Bone Marrow Morphology and Immunophenotypic Expression in De-Novo Acute Leukaemia

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Abstract

Acute leukaemias are a heterogeneous group of hematologic malignancies with diverse morphologic and immunophenotypic profiles. It's characteristics differ in clinical, morphological, immunophenotypic, genetic, and molecular perspective. Despite the increasing importance of molecular and genetic features in the classification of acute leukemias, morphological and immunophenotypic analysis remains the primary method to diagnose acute leukemia for initial evaluation and to guide specific molecular genetic tests. This study was conducted to observe the immunophenotypic patterns and their morphological expression among patients with acute leukemia, and to find the correlation between the immunophenotypic markers and the different French-American-British (FAB) sub-classifications of acute leukemias. This descriptive cross-sectional study was designed to evaluate the extent and correlation of bone marrow morphological features with immunophenotypic expression in patients with de-novo acute leukaemia (AL) and to determine discrepancies between morphology and immunophenotyping. This study was conducted in the Department of Haematology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, from January 2017 to June 2018. Fifty (50) newly diagnosed de-novo AL patients were evaluated through bone marrow morphology and flow cytometric immuno- phenotyping. The Beckman Coulter Cytomics FC 500 was used to analyze surface and cytoplasmic antigens. Data were analyzed using SPSS v23 with descriptive and inferential statistics. Among 50 patients, 30 (60%) were male and 20 (40%) female. The predominant age group was 19 to 29 years (42%). Morphologically, two-third (66%) cases were acute myeloid leukaemia (AML) and one-third (34%) acute lymphoblastic leukaemia (ALL). Among AML cases, the most frequent FAB subtypes were M1 (24%), M2 (20%), and M4 (14%); among ALL cases, L2 subtype was most common (20%). Immunophenotyping identified one-third (66%) of AML, 28% B-ALL, 6% T-ALL, and 4% mixed phenotypic acute leukaemia (MPAL). Expression positivity was highest for CD45 (94%), cMPO (66%), and HLA-DR (78%). Discrepancy between morphology and immunophenotype was observed in 12% cases. Bone marrow morphology remains essential in the initial evaluation of de-novo acute leukaemia, but immunophenotyping provides critical complementary data for accurate subtyping. A 12% diagnostic discrepancy highlights the necessity for integrated morphologic-immunophenotypic assessment to ensure precise classification and guide targeted therapy.

Keywords: *De-novo acute leukaemia, bone marrow morphology, immunophenotype, flow cytometry, mixed phenotypic acute leukaemia.*

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INTRODUCTION

Acute leukaemia (AL) represents a heterogeneous group of haematological malignancies characterized by clonal proliferation of immature haematopoietic cells with rapid clinical progression[1]. The diagnosis relies primarily on bone marrow morphology and immunophenotyping, which together establish lineage and guide therapy[2].

The French–American–British (FAB) classification introduced morphologic criteria dividing acute leukaemias into seven subtypes of AML (M0–M7) and **three subtypes of ALL (L1–L3)**[3–4]. Although the FAB system remains useful for initial diagnosis, it lacks the molecular precision required for risk stratification. Patients whose

blasts co-express both myeloid and lymphoid markers are categorized as mixed phenotypic acute leukaemia (MPAL), a rare subtype accounting for 2–5% of AL[5–6].

Morphology alone may be insufficient in distinguishing subtypes such as AML-M0 from M1 or differentiating B-ALL from T-ALL. Moreover, aberrant antigen expression and minimal residual disease (MRD) detection require flow cytometric immunophenotyping, now considered an indispensable diagnostic tool[7–9].

Given the limited data from Bangladesh, particularly regarding the extent and influence of morphological–immunophenotypic correlation in de-novo AL, this study aimed to assess bone marrow morphology with immuno-phenotypic expression patterns and identify diagnostic discrepancies in newly diagnosed cases at a tertiary care center in Dhaka.

MATERIALS AND METHODS

This cross-sectional study was conducted at the Department of Haematology, BSMMU, from January 2017 to June 2018. Fifty (50) consecutive patients with de-novo acute leukaemia diagnosed on peripheral smear were enrolled. Patients aged ≥14 years, newly diagnosed with acute leukaemia and untreated prior to enrollment, were included. Relapsed or secondary leukaemias were excluded.

