnosed on trephine biopsy with diagnostic accuracy of 96.55%, of imprint smears 91.37% and based on patient selection criteria, approximately 96 % inadequacy found on bone marrow aspirate smears.

Of 27 cases of pancytopenia, aplastic anaemia (70.37 %) was found to be the commonest cause, based on patient selection criteria's, as shown in table 2. Of 27 cases of pancytopenia (found on automated complete blood count), 100% diagnosis obtained from trephine biopsy section, 92.59% diagnosis from trephine biopsy imprint

and approximately 96 % inadequacy found from bone marrow aspirate smears. In this study three cases were showing pancytopenia on hemogram, bone marrow aspirations smears were inadequate for diagnosis and clinicians were suspecting of haematological disorders but on trephine imprint and trephine biopsy it showed normal bone marrow.

In all 15 cases of leukemia, inadequacy of BMA smears was 87% and diagnosis was done on biopsy imprint and biopsy section, as shown in table 3. Of 15 cases of leukemia, 6 (40%) cases showed reticulin fibrosis in their marrow, out of which 4 (66.66%) cases were of acute leukemia and 2 (33.33 %) cases were of chronic leukemia. Of 6 cases of leukemia associated with fibrosis, 5 (83.33%) cases showed bone marrow aspiration failure.

Of three cases of NonHodkins lymphoma, two cases showed marrow involvement, 100 % diagnosis obtained from trephine sections and 50% from biopsy imprints, as shown in table 4. Trephine biopsy done as per clinicians advice. Bone marrow trephine biopsy done in one case of Hodgkin's lymphoma as per clinician's advice, but not showed marrow involvement by both imprints and biopsy sections. Of 2 cases of multiple myeloma, 100 % diagnosis obtained from bone marrow biopsy imprints and biopsy sections. In all cases bone marrow aspirations were diluted. In 2 cases of myelifibrosis, bone marrow biopsy imprints were inadequate for interpretation and diagnosis obtained only on trephine section, which showed extensive reticulin fibrosis in both cases.

DISCUSSION

In this study, we found good correlation between bone marrow aspiration smear and trephine section for assessment of grades of cellularity. The present study observed that the diagnostic accuracy of BMB and biopsy imprints were higher in comparison to BMA in diagnosing various haematological disorders. The megakaryocytic density could be assessed reliably from trephine section alone because of variable distribution in different portions of aspiration smears and trephine imprints. Comparisons of adequacy of trephine biopsy and trephine imprints with various studies are shown in tables 5 and 6.

Trephine biopsy was found to be safe and useful procedure, well tolerated by patients. Complication like retroperitoneal haematoma haemorrhages or infection were not seen in any patients.

Comparison of cause wise distribution of cases of Pancytopenia in various studies is shown in table 7. The present study proved to be diagnostic in cases of pancytopenia and correlated well with the study of Rege et al (1992)[3]. Whereas studies of Tilak and Jain (1999)[7] and Khodke et al (2001)[8] had more number of cases of megaloblastic anaemia. The present study has increased number of cases of Aplastic anaemia because only those cases were included where bone marrow aspiration were inadequate and Aplastic anaemia is known to give inadequate aspirates. This variation in the frequency of disorders causing pancytopenia may be due to differences of methodology, selection of cases, diagnostic criteria and period of observation.

According to Bird and Jacobs (1983)[9], marked increase in reticulin in both acute lymphoblastic and acute myeloblastic leukemia may lead to dry tap. In present study 6 cases of leukemia presented with dry tap and trephine sections showed increase fibrosis. In some cases of leukemia, no satisfactory bone marrow aspirate can be obtained due to extreme cellularity and compactness of the marrow. These are the cases where bone marrow trephine biopsy has primary diagnostic value.

In the present study, 50% cases of CML showed granulocytic hyperplasia and 50% granulocytic megakaryocytic hyperplasia. According to Burkhardt et al (1982)[10], when bone marrow trephine biopsy section show granulocytic megakaryocytic hyperplasia there is greater likelihood of fibrosis, while when there is granulocytic hyperplasia there is greater likelihood of transformation to blasts crisis. This shows, in chronic myeloid leukemia, the importance of trephine section lies in characterization of proliferating cell lines and associated myelofibrosis. In present study 2 cases of CML shows reticulin fibrosis in trephine section.

According to Ellman (1976)[11] trephine biopsy of bone marrow is the best method for detecting lymphomatous bone marrow involvement. It is due to the fact that nodules and cluster of lymphoma cells can be quite dense and adherent and may be difficult to aspirate. In present study 3 cases of NonHodkins lymphoma showed aspiration failure.

