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A Clinicopathological study of 110 cases of leukemia at tertiary care center

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Abstract
Introduction: Leukemia is a hematological disorder which needs timely diagnosis and early treatment because at initially leukemic cell burden is low. So, the chances of achieving complete remission will be highest.
Aims: The present study was undertaken for correlation of patient's signs and symptoms with their laboratory findings like CBC, peripheral smear findings, immunophenotyping to know the lineage specificity and cytogenetics to know molecular abnormality.
Methods and Material: The blood samples of suspected patients were received and examined at Hematology and Surgical pathology sections of Central Diagnostic Laboratory from November 2018 to June 2020. The clinical history was obtained, CBC was performed, peripheral smear and bone marrow samples were received for morphological examination and special investigations like Immunophenotyping, Cytogenetics and fish.
Results: Out of the 110 cases, 17 were ALL, 33 were AML, 16 were CLL, 29 were CML. other 15 cases were distributed in acute leukemia, lymphoma, myelofibrosis and acute undifferentiated leukemia.
Conclusions: Present study revealed that acute leukemia was more prevalent than chronic leukemia. The most common type of leukemia was AML followed by CML, ALL and CLL. ALL was mostly observed in children whereas CML and CLL were mostly observed in adults. AML mostly observed in adults.

Keywords: Acute leukemia, all, AML, CLL, CML, immunophenotyping, fish

Introduction
Leukemia is a disease resulting from the neoplastic proliferation of haemopoietic cells. It results from mutation of a one stem cell, the progeny of which form a clone of leukemic cells. Usually there is a series of genetic alterations instead of one event. The cell during which the leukemic transformation occurs could also be a lymphoid precursor, a myeloid precursor or a pluripotent haemopoietic stem cell capable of differentiating into both myeloid and lymphoid cells.
Genetic alterations resulting in leukemic transformation often result from major alterations within the chromosomes, which may be detected by microscopic examination of the chromosomes of cells in metaphase. Such clonal evolution can lead to transformation into a more aggressive or treatment refractory form of the disease with an associated worsening of prognosis.
Leukemia is classified based on the aggressiveness of the illness and differentiation of the leukemic cells as:
1. Acute, an aggressive disease
2. Chronic, a less aggressive form.

They are further divided into lymphoid, myeloid, and mixed lineage (biphenotypic or bilineage) leukemia, the latter showing usually both lymphoid and myeloid differentiation. Distinction between lymphoid and myeloid leukemias is important as treatment/prognosis differs a lot.
The clinical manifestations of the leukemia are directly or indirectly due to the proliferation of leukemic cells and their infiltration in various tissue. Increased cell proliferation has metabolic consequences and infiltrating cells also disturb tissue function. Anemia, neutropenia and thrombocytopenia are important consequences of infiltration of the bone marrow, which successively can cause weakness, infection and hemorrhage. Infections cause

many of the main complications and most deaths in patients with acute leukemia. The combined evaluation of leukemia by morphology, cytogenetics and immunophenotyping is extremely important for an accurate diagnosis and for planning treatment. Hence this study was being conducted to diagnose and classify the type and sub-type of leukemia by a combination of morphology, cytochemistry, immunophenotyping and cytogenetics.

Materials and Methods

The present study is a prospective and retrospective study of 110 cases of leukemia. Peripheral venous blood, bone marrow aspirate samples and biopsy of suspected leukemic patients received and examined at Central Diagnostic Laboratory from November 2018 to June 2020.

The relevant clinical history was obtained in each case, routine blood counts performed and peripheral smear studied in detail. About 0.5-1ml of bone marrow sample was collected from each case in EDTA and heparinized tubes. Smears from EDTA samples were used for CBC and stained with Geimsa stain. Bone marrow aspiration smears are stained with standard Romanowsky stain (Leishman's stain) and Geimsa stain and studied for morphology of cells. CBC was performed on 5-part differentiation hematology analyser XN-550.

The cases were diagnosed by the morphology of leukemic cells (immature cells of myeloid or lymphoid series). Sometimes it is very difficult to classify acute leukemia in myeloid and lymphoid lineage accurately. In such cases modern modalities like flowcytometry, cytochemistry, cytogenetics and molecular study are required to classify the cases of acute leukemia in myeloid and lymphoid Lineage. In developing country like India, the new modalities, flowcytometry and cytochemistry are not available in most of the laboratories and in our center too.

