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Research Article

CLINICAL, HEMATOLOGICAL AND IMMUNOPHENOTYPIC PROFILE OF ACUTE LEUKEMIA IN A TERTIARY CARE CENTRE IN SOUTH TAMILNADU

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A B S T R A C T

Objectives: To study clinical, hematological and immunophenotypic profile of 60 cases of de novo acute leukemia at diagnosis.
Materilas and Methods: This is a retrospective analysis of 60 de novo cases of acute leukemia in our institution at a period of three months from June to august of 2018. Diagnosis was based on peripheral blood and bone marrow examination for morphology, cytochemistry and immunophenotypic studies. Statistical analysis was performed by the SPSS software package, version 10.
Results: Among the total of 60 de novo adult leukemia patients, 33 (55%) cases were AML and 27 (45%) cases were ALL. Among the AML cases, 26 (78.8%) were males and 7 (21.2%) were females. Among the ALL cases, 12 (44.4%) were males and 15 (55.6%) were females. The majority of our AML cases presented in the age group of 15-30 years (40.7%). The commonest FAB subtype in our study was AML-M2 at 44.4% followed by AML-M1 at 37%. In both AML and ALL, the most common complaint was fever, 66.7% and 69.7% respectively. The most common feature was lymphadenopathy (78.8%) followed by spleenomegaly (60.6%)
Conclusion: AML is the most common type of leukemia in our study, due to increased referral of cases to our tertiary care centre and also due to good response of ALL cases to treatment, and so they are being admitted less in our hospital. Immunophenotyping is necessary tool for diagnosing and initiating treatment.

INTRODUCTION

Acute Leukemias are a group of hematological neoplastic disorders characterized by proliferation and accumulation of immature hematopoietic cells in blood and bone marrow and in other tissues. These immature malignant cells are called blast cells. There is also impaired production of normal blood cells. They are further classified into Myeloid and Lymphoid lineage depending upon the character of the precursor cell seen. The two main morphological types of acute leukemia are Acute Myeloid Leukemia (AML) and Acute Lymphoblastic Leukemia (ALL). There is geographical variation in occurrences of these leukemias making it necessary for each Centre to have its own data. Incidence of AML increases with age and above the age of 65 its incidence is 30 times more than that of children. ALL is a childhood cancer but when it accounts for 20% of acute leukemia in adults, its prognosis is poor. Immunophenotyping has become the backbone for diagnosis and classification of leukemia. Every blood cell has cluster differentiation (CD) antigen, which is identified and quantified through immunophenotyping.

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The various clinical, hematological and immunophenotypic studies help in further understanding the of the disease and help in development of newer treatment strategies for better life. In this study, we retrospectively analyzed about 60 cases of de novo leukemia, both AML and ALL, in relation to its incidence, morphology, cytochemistry and immunophenotype at the time of diagnosis.

MATERIALS AND METHODS

A total of 60 adult cases of de novo leukemia which were diagnosed at at our institution, Govt Rajaji Hospital, Madurai medical college, at a period of 3 months in year 2018 were analyzed for our study. Totally 38 male patients and 22 female patients were observed. Clinical details and diagnostic workup were complete and all the patients were diagnosed by peripheral blood count, peripheral smear examination, bone marrow aspiration and immunophenotypic studies. The morphological diagnosis was supplemented by immunological diagnosis. Previously untreated patients were included in our study. No cases of chronic leukemia were eligible. Immunophenotyping was done by flow cytochemistry on Ficoll-Hypaque separated mononuclear cells from heparinised bone marrow aspirates or peripheral blood. Commercially available monoclonal antibodies were used. They are CD 34,

human leukocyte antigen (HLA) DR, CD 13, CD 33, CD 117, CD 79, CD 22, CD 10, CD 19, CD 3, CD 2, CD 5, CD 7. For all the above markers, blasts were considered positive, if 20% or more expressed the particular marker, and others were considered as negative. Statistical analysis was performed by the SPSS software package, version 10.

