ORIGINAL RESEARCH PAPER

VOLUME-6 | ISSUE-5 | MAY - 2017 | ISSN No 2277 - 8179 | IF : 4.176 | IC Value : 78.46

INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

Haematological and Immunophenotypical Study on Acute Leukemias in a Tertiary Care Centre

Pathology

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ABSTRACT

Acute leukemias are heterogeneous group of haematological malignancies characterized by clonal expansion of immature myeloid or lymphoid precursors (blasts). The present study was conducted to have haematological as well as immunophenotypical interpretation in the diagnosis of acute leukemia. This study was performed over a peroid of 1 year from April 2016 to March 2017. The complete haemograms were done and slides were prepared with peripheral blood and in selected cases bone marrow aspirates which were stained by leishman and giemsa stain. Flow cytometric immunophenotyping was done to establish the types of acute leukemias. In our study, 52 cases of acute leukemias were reported. After confirmation by immunophenotyping, there were total 59.6% cases of AML, 28.8% of B-cell ALL, 5.7% of T-cell ALL, 3.8% of bi-lineage and 1.9% cases of bi-phenotypic leukemia. It is imperative to ascertain the lineage of leukemia by both morphological features and immunophenotyping. Immunophenotyping is a sensitive means to monitor the progress of patients after chemotherapy, risk stratification and aids in detection of minimal residual disease.

KEYWORDS:

Acute myeloid leukemia, Acute lymphoblastic leukemia, Immunophenotyping

INTRODUCTION

Acute leukemias are heterogeneous group of haematological malignancies characterized by clonal expansion of immature myeloid or lymphoid precursors (blasts). The blasts progressively replace the normal haematopoietic tissue and invade other organs of the body. Therefore anemia, infection and hemorrhages due to bone-marrow failure are the most common complications of this disease and may lead to death.[1, 2, 3] Among the childhood cancers, acute leukemias are the most common ones. In children acute lymphoblastic leukemia (ALL) is the most common form accounting for 80% of cases while acute myeloid leukemia (AML) is the commonest type in adults.[4, 5] For diagnosis of acute leukemia the percentage of blasts in marrow is >20%. Morphologically, acute leukemias are classified into two types AML and ALL. AML is subclassified into M0-M7 and ALL has three subtypes L1-L3.[6] Every blood cell expresses certain cytoplasmic and surface proteins which are called cluster differentiation (CD) antigens. Every level of differentiation has a unique set of expression of CD antigens. Immunophenotyping is the identification and quantification of cellular antigens through flourochrome labeled monoclonal antibodies.[7]

MATERIALS AND METHOD

This study was carried out in the Department of Pathology, Assam Medical College and Hospital, Dibrugarh, North-East India from April 2016 to March 2017. A total of 52 cases of acute leukemias were reported in the Haematology section.

The complete haemograms were determined according to standard laboratory procedures. Slides were prepared with peripheral blood and in selected cases bone marrow aspirates which were stained by 'Leishman stain' and 'Giemsa stain' to find out the blast cells morphology in peripheral blood and bone marrow. Diagnosis of acute leukemia was made in cases where blast percentage was ≥20% (WHO guideline) and then Flow cytometric immunophenotyping was performed to distinguish between AML & ALL. The detected antigens were CD45, HLA-DR, CD34, CD13, CD33, CD117, CD54, CD41, CD61, CD2, CD3, CD4, CD5, CD7, CD8, CD10, CD19, CD22 and CD79a. For all the above markers, blasts were considered to be positive if ≥20% expressed membrane antigen and ≥10% in case of cytoplasmic antigen.. For myeloperoxidase (MPO), it was considered to be positive when more than 3% of blasts reacted positively with anti-MPO.

RESULTS

In our study, 52 cases of different patterns of acute leukemia were reported. Based on haematological parameters, 34 cases (65.4%), 11 cases (21.2%), 5 cases (9.6%) and 2 cases (3.8%) were reported as acute leukemia, AML, ALL and bi-lineage leukemia respectively. The lowest and hightest total leucocyte count were found to be 2200/mm^3 and 339000/mm^3 respectively. The lowest blast percentage was 24% and highest was 95%. The haematological diagnosis (by means of complete blood count (CBC) / peripheral blood smear (PBS) / bone marrow aspirates (BMA)) and the number of cases that fall in different ranges of haemoglobin (Hb) level, total leucocyte count (TLC), platelet count (Plt.) and blast percentage are detailed in Table 1.

