Section: Pathology

Original Research Article

A STUDY OF HEMATOLOGICAL AND BONE MARROW FINDINGS ALONG WITH CYTOGENETIC AND MOLECULAR ANALYSIS IN CHRONIC MYELOID LEUKEMIA

Meghavi Joshi[1], Kosha Panchal[2], Himani Patel[3]

[1]Associate Professor, Nootan Medical College and Research Center, Sankalchand Patel University Visnagar, India.
[2]Department of Pathologist, Ahmedabad, India.
[3]Associate Professor Nootan Medical College and Research Center, Visnagar, India.

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Corresponding Author:
Dr. Meghavi Joshi,
Email: joshi.meghavi@yahoo.com

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ABSTRACT

Background: Chronic myeloid leukemia (CML) is the commonest leukemia in India. Myeloproliferative neoplasms are the clonal hematopoietic stem cells disorders characterized by proliferation of one or more of the myeloid lineage cells by proliferation of white blood cells mainly of granulocytic cell lineages. There is a paucity data on cytogenetic and molecular analyses of Indian CML patients. **Aim**: This study aimed to document hematological parameters and bone marrow findings with further evaluation by cytogenetic and molecular analysis for BCR-ABL fusion gene. **Materials and Methods**: Patients were diagnosed as CML over the period of 3 years. Hematological findings and bone marrow findings were correlated and PAS and reticulin stain applied on bone biopsy for detecting fibrosis. The cytogenetic analysis of the cases in our study showed the presence of Philadelphia chromosome and The FISH technique was employed in the cases for the demonstration of the BCR-ABL fusion gene, molecular abnormality of CML via dual color fusion technique. **Result:** Among 100 CML patients, the mean age range of presentation was 41-50 years. males constituting 64% whereas as females were affected relatively less constituting 36%. The Male to Female ratio was 1.7:1. The mean total leukocyte count was $152x10^3$ /cumm with range being 4x103-$700x10^3$/cumm. CML chronic phase most commonly encounter 62%. Hypercellular marrow with 90-95%cellularity, Granulocytic series was significantly increased with all forms of maturation seen. The molecular findings in CML revealed the Ph chromosome in 88% of the cases.The FISH analysis for the detection of the BCR-ABL fusion genes showed positivity in 98%. **Conclusions:** Chronic myeloid leukemia is relatively common leukemia affecting Indian population. The median age is usually between 30 to 50 years with high preponderance in male. The chronic phase is the most common presenting phase but transformation is also much likely in CML, so continuous monitoring of the patients is required during the treatment. The cytogentic analysis of the CML and Molecular evidence of the BCR-ABL fusion gene which is done with a very high sensitivity rate by FISH technique.

INTRODUCTION

Leukemia that are characterized by abnormal proliferation of the hematopoietic cells.[1] Leukemia are the group of disorders that are characterized by abnormal production of blood cells which can be of the various blood forming cells such as of the myeloid or lymphoid lineages.[2] They arise from mutations of the stem cells leading to clonal proliferation of particular lineage. These genetic alterations also are associated with chromosomal alterations resulting in leukemic transformation. Myeloproliferative neoplasms are the clonal hematopoietic stem cells' disorders characterized by proliferation of one or more of the myeloid lineage cells either the granulocytic, erythroid or the megakaryocytic and mast cells characterized by proliferation of white blood cells mainly of granulocytic cell lineages.[3]

Aim and Objectives

- To evaluate hematological Parameters in patients of CML.
- To Study of the Bone Marrow Aspiration (BMA), Trephine Biopsy findings and compare

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diagnostic utility of simultaneous Bone Marrow Aspiration and Biopsy.
- To study Cytogenetic pattern in CML.

MATERIALS AND METHODS

The study included the 100 cases diagnosed clinically, morphologically as CML on bone marrow aspirate findings and in correlation with the peripheral blood findings. Those cases were primarily included in which trephine biopsy was also performed either primarily or during the follow up. The treatment receiving cases who presented during this period were also included.