RESULTS

Among the total of 60 de novo adult leukemia patients, 33 (55%) cases were AML and 27 (45%) cases were ALL. Among the AML cases, 26 (78.8%) were males and 7 (21.2%) were females. Among the ALL cases, 12 (44.4%) were males and 15 (55.6%) were females.

Table 1 clinical and hematological parameters of Acute Myeloid Leukemia patients:Parameter frequency (%)

PARAMETER	FREQUENCY (%)
AGE(YEARS)	
15-30	11 (40.7%)
31-50	8 (29.6%)
51-65	7 (25.9%)
66<	1 (3.7%)
SYMPTOMS	
FEVER	18 (66.7%)
GUM BLEEDING	6 (22.2%)
GUM HYPERTROPHY	3 (11.1%)
EPISTAXIS	6 (22.2%)
BREATHLESSNESS	1 (3.7%)
LEUKEMIA CUTIS	1 (3.7%)
ABDOMEN PAIN	1 (3.7%)
BONE PAIN	2 (7.4%)
MALENA	1 (3.7%)
SIGNS	
HEPATOMEGALY	8 (29.6%)
SPLEENOMEGALY	13 (48.1%)
LYMPHADENOPATHY	6 (22.2%)
PETECHIA, PURPURA	3 (11.1%)
BONE TENDERNESS	3 (11.1%)
HEMOGLOBIN (gm/dl)	
<5	15 (55.6%)
5-10	11 (40.7%)
>10	1 (3.7%)
WHITE BLOOD CELLS	
(cells/cu mm)	4 (14.8%)
<5000	15 (55.6%)
5000-50000	5 (18.5%)
50000-100000	3 (11.1%)
>100000	
PLATELET COUNT	
(cells/cu mm)	16 (59.3%)
<50000	10 (37%)
50000-100000	1 (3.7%)
>100000	
BLAST CELLS IN	
BONE MARROW	16 (59.3%)
20%-90%	11 (40.7%)
>90%	
BLAST CELLS IN	
PERIPHERAL SMEAR	
<20%	2 (7.4%)
20%-90%	17 (63%)
>90%	8 (29.6%)

Clinical Features

The majority of our cases presented in the age group of 15-30 years (40.7%). Most of our patients had fever (66.7%) as a major complaint during presentation and splenomegaly (48.1%) was the most common finding. 12 (44.4%) patients presented with bleeding at the time of presentation. One patient had leukemia cutis at the time of presentation and it was acute myelomonocytic type. (Table 1)

FAB subtype

The commonest FAB (French American British classification) subtype in adults in our series in AML-M2 at 44.4% followed by AML-M1 at 37%. There are no cases of M5, M6, M7 in our study.

Table 2 distribution of Acute Myeloid Leukemia according to fab subtype

Fab subtype	Number of patients (%)
M1	10 (37%)
M2	12 (44.4%)
M3	2 (7.4%)
M4	3 (11.11%)

Laboratory parameters

The lowest WBC count was $2.1x10^9$/L and highest count was $160x10^9$/L. Both these observations were seen in AML-M1. The percentage of blast in bone marrow was least in AML-M3, about 40%. The percentage of blast in peripheral smear was low in AML-M4 which is 25%. Pancytopenia was observed in 4 (14.8%) patients. The hemoglobin level ranges from 7.6 gm/dl to 3.4 gm/dl. Platelet count varied from least count of $4.0x10^9$/L and $150x10^9$/L. Table 1

Table 3 Immophenotype Profile of Acute Myeloid Leukemia Patients

Fab Subtype	HLA-DR(%)	CD34(%)	CD13(%)	CD33(%)	CD117(%)	MPO(%)
M1	0(0)	0(0)	10(100%)	10(100%)	10(100%)	6(60%%)
M2	7(58.3%)	12(100%)	12(100%)	0(0)	7(58.3%)	10(83.3%)
M3	0(0)	0(0)	1(50%)	2(100%)	2(100%)	0(0)
M4	0(0)	3(100%)	3(100%)	3(100%)	0(0)	1(33.3%)