Bone Marrow Examination: Morphologic classification was done following FAB criteria.

Immunophenotyping: Performed using Beckman Coulter Cytomics FC 500 flow cytometer, analyzing cell surface and cytoplasmic antigens with fluorochrome-conjugated antibodies (CD3, CD5, CD7, CD10, CD13, CD19, CD22, CD33, CD45, CD79a, CD117, cMPO, HLA-DR, TdT).

Standard single-cell suspension techniques and quality controls were applied.

Data Analysis: SPSS v23 was used. Quantitative data were summarized by mean ± SD; qualitative data by frequency and percentage. Z-test was applied, with $p < 0.05$ considered statistically significant.

Ethical Considerations

Ethical approval was obtained from the BSMMU institutional review board. Written informed consent was obtained from all participants.
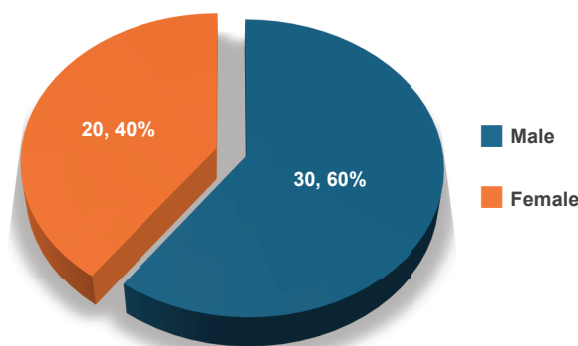
RESULTS

Figure- 1: Sex Distribution of the Study Subjects (n=50)

Figure 1 pie chart displays the sex distribution of the patients; a total of 50 patients were included in this study according to selection criteria. Among the patients 30 (60%) were male and 20 (40%) were female.
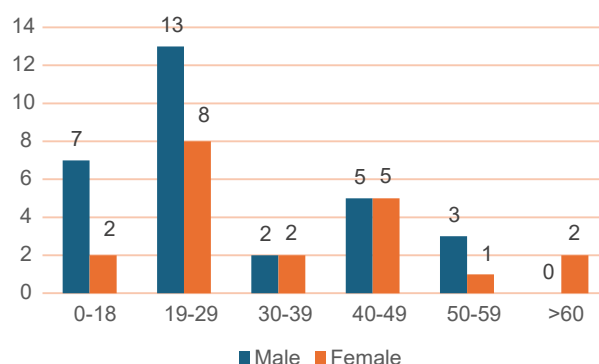

Figur- 2: Age Distribution of the Study Subjects (n=50)

Figure 2 presents the distribution of the patients; majority (42%) of the respondents was found in the age group of 19-29 years.
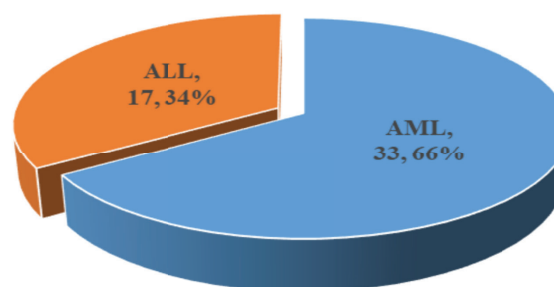

Figure- 3: Distribution of morphologic types of acute leukaemia (n=50)

Figure 3 shows the distribution of morphologic types of acute leukaemia of BM; here 33 (66%) patients were AML and 17 (34%) patients were ALL by morphologic assessment of BM.
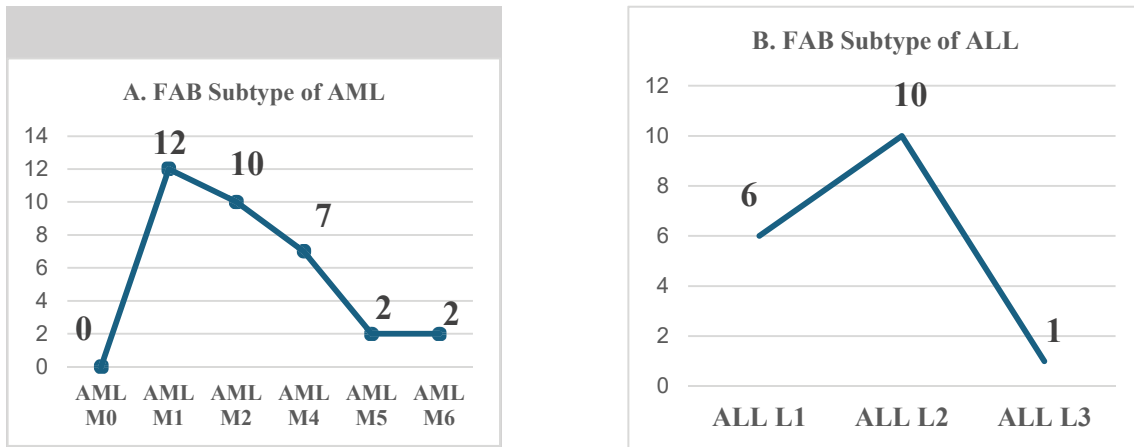
Figure- 4 (A & B): Distribution of FAB sub type (morphological) of acute leukaemia (n=50)