The investigations that these patients underwent include: Complete blood counts, Peripheral smear examination, Bone marrow aspiration and biopsy, Cytogenetic analysis. Radiological investigation and the clinical details retrieved from the case papers.

Peripheral smear and Bone marrow aspiration is prepared and stained by Wright's stain. Bone biopsy kept in formalin has to be processed further in the form of decalcifying the tissue and further process and stained with haematoxylin and Eosin stain.

RESULTS

Thus, the mean age range of presentation was 41-50 years with the mean age of Presentation being 38 years. Only 2% of the cases were present below the age of 10 years that is in pediatric population with 7% cases in the age of 11-20 years.[table.1] Thus, it was found majority of the affected patients were males constituting 64% whereas as females were affected relatively less constituting 36%.The Male to Female ratio was 1.7:1.[table.2]

The mean Hb was 8.5 gm/dl with the range being 5.0 -12.5 gm/ dl. The mean total leukocyte count was 152x103 /cumm with the mean range being 4x103-700x103/cumm. The mean platelet count was 310x103/cumm with the, mean range being 10x103-1487x103/cumm. [table.3] The morphological presentation of the CML was in three forms: The CML presented in three phases the chronic, accelerated and the blast crisis phase.[table.4,5]

The cases of CML-Blast crisis which constituted 26% of the cases, Immunophenotyping was done for typing of the blast lineage: Myeloid or Lymphoid. The results were 16 cases showed Myeloblastic crisis, 09 cases showed Lymphoblastic crisis and one case showed mix blast crisis. Thus, 62% cases were Myeloblastic crisis, 35% cases were lymphoblastic crisis,3% Mixed blast crisis. As the cases included also, those cases which presented post treatment that is during follow up there was presence of transformed cases from one to the other phase.21% cases showed this type of transformation.[table.6]

The peripheral smear findings showed increased total leukocyte count with predominantly myeloid series hyperplasia of all ranges of maturation along with basophils, eosinophils and blast population. (Fig.1) The Bone marrow aspirate along with bone biopsy was performed in 79% of the cases primarily whereas in remaining cases 21 % the biopsy was performed during the followup. The Bone marrow aspirate findings revealed: Hypercellular marrow with 90-95%cellularity. (Fig.2), Normocellular to hypocellular marrow in follow up cases. Granulocytic series was significantly increased with all forms of maturation seen ranging from myelocytes, metamyelocytes ,band cells , polymorphs, eosinophils and basophils.(Fig.2)Blast % varied according to the phase of CML ranging from 2% to as high as 90% in case of blast crisis.(Fig.3).Normal to increased megakaryocytes with presence of small, hypolobated forms occasionally reffered to as the micromegakaryocytes or Dwarf forms.(Fig 7)The erythroid lineage was markedly suppressed in majority of the cases.

The bone biopsy findings were: Concordant with the aspiration findings as above in terms of cellularity, proliferation of the granulocytic series, megakaryocytic lineage.(Fig 4 and Fig 5)The additional findings evaluated on the bone biopsy were above findings in case of dry aspirate, assess cellularity in cases of diluted marrow or unexplained peripheral cytopenias, and presence of fibrosis.(Fig.8) The megakaryocytic lineage increased and dwarf forms(Fig 7) were highlighted by special stain PAS.PAS stain was carried out in 19 cases which showed where the increased and micromegakaryocytic forms stained pink within the glycogen containing cytoplasm of the megakaryocytes.

The molecular findings in CML revealed the presence of the Ph chromosome which is classically found in CML. The findings showed the Ph chromosome in 88% of the total cases, while morphological diagnosis of CML wherein Ph was found to be negative was in 6% of cases. In 3% of the cases Ph was not done while the morphological diagnosis was CML. Other 3% cases showed Non-Informative karyotype on analysis. [table.7]

The FISH analysis was performed for the detection of the BCR-ABL fusion genes demonstration which showed positivity for fusion in 98% of the cases. Only 2% of cases were negative. The cases which did not show Philadelphia showed the presence of BCR-ABL fusion which is the basic molecular abnormality in CML. Thus, the FISH technique is more sensitive than the conventional karyotyping. The reporting of the BCR-ABL fusion gene was done in the forms of various signals that were recorded via dual colour fusion probes.