Mpo- Myeloperoxidase

Immunophenotyping

In our study, HLA DR was highest in case AML-M2 and was less common in others. CD34, CD117, MPO, HLA DR are absent in M3, acute promyelocytic leukemia. It was positive in M2. Table 3

Table 4 Clinical And Hematological Parameters of Acute Lymphoblastic Leukemia Patients

PARAMETER	FREQUENCY (%)
AGE(YEARS)	
15-30	26 (78.8%)
30-65	6 (18.2%)
>66	1 (3%)
SYMPTOM	
FEVER	23 (69.7%)
BLEEDING GUMS	4 (12.1%)
EPISTAXIS	2 (6.1%)
BREATHLESSNESS	6 (18.2%)
TESTICULAR SWELLING	1 (3.03%)
MALENA	2 (6.1%)
SIGNS	
LYMPHADENOPATHY	26 (78.8%)
HEPATOMEGALY	14 (57.6%)
SPLEENOMEGALY	20 (60.6%)
BONE TENDERNESS	27 (81.8%)
PLEURAL EFFUSION	2 (6.1%)
PERICARDIAL EFFUSION	1 (3%0
SVC OBSTRUCTION	1 (3%)
CARDIAC TAMPONADE	1 (3%)
RETINAL HEMMORHAGE	1 (3%)
HEMOGLOBIN (gm/dl)	
<5	7 (21.2%)
5-10	19 (57.6%)
>10	7 (21.2%)

WHITE BLOOD CELLS (cells/cu mm)	8 (24.2%)
<5000	16 (48.4%)
5000-50000	8 (24.2%)
50000-100000	1 (3%)
>100000	
PLATELET COUNT (cells/cu mm)	18 (54.5%)
<50000	8 (24.2%)
50000-100000	7 (21.2%)
>100000	
BLAST IN BONE MARROW	
<20%	3 (9.1%)
20-90%	12 (36.4%)
>90%	18 (54.5%)
BLAST IN PERIPHERAL SMER	9 (27.3%)
<20%	14 (42.4%)
20%-90%	10 (30.3%)
>90%	
CELL LINEAGE	
B ALL	14 (42.4%)
T ALL	19 (57.6%)

Clinical Feature

In ALL most common complaint was fever 69.7% and one patient presented with testicular swelling. In our study the most common feature was lymphadenopathy 78.8% followed by splenomegaly 60.6%. Most importantly two patients (6.1%) presented with pleural effusion and one patient presented with pericardial effusion, acutely as cardiac tamponade.11 patients (33.3%) presented with bleeding as initial manifestation. T cell ALL is the most common type causing Pleural effusion and Pericardial effusion in our study. (Table 4)

Laboratory Parameter

The highest leucocyte count $150x10^9$/L and the lowest was $3x10^9$/L. Pancytopenia was observed as an initial picture in 7 (21.1%) patients. Blast cells in peripheral smear was less than 20% in 9 (27.3%) patients and bone marrow blast cells were more than 90% in 18 (54.5%) patients. Despite normal leucocyte count, fever was present in 6 (18.1%) patients. In patients with testicular swelling, pleural effusion and pericardial effusion, the bone marrow had blast cells more than 20% and flow cytometry of the bone marrow aspirate confirmed ALL as the diagnosis.

Table 5 Immunophenotypic Profile of Acute Lymphoblastic Leukemia Patients

STEM CELL (%)		B CELL (%)				T CELL (%)			
HLA DR	CD34	CD79	CD22	CD10	CD19	CD3	CD2	CD5	CD7
12(36.4%)	10(30.3%)	2(14.2%)	10(71.4%)	14(100%)	14(100%)	19(100%)	19(100%)	12(63.2%)	11(57.9%)

Immunophenotyping

Among B cell markers, CD 10 and CD 19 has been associated with all cases. Among T cell markers CD 2 has been associated with all cases. HLA DR and CD 34 has been associated with 36.4% and 30.3% of cases respectively.