Statistical analysis

Descriptive statics [mean (SD), frequency (%)] was used. Clinical and pathological findings were assessed through proportion.

Results

Total 110 cases studied during the period, from November 2018 to June 2020, in the Central Diagnostic Laboratory. Of the 110 cases studied, 17 were diagnosed as acute lymphoid leukemia, 33 were diagnosed as acute myeloid leukemia, 16 were diagnosed as chronic lymphoid leukemia, 29 were diagnosed as chronic myeloid leukemia, and other 15 cases were distributed in acute leukemia, lymphoma, myelofibrosis and acute undifferentiated leukemia [Table 1]. An initial demographic patient profile was rendered based on age and gender distribution.

The youngest patient encountered in the present study was a one-year-old male toddler and the eldest patient encountered was a ninety-six-year-old female. The present study showed a male preponderance with the Male: Female ratio of 1.34:1. Majority of the males belonged to the age group of 21 to 30 years (17.46 %), 61-70 years (17.46 %) and majority of the females belonged to the age group of 51-60 years (19.1 %) [Table 2]. The most common clinical manifestation was generalised weakness followed by fever [Table 3]. Other sign/symptoms include nausea and vomiting, epistaxis, excessive sweating, chest pain and cough, headache, giddiness, tachypnoea, oedema in both legs, dyspnoea on exertion, easy bruising. Very high total leukocyte count was seen mostly in CML cases. Low total leukocyte count was seen in 2 cases of ALL. Haemoglobin value below 11g/dl in children, below 12g/dl in females and below 13g/dl in males is considered as anemia. Low platelet count was seen in majority of CLL followed by AML cases. High blast cell count was seen in AML [Table 4].

Table 1: Age wise distribution of the cases

Age	ALL	AML	CLL	CML	No	(%)
0-10	4	1	0	1	6	5.4
11-20	5	2	0	0	7	6.3
21-30	4	5	0	3	12	10.9
31-40	1	6	1	5	13	11.8
41-50	2	3	2	7	14	12.7
51-60	0	8	1	5	14	12.7
61-70	1	5	7	4	17	15.4
71-80	0	2	3	4	9	8.18
>80	0	1	2	0	3	2.72
Total	17	33	16	29	95*	-

*Out of 110 cases 15 cases were distributed in acute leukemia, lymphoma, myelofibrosis and acute undifferentiated leukemia

Table 2: Age and gender wise distribution of the cases

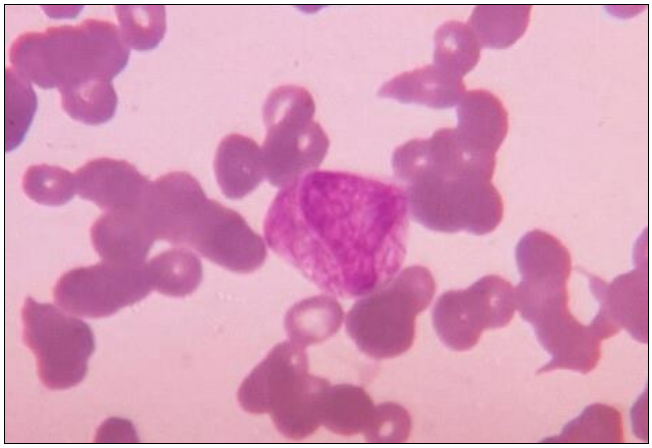
Age in years	Gender		Total
	Male No (%)	Female No (%)	
0-10	3 (37.5%)	5 (62.5%)	8
11-20	6 (75.0%)	2 (25.0%)	8
21-30	11 (68.8%)	5 (31.3%)	16
31-40	6 (42.9%)	8 (57.1%)	14
41-50	8 (50.0%)	8 (50.0%)	16
51-60	9 (50.0 %)	9 (50.0 %)	18
61-70	11 (64.7%)	6 (35.3%)	17
71-80	7 (70.0 %)	3 (30.0 %)	10
>80	2 (66.7%)	1 (33.3%)	3
Total	63 (57.3%)	47 (42.7%)	110