According to Krzyzaniak et al (1988)[12], fibrosis in cases of multiple myeloma is because of local action of lymphokines released by plasma cells. Thus bone marrow trephine biopsy may be required to histologically confirm concurrent simple marrow fibrosis. Present study correlated well with above study, as in both cases of multiple myeloma, bone marrow aspirations were diluted. According to Sabharwal et al (1990)[13], biopsy is useful for differentiating myelomatous plasmacytosis from non myelomatous plasmacytosis, since compact masses of plasma cells with no stroma is a crucial histological feature for such differentiation. According to Singhal et al (2004)[14] histomorphology of multiple myeloma correlated with prognosis. Cases with poorly differentiated myeloma cells i.e. plasmablastic, diffuse pattern of involvement and dense fibrosis had survival less than one year.

CONCLUSION

The results of our study shows that trephine sections and trephine imprints alone or in combination offer significantly more information as compared to aspiration smear. But bone marrow trephine biopsy is not a substitute but a complementary procedure to bone marrow aspiration. It is the histomorphological study of trephine biopsy that gives well preserved marrow architecture with its all cellular and stromal components. Hence trephine biopsy becomes mandatory for the diagnosis of aplastic anaemia, leukaemias and Myelofibrosis yielding dry aspirate on bone marrow aspiration. The evaluation of all three sets of smears comprises the complete work up for proper bone marrow interpretation and to reach final diagnosis.

Table 1: Distribution of cases according to diagnosis

Sr. No.	Category	Subcategory	No. Of cases (%)	Total (%)
1	Anaemia	Aplastic	19 (33.92 %)	24 (42.85%)
		Megaloblastic	04 (7.14 %)	
		Haemolytic	01 (1.78 %)	
2	Leukemia	AML	06 (10.71%)	15 (26.78 %)
		ALL	02 (3.57 %)	
		CML	04 (7.14 %)	
		PCL	01 (1.78%)	

		Acute leukemia (unclassified)	02 (3.57%)	
3	Miscellaneous	Myelofibrosis	02 (3.57 %)	17 (30.35%)
		Multiple myeloma	02 (3.57%)	
		MDS	01 (1.78 %)	
		NHL	02 (3.57 %)	
		Gauchers disease	01 (1.78 %)	
		CLD	01 (1.78%)	
		Normal bone marrow	08 (14.28 %)	
TOTAL			**56 (100%)**	**56(100%)**

Table 2: Findings of bone marrow aspirate, trephine biopsy imprint (BI) and trephine biopsy section (BS) in cases of Pancytopenia

Cases	**Total no. of positive**	**Bone marrow aspirate smear**	**Positive %**	
		Inadequate	BI	BS
Aplastic anemia	19	19	18	19
Megaloblastic anemia	03	02	03	03
AML	01	01	01	01
ALL	01	01	01	01
NBM	03	03	02	03
Total	27(100%)	26 (96.30%)	25(92.59%)	27 (100%)

Inadequate bone marrow aspirate smears: dry tap/ diluted with peripheral blood.
NBM : Normal Bone Marrow

Table 3: Findings of bone marrow aspirate, biopsy imprint (BI) and biopsy Sections (BS) in cases of Leukemia

Cases	**Total no of Positive**	**Bone marrow aspirate smear**	**Positive %**	
		Inadequate	BI	BS
AML	06	06	06	06
ALL	02	02	02	02
CML	04	02	04	04
AL(Unclassified)	02	02	02	02
PCL	01	01	01	01

Total	15(100%)	13(86.67%)	15(100%)	15(100%)

Inadequate: dry tap/ diluted marrow
PCL : Plasma Cell Leukemia

Table 4: Findings of bone marrow aspirate (AS), biopsy imprint (BI) and biopsy sections (BS) in cases of Lymphoma and Multiple Myeloma

Cases	Total cases	Positive cases	Positive cases (%)		
			AS	BI	BS
Non-Hodgkins lymphoma	03	02	0	01(50%)	02(100%)
Hodgkins Lymphoma	01	0	0	0	0
Multiple myeloma	02	02	0	02(100%)	02(100%)

Table 5: Comparison of adequacy of trephine biopsy with various studies

Authors	Year	Total number of biopsies	Positive biopsies	% of adequacy
Bearden et al[1]	1974	205	203	99.02
Block Mathew[2]	1976	211	208	98.57
Rege et al[3]	1992	110	90	81.81
Varma et al[4]	1993	767	535	69.75
Present study	2011	58	56	96.55