DISCUSSION

Acute leukemias are group of heterogenous disorders with respect to morphological, immunophenotypic and cytogenetic features. In our study a total of 60 de novo cases of adult leukemia are studied with maximum being AML (55%) and ALL (45%). The incidence of adult leukemia is lower in Latin America and Asia compared to North America, West Europe and Oceania [(1)]. The present study is similar to Khalid Hassan *et al* [(2)], which has incidence of AML 54.7% and ALL 45.2%.

The most common age group of AML patients in our study belong to age group of 15 years to 30 years (40.7%). Least is above 66 years (3.7%). The most common age group among ALL patients belong to 15 years to 30 years (78.8%). The ratio of male to female incidence in AML is 3:1 and in ALL is 1:8. AML has the lowest survival rate among all leukemias. It constitutes to about 25% in adult age group [(8)].

The most common clinical presentation in our study is fever 66.7% in AML and 69.7% in ALL. In Ghosh S *et al*, KaKepoto *et al* the most common presentations were found to be fever and generalized weakness [(3,4)]. The most common presentation of AML is AML M2(44.4%) which is similar to Asif MJ *et al* and Butt Fl *et al* [(5,6)]. In Gupta N *et al*, ALL constituted 81.7% of all leukemia cases, among that B ALL and T ALL was 95.2% and 18.3% respectively [(7)]. But this does not correspond with our study. In our study, T ALL was 57.6% and B ALL was 42.4%. we have more incidence of T ALL which is opposite to that found in other parts of the country. Patient had initial presentation pleural effusion and pericardial effusion was 6.1% and 3 % in our study, all of them belonged to T ALL. Leukemia cutis was initial presentation in 1 patient, belong to AML M4.

In leukemia, extra medullary infiltration by leukemic cells, may lead to manifestations like lymphadenopathy, splenomegaly, hepatomegaly and gum hypertrophy. Hepatomegaly was observed in 29.6% of AML and 57.6% of ALL patients. Splenomegaly was observed in 48.1% of AML and 60.6% of ALL cases. Lymphadenopathy was seen in 78.8% of ALL and 22.2% of AML cases. Testicular swelling was seen in 3% of ALL cases, all of them belong to T ALL. Gum hypertrophy was seen in 11.1% of AML cases, all of them belong to AML M4. Bone tenderness was found in 11.1% of AML cases and 81.1% of ALL cases.

Hematopoeitic disturbances are the most common findings in laboratory investigations. Pancytopenia was found in 14.8% in AML and 21.1% of ALL patients. In Chang *et al*, pancytopenia was seen in 19.6% of cases [(9)]. This is associated with susceptibility to infection and bleeding manifestation.

In our study, bleeding manifestations like gum bleeding, epistaxis were both seen in 22.2% of cases of AML and 12.1% and 6.1% of ALL cases respectively. Anemia Is a constant feature in equally AML and ALL, with incidence of 96.3% and 78.8% respectively, which is similar to Ghosh S *et al*. Hyperleukocytosis which is defined as total leukocyte count of more than $100x10^9$/L. This is more common in AML than ALL. In our study, 11.1%of AML cases, which is similar to Chang *et al* and 3% of ALL had hyperleukocytosis. Thrombocytopenia was seen in 96.3% of AML cases, which is similar to Chang *et al* and 78.7% of ALL cases.

Immunophenotypic feature of B ALL is defined by the presence of either strong CD 19 along with one other strongly expressed B cell marker (CD79a, CD22, CD10) or weak CD19 and two others strongly expressed B cell markers [(10)]. In our study CD19 and CD10 were 100%. CD10 negativity in B ALL

is negative prognostic factor, associated with low rate of complete remission.