Table 3: Frequency of clinical symptoms and signs

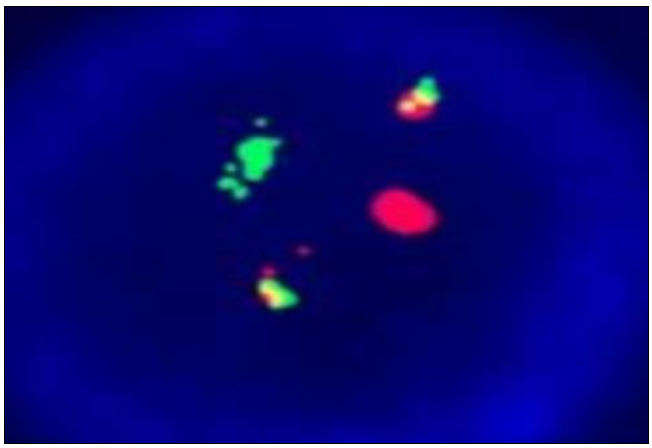
Signs/Symptoms	Total	Frequency (%)
Fever	41	32.27
Generalised weakness and easy fatigability	79	71.81
Abdominal pain	19	17.27
Pallor/anemia	10	9.09
Hepatosplenomegaly	9	8.18
Hepatomegaly	2	1.81
Splenomegaly	9	8.18
Lymphadenopathy	7	6.36
Loss of appetite	5	4.54
Loss of weight	7	6.36
Others	30	27.27

Table 4: Frequency of laboratory indices in leukemic patients

Parameter	Range	ALL		AML		CLL		CML	
		No	%	No	%	No	%	No	%
Total WBC Count/ µL	<4000	2	11.8	6	18.2	0	0	1	3.4
	4000-11000	2	11.8	4	12.1	0	0	0	0
	11100-25000	6	35.3	2	6.1	3	18.8	0	0
	25100-50000	2	11.8	7	21.2	3	18.8	2	6.9
	50100-100000	2	11.8	4	12.1	5	31.3	4	13.8
	>100000	3	17.6	10	30.3	5	31.3	22	75.9
Hb (Gm/dl)	<6	7	41.2	12	36.4	1	6.3	3	10.3
	6.1-9	6	35.3	16	48.5	5	31.3	14	48.3
	>12	4	23.5	5	15.2	10	62.5	12	41.4
Platelet count µL	<20000	6	35.3	11	33.3	2	12.5	0	0
	20000-50000	6	35.3	13	39.4	0	0	2	6.9
	50100-100000	2	11.8	3	9.1	3	18.8	4	13.8
	>100000	3	17.6	6	18.2	11	68.8	23	79.3
% of blasts in peripheral blood on first presentation	Absent	5	29.4	3	9.1	13	81.3	6	20.7
	<20%	2	11.8	2	6.1	2	12.5	22	75.9
	20-50%	5	29.4	12	36.4	0	0	0	0
	51-80%	4	23.5	8	24.2	1	6.3	1	3.4
	>80%	1	5.9	8	24.2	0	0	0	0



(a)



(b)

Fig 1: AML-M3. (a) AML-M3 with "faggot cells" having numerous Auer rods in the cytoplasm- Giemsa stain (X1000) in peripheral blood smear. (b) FISH findings are consistent with acute promyelocytic leukemia (AML M3). There is an evidence of PML/RARα fusion gene in 99% cells studied

Discussion

In the present study 110 cases of leukemia were evaluated by morphology, cytochemistry, cytogenetics, FISH and immunophenotyping to arrive at a final diagnosis. The duration of the study was from November 2018 to June 2020, in the Hematology and Surgical pathology sections of Central Diagnostic Laboratory, Shree Krishna Hospital, Karamsad.

In our study majority of the cases were of AML (33) followed by CML (29), ALL (17), and then CLL (16) and 15 cases included in category of others. Vinsheth *et al.* [1] in their study found CML as the commonest leukemia (110) followed by AML (18) and ALL (10). Kaushal *et al.* [2] too found CML as the commonest leukemia (14) followed by ALL (9). Agase *et al.* [3] in their study found CML as the commonest leukemia (35) followed by ALL (23), AML (11) and CLL (1). Rajnikant *et al.* [4] in their study found CML as the commonest leukemia (31) followed by AML (25), ALL (21) and CLL (2). Our study is almost in concordance with other studies except for higher number of AML cases which could be due to population bias.