Table 6: Comparison of adequacy of trephine biopsy imprint with various study

Authors	Years	Total number of biopsy	Positive imprint smears	% of positive imprints
James et al[5]	1980	310	310	100
Varma et al[4]	1993	535	479	89.53
Aboul- Nasr et al[6]	1999	173	172	99.42
Present study	2011	58	53	91.37

Table 7: Cause wise distribution of cases of Pancytopenia in various studies

Causes		Authors		
	Rege et al[3] (1992)	Tilak and Jain[7] (1999)	Khodke et al[8] (2001)	Present study 2011

Aplastic anaemia	61.90%	7.79%	14%	70.37%
Megaloblastic anaemia	4.76%	68.83%	44%	11.11%
Acute leukemia	9.52%	1.3%	2%	7.41%
Miscellaneous	19.06%	22.08%	38%	11.11% ((NBM)

NBM - Normal Bone Marrow

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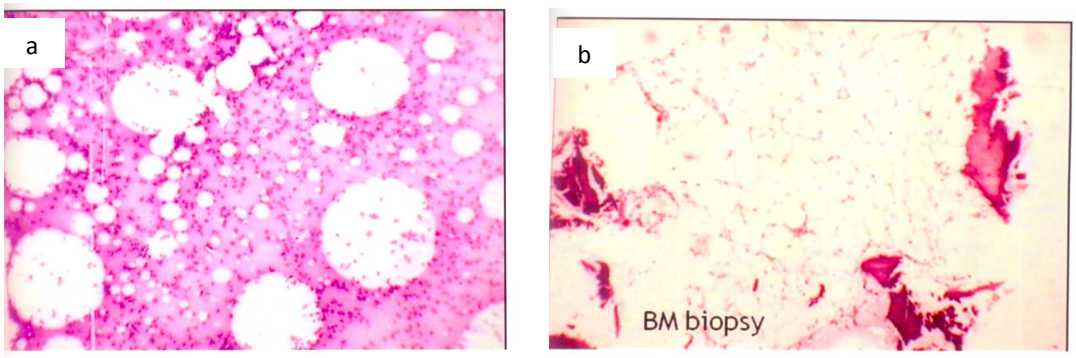

a. (Leishman stain, 400x) b. (H & E stain, 100x)

Fig a and b : Bone marrow biopsy imprint (a) and biopsy section(b) in aplastic anaemia showing marked reduction of haematopoietic cells and section showing predominantly fat spaces.

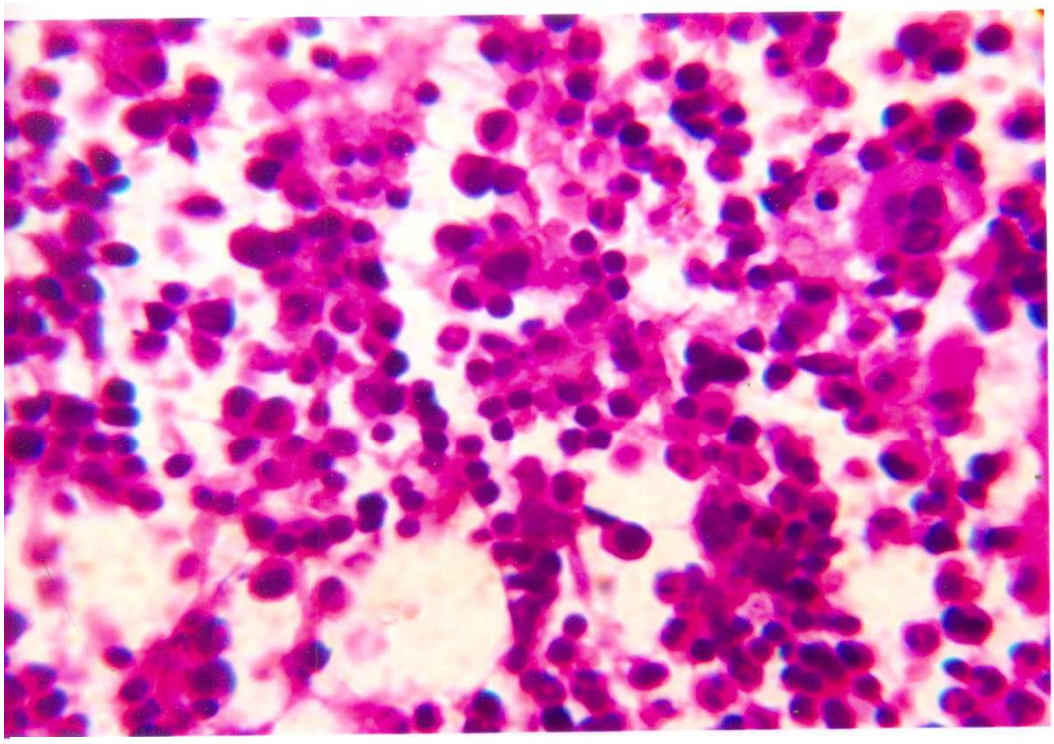

Fig c. Bone marrow trephine biopsy section showing plasma cells in multiple myeloma.
(H & E stain, 400x)