Table1: Haematological findings of all cases presenting as acute form of leukemia

Heamatological parameters	Acute leukemia	AML	ALL	Bi-lineage leukemia	Total
	No. of cases				
Hb level in g/dl					
<5	16	5	4	0	25
5 - 10	18	6	1	2	27
TLC/cu. mm					
<50000	10	2	0	0	12
50000 - 100000	21	3	1	0	25
>100000	3	6	4	2	15
Plt. count/cu. mm					
<20000	11	5	2	0	18
20000 - 50000	14	5	3	1	23
>50000	9	1	0	1	11
Blast (%) in PBS/BMA					

20% - 50%	9	2	0	0	11
50% - 80%	5	4	2	2	13
>80%	20	5	3	0	28

In all cases, immunophenotyping by flow cytometry was performed to establish the pattern of acute leukemia. Markers for diagnosis of AML were usually CD13, CD33 and MPO. CD14 and CD64 positivity signifies monocytoid variant of AML (M4 / M5). Positive markers for diagnosis of B cell-ALL were CD19, CD20, CD79a etc. Positive markers for diagnosis of T cell-ALL were CD2, CD3, CD5, CD7 etc. Majority of blast in both AML and ALL exhibited positivity for HLA-DR.

Out of 34 cases which were haematologically interpreted as acute leukemia, after immunophenotyping 20 cases were diagnosed as AML, 11 cases as B-cell ALL, 2 cases as T-cell ALL, and 1 case as bi-phenotypic leukemia. The bi-phenotypic and bi-lineage cases expressed both myeloid as well as lymphoid markers. All cases which were haematologically diagnosed as AML (11 cases) and ALL (5 cases) were confirmed by immunophenotyping.

After confirmation by Flowcytometry (immunophenotyping), There were total 31 (59.6%) cases of AML, 15 cases (28.8%) of B-cell ALL, 3 cases (5.7%) of T-cell ALL, 2 cases (3.8%) of bi-lineage and 1 case (1.9%) of bi-phenotypic leukemia which are detailed in Table 2.

Table2: Distribution of haematological diagnosis with that of immunophenotypical diagnosis

Heamatological diagnosis		Immunophenotypical diagnosis	
Diagnosis	No. of cases	Diagnosis	No. of cases
Acute leukemia	34	AML	20
		B-cell ALL	11
		T-cell ALL	2
		Bi-phenotypic	1
AML	11	AML	11
ALL	5	B-cell ALL	4
		T-cell ALL	1
Bi-lineage	2	Bi-lineage	2

In AML, the commonest age group affected was 21 - 40 years (48.4%). The youngest patient was 3 year old while the oldest patient was of 70 year old. In ALL, maximum number of cases were observed in the age group of 0 –10 years (72.2%). The youngest patient was 1 year old while the oldest patient was of 67 year old.

In AML, males and females were almost equally affected (male:female = 0.8:1). In ALL, overall the males have formed significant majority of the patients. 13 cases (72.2%) were male, while the females contributed only 27.8% of the cases.

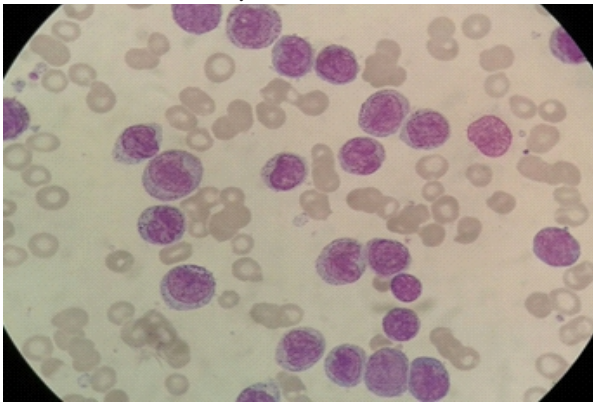

Figure 1: AML-M4; Peripheral blood smear: Both myeloblast and monoblast are seen (Giemsa stain, 10x100X)