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Table 1: Age Distribution

Age range(in years)	No. of Cases with %
5-10	2(2%)
11-20	7(7%)
21-30	25(25%)
31-40	19(19%)
41-50	28(28%)
51-60	14(14%)
61-70	4(4%)
>70	1(1%)
Total	100cases(100%)

Table 2: Sex Distribution of the Cases

SEX	NUMBER OF CASES(%)
MALES	64(64%)
FEMALES	36(36%)
Total	100(100%)

Table 3: Haematological parameters

Parameters	Mean	Range
Hb(gm/dl)	8.5	5-12.5
TLC(x10^3/cumm)	152	4.0-700
Platelet(x10^3/cumm)	310	10-1487
Blast%	10	3-90
Basophils	9	2-30

Table: 4 Morphological presentation of the CML

Phase of CML	Number of cases
CML-CP	62
CML-AP	12
CML-BC	26
Total	100

TablE 5: The association of the three phases with the mean value and range of hematological parameters

	Mean of Parameters		
CML-Phase	**Hb (gm/dl)**	**TLC($x10^3$/cumm)**	**Platelets($x10^3$/cumm)**
CML-CP	8.8	177	338
CML-AP	7.9	62.4	380
CML-BC	7.1	103	112

Table 6: Transformed cases from one to the other phase

Transformed phase	No of cases
CML-CP to CML-AP	07
CML-CP to CML-BC	10
CML-BC to CML-CP	04
Total cases	21

Table 7: Philadelphia chromosome

Philadelphia status	No of cases with %
Ph positive	88(88%)
Ph negative	06(6%)
Not done	03(3%)
Non informative	03(3%)
Total	100(100%)

Table 8: Age distribution in different studies

Study	Mean age in years	Age range
Daley et al[6]	37	14-81
Mottalib et al[7]	37.4	31-40
Ahmed et al[8]	38	18-65
Present study	38	40-50

Table 9: Sex distribution in different studies

Study	Males(%)	Females(%)	M:F ratio
Ahmed et al[8]	68	32	2.2:1
Mottalib et al[7]	67	33	2:1
Daley et al[6]	71	29	2.3:1
Present study	64	36	1.7:1

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Table 10: Hematological parameters of other studies

Study	Parameters(mean and range)		
	Hb (gm/dl)	TLC(X103/cumm)	Platelets(x103/cumm)
Present study	8.5(5-12.5)	152(4-700)	551(10-1487)
Bansal et al[10]	9-11	46-186	
Ahmed et al[8]	9.94	214(17-700)	551(100-1500)

Table 11: Phases of CML in different studies

Study	Phases (%)		
	CML-CP	CML-AP	CML-BC
Present study	62	12	26
Mottalib et al[7]	82	11	5
Daley et al[6]	78	17	5
Ahmed et al[8]	77	15	8

Table 12: Various signals obtained by FISH in our study were similar to the signal patterns in other studies

Signals	Present study	Lim T Het et al[11]	Jain et al[12]
1O1G2Y	82%	71%	74%
1O1G1Y	7%	10%	11%
2O2G1Y	3%	9%	1%
1O2G1Y	5%	3%	4%
1O1G3Y	2%	3%	6%
2O1G1Y	3%	2%	2.5%