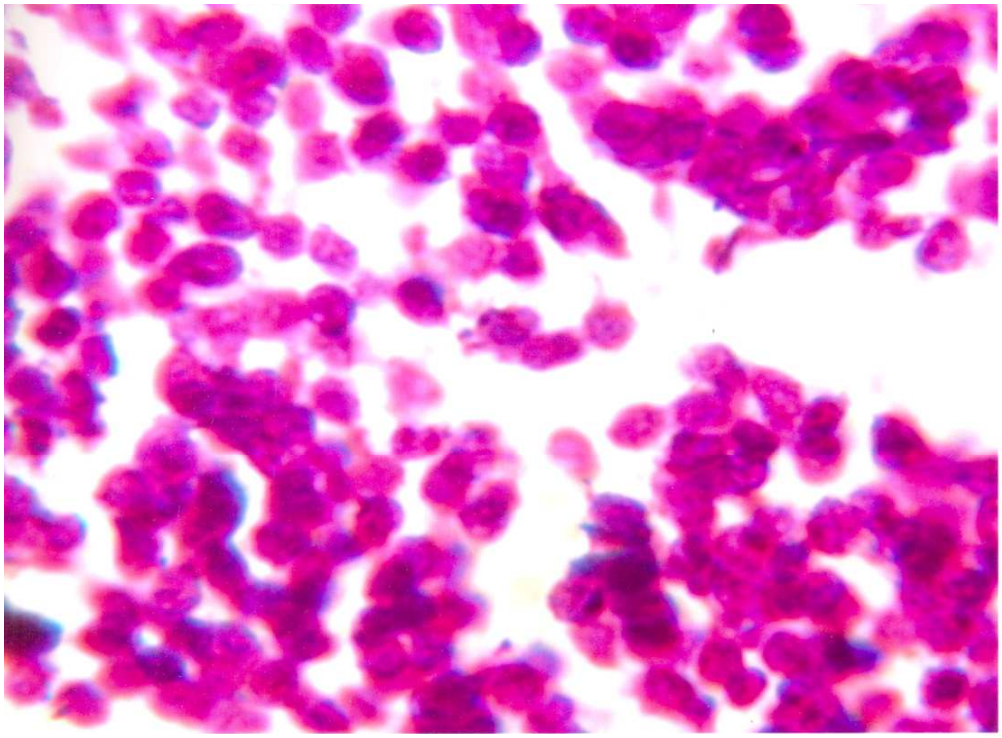

Fig d.: Bone marrow biopsy section showing monotonous population of large lymphoid cells in patient with known case of non-Hodgkin's lymphoma.
(H & E stain, 1000x)

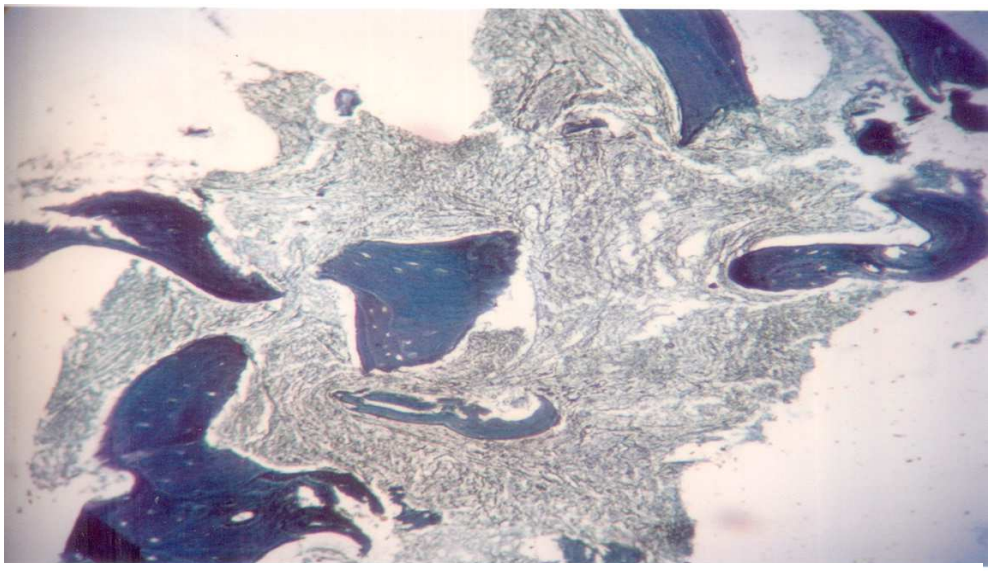

Fig e:s Bone marrow biopsy section in myelofibrosis showing extensive reticulin fibrosis (Reticulin stain, 100x).