Figure 4 (A & B) states the distribution of FAB sub type (morphological) of acute leukaemia; here, among AML cases, figure 4A uncover M1 (24%), M2 (20%), and M4 (14%) were most frequent; figure 4B shows for ALL, L2 (20%) predominated.
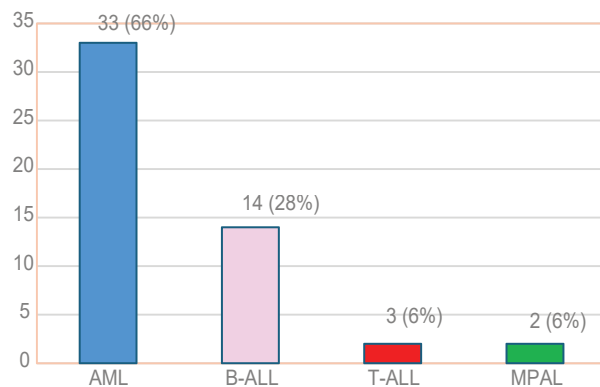
Figure 5 illustrates the Immunophenotypic distribution of acute leukaemia; here, Flow cytometry identified 33 (66%) AML, 14 (28%) B-ALL, 3 (6%) T-ALL, and 2 (4%) MPAL.

Immunophenotypic marker Positivity in FAB Sub-Classification of AL. There is positivity of CD3 in 10 (20%), CD5 in 9 (18%), CD7 in 11 (22%), CD10 in 13 (26%), CD19 in 15 (30%), CD22 in 9 (18%), CD79a in 17 (34%), CD13 in 28 (56%), CD33 in 31 (62%), CD117 in 21 (42%), cMPO in 33 (66%), CD45 in 47 (94%), HLADR in 39 (78%) and TdT in 10 (20%) were found. (Table-1).

Figure- 5: Immunophenotypic distribution of acute leukaemia (n=50)

Table 1 states the distribution of immunophenotypic marker positivity in FAB subtypes. Marker positivity was most frequent for CD45 (94%), CD33 (62%), cMPO (66%), and HLA-DR (78%)

Table- I: Distribution of immunophenotypic marker positivity in FAB subtypes (n=50)

| FAB	CD3	CD5	CD7	CD10	CD19	CD22	CD79a	CD13	CD33	CD117	cMPO	CD45	HLA DR	TdT
AMLM1	2	1	3	1	0	1	3	8	11	8	11	12	7	1
AMLM2	2	1	3	0	1	2	1	8	7	6	9	10	8	0
AMLM4	2	0	1	0	1	0	0	6	7	2	7	7	7	1
AMLM5	0	0	0	0	0	0	0	2	2	0	2	2	2	0
AMLM6	1	0	1	0	0	0	0	2	2	2	2	2	2	0
ALLL1	1	0	1	5	5	1	5	1	1	1	1	4	5	3
ALLL2	2	7	2	7	7	5	7	1	1	2	1	10	7	5
ALLL3	0	0	0	0	1	0	1	0	0	0	0	0	1	0
Total	10	9	11	13	15	9	17	28	31	21	33	47	39	10
	(20%)	(18%)	(22%)	(26%)	(30%)	(18%)	(34%)	(56%)	(62%)	(42%)	(66%)	(94%)	(78%)	(20%)