In our study, out of 33 cases of AML, AML-M3 with 5 cases was the commonest followed by M5 with 3 cases and M3 with 1 case. This is in league with the study done by Ghosh *et al.* [5] with AML-M2 being the commonest subtype in adults (32%) and also in children (42%).

In the present study there was a male preponderance as in other studies. 57.3 % of cases were males and 42.7 % females, the male: female ratio being 1.34:1. Kaushal *et al.* [2] in their study reported male: female ratio of 1.16:1. Rajnikant *et al.* [4] in their study reported male: female ratio of 0.73:1. Alpana *et al.* [6] in their study reported male: female ratio of 2.77:1.

Different types of leukemia have different clinical presentation. In our study, the most common clinical presentation of patients was generalised weakness followed by fever (consistently seen in cases of AML). The study by Kaushal *et al.* [2] found splenomegaly as the commonest presentation (81%) followed by hepatomegaly (69%) and fever (61%). In the study done by Radha *et al.* [7] low-grade fever were the commonest clinical findings (89.15%) followed by progressive pallor (77.5%).

In present study high total leukocyte count was found in CLL followed by CML, AML and ALL**.** Kaushal *et al.* [2] found high total leukocyte count in ALL followed by CML and AML. Munir *et al.* [8] found high total leukocyte count in AML followed by CML and ALL. Agase *et al.* [9] found high total leukocyte count in AML and CML followed by ALL

and CLL. Rajnikant *et al.* [4] found high total leukocyte count in AML and CML followed by ALL and CLL.

In our study, chromosomal study was done in 34 cases among which abnormalities were present in 26 out of 110 cases (23.6%). In out of 8 cases, 6 cases showed no any chromosomal abnormality. In rest of 2 cases, cytogenetic analysis was not possible due to unavailability of metaphase

Akbar Safaei *et al.* [10] found chromosomal abnormalities in 142 out of 168 cases (84.5%) and in the study by Kaushal *et al.* [2] found 8 cases out of 26 cases had chromosomal abnormalities (30.7%). Detection of chromosomal abnormalities depends on multiple factors like, type of leukemia, percentage of blast cells in the sample, the technical expertise in culture preparation and banding and on the expertise of the analysing cytogeneticist. These could be the reason for variation in results by different study groups.

In our study, out of 33 cases of AML, chromosomal study was done in 8 (24.2%) cases in which chromosomal abnormality was found in 3 cases among which 2 cases showed deletion of chromosome 7 and one case showed reciprocal translocation between the long arms of one of the chromosomes 11 and 22, between the regions q23 and q11.2 respectively, found in 65% of the metaphases studied, suggestive of Philadelphia positive chromosome complement. In other 2 cases no chromosomal abnormality was detected. In one case study was not possible due to unavailability of metaphase**.** Moorman *et al.* [11] in their study on AML, found chromosomal abnormality in 76% of cases (593 out of 779).

In our study, out of 17 cases of ALL, chromosomal study was done in 2 (11.7%) cases, in one case BCR-ABL was not detected and in other case it was not possible due to unavailability of metaphase**.** In the study done by Settin *et al.* [12] in 40 out of 63 (63.4%) cases they found that chromosomes 9, 11 and 22 were the most frequently involved.

The study done by Praveena *et al.* [13] showed accurate subtyping of AML cases in 5 out of 20 cases of AML (25 %). The study done by Zheng *et al.* [14] showed accurate subtyping of AML cases in 33 out of 122 cases of ALL (26.2 %).

The study done by Zhong *et al.* [15] showed accurate subtyping of ALL cases in 15 out of 30 cases of ALL (50 %). The study done by Chana *et al.* [16] showed accurate subtyping of ALL cases in 8 out of 15 cases of ALL (53.3 %).

The variation in the accuracy of ALL diagnosis by cytogenetics in our study is probably due to suboptimal quality of the bone marrow samples we received mostly from the pediatric age group. Some of our cases showed nonspecific findings and others showed normal karyotype and hence accurate subtyping was not possible in some of these.

Out of the 29 cases of CML in our study, 23 cases (79.3%) showed Philadelphia positivity including one case showing complex karyotyping with multiple abnormality including deletion of long arm of 3, 4,11 and short arm of chromosome 10, translocation between 7 and unknown chromosome, loss of 15, 17,18 21 and chromosome Y, final diagnosis given as CMML.