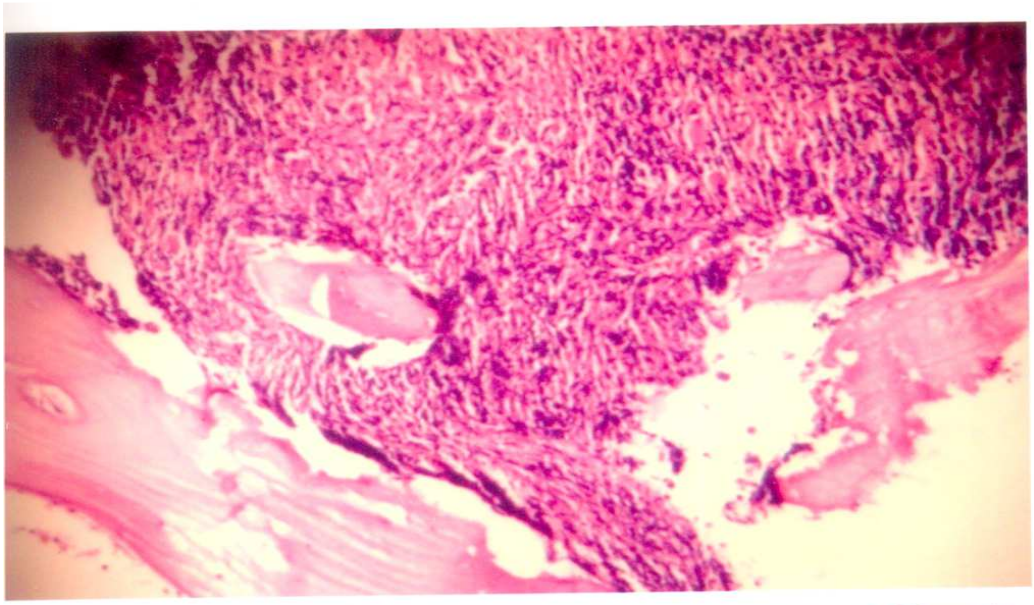

Fig. f : Bone marrow biopsy section in chronic myeloid leukemia showing hypercellularity with myeloid hyperplasisa and occasional blasts cells with myelofibrosis
(H&E stain, 100X)

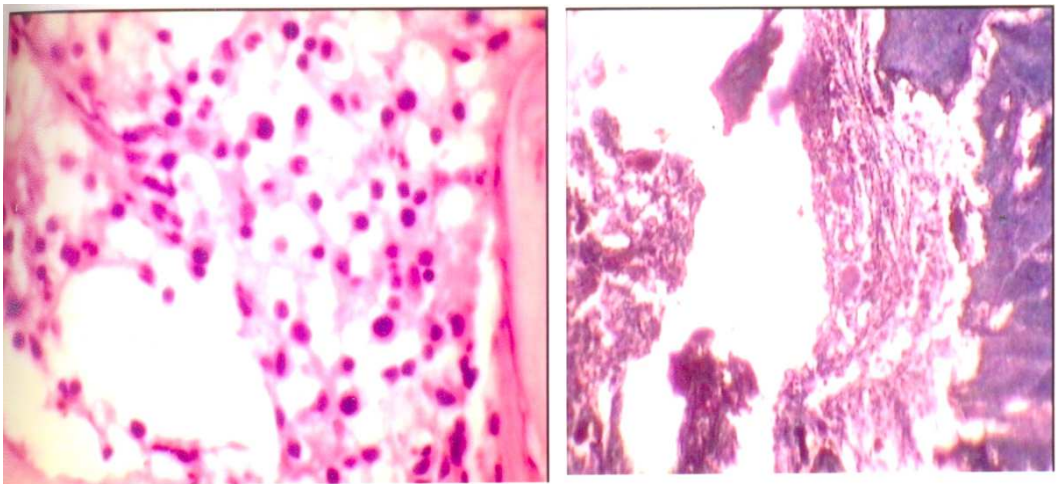

Fig. g : Bone marrow biopsy sections in plasma cell leukemia showing plasma cells with retuculin fibrosis.
(H&E and Reticulin stain, 400X)