Morphology–Immunophenotype Correlation
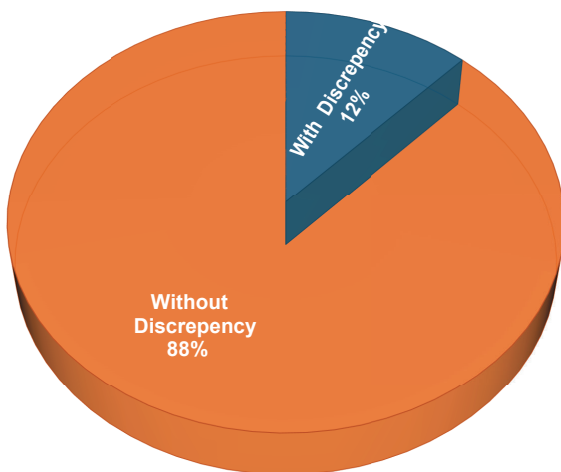
Figure- 6: Frequency of morphological–immunophenotypic discrepancy

Figure 6 unwrap the frequency of morphological–immuno- phenotypic discrepancy. A strong concordance was observed in 44 (88%) cases, whereas 6 (12%) showed diagnostic discrepancy between morphology and immuno-phenotype.

DISCUSSION

In this study, from January 2017 to June 2018, carried out in the department of Haematology, BSMMU, out of 50 patients of newly diagnosed untreated de-novo Acute Leukaemia patients from 14 to 65 years of age, there were 30 (60%) male and 20 (40%) female patients. There were 33 (66%) AML patients and 17 (34%) ALL patients. Majority (42%) of the respondents was found in the age group of 19-29 years. In this study shows on BM morphology 33 (66%) patients were AML and 17 (34%) patients were ALL. Among the ALL, there were 14 (28%) B ALL patients and 3 (6%) T ALL patients. The most frequent FAB subtype is AML M1 12 (24%), AML M2 10 (20%) and ALL L2 10 (20%) patients. It is observed that there are 2 (4%) patients of AL have got the immunophenotypic variety of MPAL. Among them morphologically 01 patient was from AML M1 and another 01 was from AML M4. In Jordan JRMS September 2015 Abbasi N, Kamal N et al. showed 48.2% of ALL and 51.8% of AML. Among the cases of ALL, 79.3% were identified as B-ALL and 20.7% as T-ALL. [10]

There were 33 (66%) AML patients, 14 (28%) B ALL patients and 3 (6%) T ALL patients. There is positivity of CD3 in 10 (20%), CD5 in 9 (18%), CD7 in 11 (22%),

CD10 in 13 (26%), CD19 in 15 (30%), CD22 in 9 (18%), CD79a in 17 (34%), CD13 in 28 (56%), CD33 in 31 (62%), CD117 in 21 (42%), cMPO in 33 (66%), CD45 in 47 (94%), HLADR in 39 (78%) and TdT in 10 (20%) were found. In this Study, there was 6 (12%) cases where there was discrepancy of morphological and immunophenotypic diagnosis of AL. Similar study was done by Gupta et al. (2015) where in 73% cases of acute leukemia found similarity in morphological appearance and immunophenotyping and remaining 27% cases shows discrepancy between morphological findings and immunophenotyping expression. Diagnosis in these 27% patients changed after immunophenotyping. [11]

CONCLUSIONS

In this study, it was observed that on bone marrow morphology 66% patients were AML and 34% patients were ALL. After Immunophenotyping study, among the ALL patiewnts, there were 14 (28%) B ALL patients and 3 (6%) T ALL patients. On bone marrow morphology, the most frequent FAB subtype is AML M1 24%, AML M2 20% and ALL L2 20% patients. There are 2 (4%) patients of AL have got the immunophenotypic variety of MPAL. Among them morphologically 01 patient was from AML M1 and another 01 was from AML M4. In this Study, there was 12% cases where there was discrepancy of morphological and immunophenotypic diagnosis of AL.

LIMITATIONS

Only a few selected available immunophenotypic markers of myeloid, lymphoid and other origin were used to see the pattern of Immunophenotypes. Extensive Immuno-phenotypic panel for the diagnosis of AL could not be done. Further follow up could not be done to see the remission status and to assess the prognostic significance.

DECLARATION

This topic is partially published in Haematology Journal of Bangladesh with similar demographic data in 2022.

FINANCIAL DISCLOSURE

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