The study done by Kasakyan *et al.* [17] showed Ph positivity in 26 out of 32 cases of CML (81.2 %). The study done by Stoll *et al.* [18] showed Ph positivity in 45 out of 52 cases of CML (86.5 %). The study done by Bernstein *et al.* [19] showed Ph positivity in 61 out 68 cases of CML (89.7%) and identified 40 cases by banding alone.

The study done by Finn *et al.* [20] showed chromosomal abnormality in 14 out of 26 cases (53%). The study done by Cuneo *et al.* [21] showed chromosomal abnormality in 14 out of 57 cases (24.5%). The study done by Marco *et al.* [22] showed chromosomal abnormality in 39 out of 580 cases (9%).

In our study, none of the CLL cases showed chromosomal abnormalities in contrast to other studies. This could be due to FISH technique that was used along with conventional karyotyping in other studies.

Out of the 16 cases of CLL, in our study, one cases showed normal karyotype and in other case the result was unsatisfactory.

Cytogenetics also helped us in identifying additional chromosomal abnormalities like double minutes (+dmins), ring chromosomes and variant translocations. Double minutes indicate a higher degree of aggressiveness of malignancy and signify a worse prognosis. The significance of ring chromosomes has not exactly been proven. Variant translocations in CML have been described by other authors. According to Reichard *et al.* [23] patients with variant and classic Philadelphia producing translocations are clinically, hematological and prognostically identical.

FISH was done in 2 cases of AML in which one case was of acute promyelocytic leukemia in the present study. In another case Philadelphia positive chromosome complement was found in AML [Fig-1].

The study done by Xiao *et al.* [24] showed 96.4% accuracy rate in diagnosis of acute leukemia by flow cytometry. The study done by Scherrer *et al.* [25] showed 99.1% accuracy rate in diagnosis of acute leukemia by flow cytometry.

In our study, flow cytometry was performed in 49 cases and it provided us a definitive diagnosis in 47 cases. In rest of the 2 cases, one case showed acute undifferentiated leukemia and, in another case, definite diagnosis was not possible because of poor specimen quality so no definite lineage analysis or gating strategy possible.

Out of 110 cases, cytogenetic examination was performed in 34 cases and it provided us a definitive diagnosis in 32 cases. In rest of 2 cases, cytogenetic analysis was not possible due to unavailability of metaphase

So, finally using morphology, cytogenetics, immunophenotyping and FISH findings accurate diagnosis was arrived in 95 cases (81.8%). In the remaining 15 cases, 12 were diagnosed as acute leukemia, 1 was diagnosed as acute undifferentiated leukemia but definite subtyping was not possible as the cytogenetic study did not show any specific abnormality of any subtype, other 2 were diagnosed as diffuse large B cell lymphoma/ Burkitt lymphoma and myelofibrosis (grade II) respectively.

Immunophenotyping alone gave a definitive diagnosis in 16 cases (14.5%) where other investigations were not sufficiently informative.

Thus, in our study on leukemia, cytogenetics and immunophenotyping played a vital role along with the cell morphology in determining the type and subtype of leukemia.

Conclusions

The incidence of various types of leukemia varies in different age groups; there are racial and ethnic differences too. There could also be population bias which occurs in a tertiary care center like our institute, leading to variation

within the prevalence of various types of leukemia.

Present study revealed that acute leukemia was more prevalent than chronic leukemia. The most common type of leukemia was AML followed by CML, ALL and CLL. Leukemia was more commonly seen in male patients. Age has significant effect on type of leukemia. ALL was more commonly observed in children whereas CML and CLL were mostly observed in adults. The incidence of AML was higher in adults as compare to children and decreased towards older age. The majority of patients had lower Haemoglobin, high leukocyte count and lower platelet count. Generalized weakness, low grade fever, abdominal pain were most common symptoms in patients.

Cytogenetics and immunophenotyping play a vital role in elucidating the genetic abnormality associated with each type and subtype of leukemia and in tracing the lineage specificity of leukemia. It helps in arriving at a final diagnosis especially in cases with ambiguous morphology on peripheral blood smear and bone marrow examination.

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