Immunophenotypic feature of T ALL is defined by the presence of cytoplasmic CD3 in leukemic blast. So, based on different subsets of markers, pro T ALL is defined by CD3, CD7 positive and pre T ALL by CD3, CD7, CD5/CD2 positive and mature T ALL CD3, CD1a positive [(10)]. In our study, pro T ALL comprise of 5.26%, Pre T ALL 57.89% and mature T ALL 31.5%. Bad prognosis in T ALL is confirmed by negativity to CD1a, positivity for CD34, and presence of myeloid antigen.

Immunophenotypic characteristic of AML is diagnosed based on the presence of either myeloperoxidase or at least two markers of monocytic differentiation (NSE, CD11c, CD14, CD64 or lysozyme in leukemic blast [(10)]. Among our 33 AML cases, 31 are non-APL and 2 cases are APL. CD34 is a stem cell marker, is a negative prognostic indicator for overall survival and remission achievement in AML which is present in 45.45% of the cases [(11)]. The expression of CD13, CD33, CD117 in our study core late to 78.7%, 21%, 57%. In our study APL cases had no expression of HLA DR, CD34 and 100% expression of CD117. AML with CD34 and HLA DR negativity carry a poor prognosis.

Griffin *et al* has proven that it is also necessary to do immunophenotyping before starting on treatment as more than 25% of patients would not respond or later relapse if treatment is initiated on morphological diagnosis [(12)].

CONCLUSION

AML is the most common type of leukemia in our study, due to increased referral of cases to our tertiary care center and also due to good response of ALL cases to treatment, they are being admitted less in our hospital. Immunophenotyping is necessary tool for diagnosing and initiating treatment.

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References

1. Ghosh S, *et al*., Haematologic and immunophenotypic profile of acute myeloid leukemia: an experience of Tata Memorial Hospital. Indian J Cancer 2003;40:71.
2. Khalid Hassan, *et al*., A Morphological Pattern of 234 Cases of Leukemias. JPMA. 1993;44:145.
3. Ghosh S *et al*., Haematologic and immunophenotypic profile of acute myeloid leukemia: an experience of Tata Memorial Hospital. Indian J Cancer 2003;40(2):71-6.
4. Kakepoto GN, *et al*., Long-term outcomes of acute myeloid leukemia in adults in Pakistan. J Pak Med Assoc 2002;52:482-6.
5. Asif MJ, *et al*., Acute leukaemia in adults: Morphological profile of 101 patients. Ann King Edward Med Uni 2000;6-4:343-8.
6. Butt FI, *et al*., A Review of 170 cases of acute leukemias. Ann King Edward Med Uni 1999;5(1):1-3.
7. Gupta N. *et al*., Spectrum and immunophenotypic profile of acute leukemia: a tertiary center flow cytometry experience. Mediterr J Hematol Infect Dis 2019,11(1):e2019017.
8. Pawar RN, *et al*., Mixed-phenotypic acute leukemia series from tertiary care center. Indian Journal of Pathology and Microbiology. 2017 Jan 1;60(1):43.
9. Chang F, *et al*., Clinical and Hematological Profile of Acute Myeloid Leukemia (AML) Patients of Sindh. J Hematol Thrombo Dis 2016;4:239.
10. Swerdlow S, *et al*., WHO Classification of Tumors of Hematopoietic and Lymphoid Tissues 4th Ed.(2008).
11. Paietta E, *et al*., Immunobiology of acute leukemia. In Neoplastic Diseases of the Blood 2013 (pp. 241-283). Springer New York. https://doi.org/10.1007/978-1-4614-3764-2_17.
12. Griffin JD, *et al*., Use of surface marker analysis to predict outcome of adult acute myeloblastic leukemia. Blood 1986;68:1232-41.

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