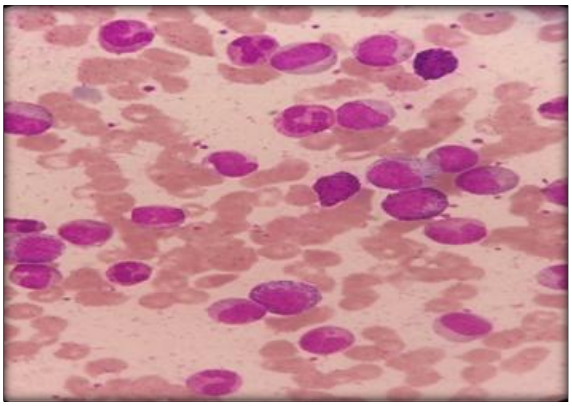

Figure 1: 100xOil immersion lens: CML-CP (Peripheral Smear)

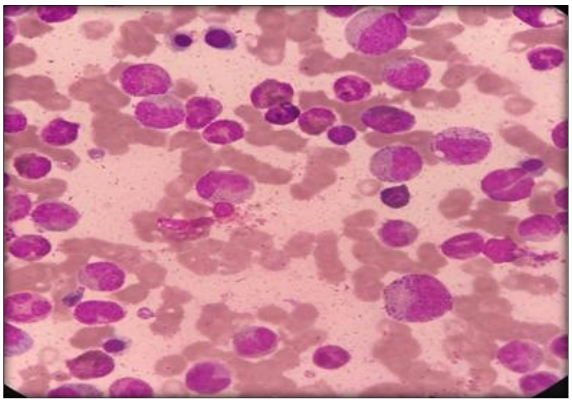

Figure 2: 100x Oil immersion Lens: CML-CP (Bone marrow aspiration)

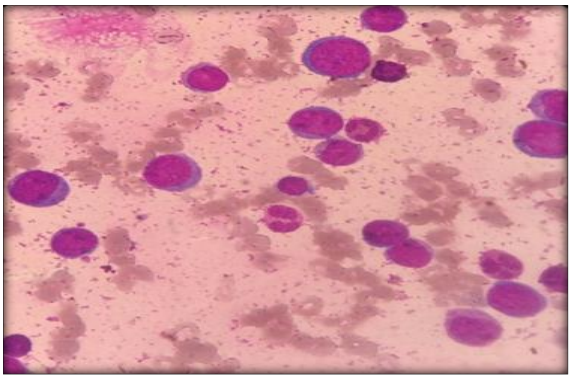

Figure 3: 100X Oil immersion lens: CML-BC (Bone marrowAspiration)

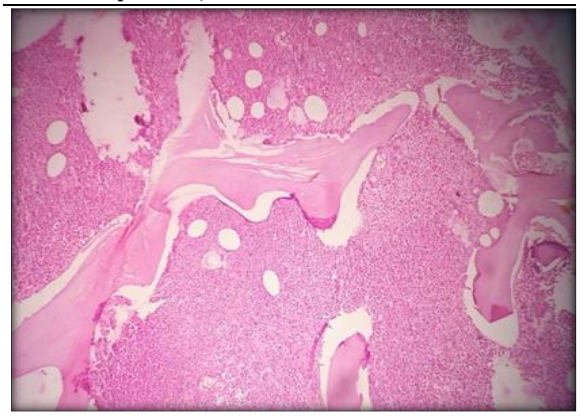

Figure 4: 10x Objective lens: CML-CP, Bone Biopsy

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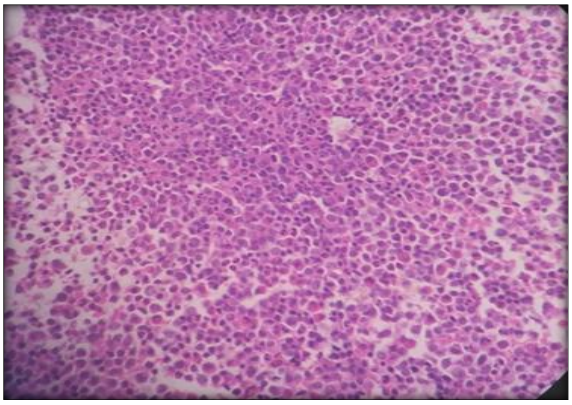