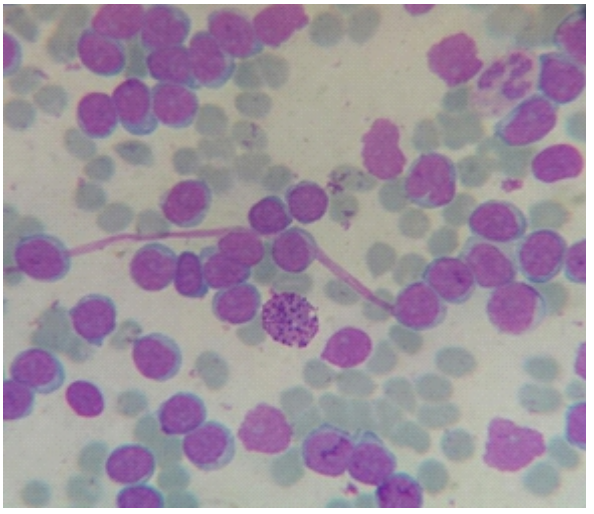

Figure 2: ALL in peripherel blood smear. More than 80% blast noted.

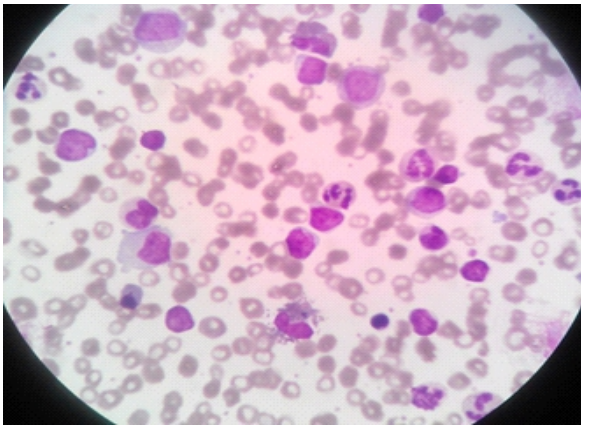

Figure 3: Bi-lineage leukemia. Both myeloblast and lymphoblast seen in PBS

DISCUSSION

In this study, anaemia was a constant feature in all cases of which 48% had severe anemia with Hb concentration of <5.0 g/dl. 77% of patients had TLC >50,000/cu.mm, while in western study only 40-50% patients presented with such high TLC.8 35% of patients had severe thrombocytopenia with platelet count <20,000/cu.mm and 79% had platelet count of <50,000/cu.mm. The results of the study were comparable to local studies but were more marked as compared to western countries, which can be explained on late presentation as the degree of anemia, leucocytosis and thrombocytopenia are directly proportional to severity of bone-marrow failure.9

Immunophenotypic observations of AML in our study (HLA-DR, CD13, CD33, MPO) were similar to those observed by Khalidi HS et al.10 and Ghosh S et al.11 in their study. Similarly, the aberrant expression of lymphoid antigens (CD22, CD79a) in AML was similar to the observations done by Ghosh S et al.11

In the present study the immunophenotypic diagnosis of B-cell ALL was seen in 83% and T-cell ALL was seen in 17% cases which was similar to observations of Shanta V et al.12 and Magrath I et al.13

Considering the different types of acute leukemia it was observed that AML was more common (59.6%) than ALL (34.6%) which closely resembled to results reported from Kenya in Africa.14

CONCLUSION

Present study revealed that AML is more prevalent than ALL. ALL is more commonly observed in children whereas the incidence of AML is higher in adults as compared to children and decreases towards older age. It is also concluded that immunophenotyping is an

important diagnostic tool to differentiate between AML and ALL in morphologically challenging cases. From immunophenotyping it is possible to subtype as well as to establish the lineage of the leukemia. It is also a sensitive means to monitor the progress of patients after chemotherapy, risk stratification and aids in detection of minimal residual disease.

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