Figure 5: 40x Objective lens: CML-CP, Bone marrow biopsy

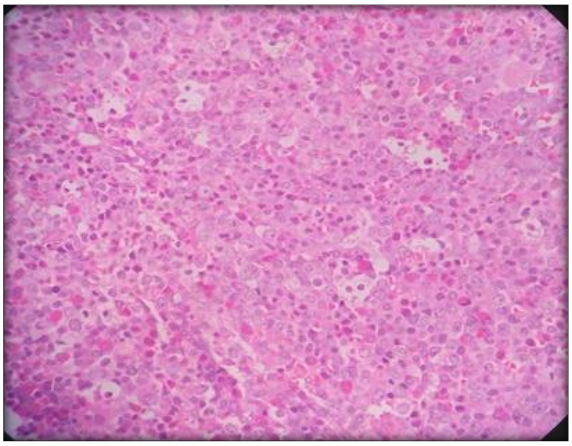

Figure 6: 40x Objective lens: CML-BC, Bone marrow biopsy

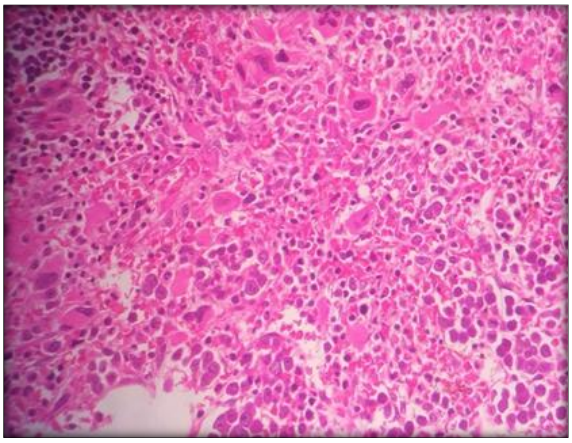

Figure 7: 40x Objective lens: Increased megakaryocytes, Bone marrow biopsy

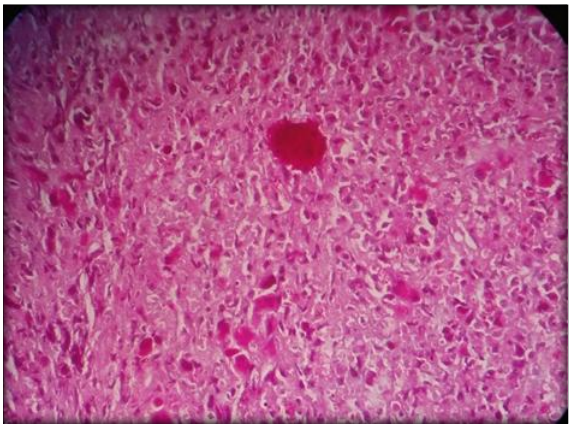

Figure 8: 40x Objective lens, PAS stain for megakaryocytes, Bone marrow biopsy

DISCUSSION

On the basis of the above results and observations in this study, The Mean age of presentation of CML in our study 38 years. Thus, CML usually presents at middle age with maximum cases presenting within a range of 30- 50 years which can correlate with other study [table 8]. The occurrence of CML is relatively less common in pediatric population and very elderly individuals beyond 70 years.[5] Sex distribution of the cases in our study showed a slight male predominance with 64% of males and 36% females. The male to female ratio was 1.7:1. (table - 9)Thus, in CML it was corresponding to other studies that overall the ratio of males to female being affected is high and proving a higher incidence in males.

Clinically majority of patients had fever, malaise, weakness, abdominal pain. The other most important finding on these patients was presence of moderate to gross splenomegaly which was clinically palpable as well as radiologically evident. Splenomegaly was detected in 90% of the patients along with evidence of hepatomegaly in 30% of these cases. In other studies also splenomegaly,[8] was found to be present in 82 to 87% of the cases . So, these findings are consistent with the literature stating splenomegaly one of the diagnostic criteria for CML.

The study of various haematalogical parameters9 revealed that; The mean presentation of Haemoglobin value is 8.5 gm/dl with the range varying from 5.0 to 12.5 gm/dl, mean of the total leukocyte count is 152x103/cumm with the range varying from 4.0 to 700x103/cumm, meanof the platelet count is 310x103/cumm with the range varying from 10 to 1487x103/cumm. It was corresponding to other studies in table.[10]The mean number of blasts in our study is 9 varying from 2 to 91 depending on the various phases. In Ahmed et al study the mean blast% were 9% with range varying from 2 to 50%.In Motallib et al, the mean blast% were 10% with range ranging from 2 to 30%.[7]

Thus, mean values of hemoglobin, TLC, platelets and blast% were close to the other study findings

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but with respect to others blast% range was on a higher side (91%). The phase distribution of CML in our study is the Chronic phase constituting 62%, Accelerated phase constituting 12% and Blast crisis phase constituting 26% which are comparable to other studies in table.[11]

Thus, it is found that majority of the patients of CML belong to the chronic phase followed by accelerated and blast crisis phase but in our study the blast crisis distribution was higher as compared to other studies either in primary diagnosis or during follow up.In case of blast crisis, in our study myeloblastic crisis constituted 62%, lymphoblastic 35% and mixed crisis in 3%.In Daley et al,[6] in case of Blast crisis morphologically 53% cases were myeloblastic,30% lymphoblastic and remaining 17% definite typing was not done. This ascertains the findings that the myeloblastic crisis are more common than the lymphoblastic crisis in the CML-BC phase.

The bone marrow aspirate and biopsy findings were according to the WHO criteria showing;.[3,4] Hypercellular marrow with granulocytic series predominance. Adequate, increased or dwarf forms of megakaryocytes. Relatively suppressed erythroid lineages, Thus, increased M: E ratio. Varying % of Blast classifying the disease into its defined phase distribution and Varying % of Basophils which a characteristic finding of CML.

The cytogenetic analysis of the cases in our study showed the presence of Philadelphia chromosome in 88% of the cases, which is classic abnormality of CML. The Ph negative, non-informative karyotype and cases where the Investigation was not done constituted remaining 12%. In the Ahmed et al study, 86% cases were Philadelphia positive. In Lim T het al study, 87%cases were Philadephia positive.[11]

The FISH technique was employed in the cases for the demonstration of the BCR-ABL fusion gene, molecular abnormality of CML via dual color fusion technique. The BCR-ABL fusion gene was recorded in form of yellow fusion signals with orange and green signals representing ABL and BCR gene.[12,13] The characteristic pattern demonstrated was 1O1G2Y signals in 82% of the cases the classic positivity for fusion. Various signals obtained by FISH in our study were similar to the signal patterns in other studies. table.[12]

CONCLUSION

Chronic myeloid leukemia is relatively common leukemia affecting Indian population with median age of being affected is usually between 30 to 50 years with variable but uncommon presentation in children and older individuals. The male sex is affected with high preponderance than females. The chronic phase is the most common presenting phase (62%) with accelerated and blast crisis phase occurring variably. The disease transformation is also much likely in CML, so continuous monitoring of the patients is required to predict this kind of transformation during the treatment. The diagnosis of CML can be made by peripheral blood findings which show the granulocytic predominance, Basophilia, variable number of blasts but it is supported by the bone marrow aspirate and trephine biopsy findings and also clinical parameters like marked splenomegaly. The other important need for aspirate is for the cytogenetic analysis of the CML and the diagnostic parameter is molecular evidence of the BCR-ABL fusion gene which is done with a very high sensitivity rate by FISH technique. The cytogenetic study is also required during follow-up to monitor the treatment response and establishing the remission criteria.

Conflict of Interest: The authors declare no conflict of interest

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