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Expression of Aberrant Immunophenotype Markers in Acute Myeloid Leukaemia in a Tertiary Care Hospital

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ABSTRACT

Background: *Detection of aberrancies in newly diagnosed Acute Myeloid Leukaemia (AML) patients by flow cytometry is of diagnostic and prognostic importance. This study assessed the frequency of expression of aberrant Immunophenotype in newly diagnosed AML patients.* ***Objectives:*** *To assess the frequency of expression of aberrant immunophenotype in patients with acute myeloid leukaemia.* ***Method:*** *This observational cross-sectional study was conducted in the Department of Haematology, BSMMU, Dhaka, for 12 months duration following ethical approval. Total 100 newly diagnosed AML patients were analysed by multiparametric flow cytometry using a panel of monoclonal antibodies. Antigen expression was rated as positive when the percentage of positive blast cells was ≥20%.* ***Result:*** *Aberrant lymphoid markers were found in 56% AML cases, wherein 26% had B-cell markers only, 23% had T-cell markers only and 7% had presence of both T-cell and B-cell markers. CD7 (23%) was the most common aberrant marker, followed in decreasing order CD10 (22%), CD19 (11%), CD4 (6%), CD2 (4%) and CD20 (1%).* ***Conclusion:*** *More than half of AML patients had aberrant lymphoid phenotypes. Though aberrance of B-cell markers and T-cell markers are almost same, CD7 was the most common aberrant immunophenotype expressed in AML patients. Further study can be undertaken to correlate prognostic and therapeutic response with these aberrancies.*

Key words: *Acute myeloid leukaemia, flow cytometry, aberrant immunophenotype, lymphoid markers.*

Introduction

Acute Myeloid Leukaemia (AML) is a heterogeneous disorder characterized by clonal expansion of myeloid precursors in peripheral blood, bone marrow and/or other tissues, presenting with a diversity of phenotypes.[1] Although AML can be correctly diagnosed by bone marrow morphology and immunocytochemical analysis, flow cytometric immunophenotyping plays an important role in accuracy of diagnosis.[2] Each leukaemia type has characteristic set of immunophenotype markers. 'Aberrant phenotypes' or 'Aberrant expression of immunophenotype markers' can be evidenced by flow cytometry through Immunophenotyping.[3] Abnormal expression or loss of expression of cell specific lineage marker not associated with specific cell type is known as aberrant expression of immunophenotype.[4] Normally, CD 13, CD33, CD117, Anti-MPO, CD14, CD15, CD41, CD55, CD59, CD61, Glycophorin are lineage specific marker for AML.[4] While the most frequently seen aberrant CD markers are the CD7, CD4, CD9, CD8, CD2, CD3, CD5, CD19, CD10, CD22, CD56. The frequency of expression may be as high as 88%. It varies with each CD marker in AML patients.[5] CD7 is the most frequently expressed lymphoid markers in AML (about 16%). The next most frequently expressed lymphoid-associated markers are CD19, CD2, CD3, CD5, CD10, CD8.[6] Immunophenotype may create confusion with mixed phenotype acute leukaemia. 2008 WHO criteria for assigning lineage for mixed phenotype acute leukaemia and European Group for Immunological Characterization of acute leukaemia (EGIL) scoring are usually used to differentiate aberrant phenotype & mixed phenotype acute leukaemia.[7] Acute myeloid leukaemia (AML) patients may express a single, two or more lymphoid-associated antigens without fulfilling the criteria for acute mixed-lineage leukaemia. Of the cases with 2 or more lymphoid-associated markers, they simultaneously may express both T and B-cell-associated markers or alone may express two B-cell associated markers or two T-cell associated markers.[6] Aberrant immunophenotype expression may be associated with adverse outcomes such as lower disease-free survival, overall survival and requiring aggressive treatment.[8] Detection of aberrant immunophenotype is of extreme importance in the decision of a tailored treatment regimen and thus the eventual outcome of the patients and detection of residual disease.[8] Detection of aberrance is also important to see association with cytogenetic abnormalities.[8] Thus, the knowledge on aberrant immunophenotype expression should be kept in mind while dealing with acute myeloid leukaemia cases to plan an appropriate management protocol. Considering the paucity of the literature, this study was planned to evaluate the expression of aberrant immunophenotype in acute myeloid leukaemia among the patients attended in Bangabandhu Sheikh Mujib Medical University.

Materials and Methods

This observational cross-sectional study was conducted in Department of Haematology, Bangabandhu Sheikh Mujib Medical University (BSMMU), from July-2021 to June-2022 following ethical clearance from institutional review board (IRB). The study population were the patients of newly diagnosed acute myeloid leukaemia of any age and gender who were subjected to do immunophenotyping and were interested to participate, excluding the patients of acute promyelocytic leukaemia, mixed phenotyping acute leukaemia, transformed and therapy related acute myeloid leukaemia. The sample size of this study was determined by following equation-

Total study subjects (n) = $n = \frac{z^2pq}{d^2} = \frac{(1.96)^2 x0.583x(1-0.583)}{(0.1)^2}$ =93.35 (Prevalence: 0.583 as p=58.3%) [4]

So, the estimated sample size was 94. Considering 10% drop off and after describing the aim, purpose and procedure of the study, a total of 100 patients

were enrolled and interviewed for the study. Written informed consent or assent was taken from those who agreed to participate in the study. After taking informed written consent, the participants were interviewed about socio-demographic parameters (age, gender, occupation, living standard, monthly income etc.), about the disease and duration of disease, co-morbid disease and their laboratory profile. Complete blood count, peripheral blood film examination, bone marrow examination and Flow cytometry for acute leukaemia panel were done. Diagnosis of acute leukaemia was based on the presence of blast cells ≥20% in bone marrow film according to WHO criteria, together with presence of immunophenotyping results consistent with acute leukaemia. For flow cytometry, peripheral blood sample or bone marrow aspirated blood sample was used for different patients according to feasibility.

Three millilitre of venous blood was aseptically collected from each patient, dispensed into a tube containing K- ethylene diamine tetra-acetic acid (K-EDTA) at a concentration of 1.2 mg/ml, to be used for CBC, preparation of Leishman-stained smears and flow cytometry if peripheral blood WBC count 10,000 or more. Bone marrow aspirated blood was used for spreading smears for morphological examination after Leishman staining and also was collected in a sterile tube containing K-EDTA for the flow cytometry.

Immunophenotyping of blast cells in bone marrow aspirated blood or peripheral blood samples were done by using 3-laser, 10-colour multiparameter flow cytometry in Department of Haematology, Bangabandhu Sheikh Mujib Medical University, Shahbag, Dhaka.

Antibodies specific for various cellular antigens was considered with different fluorochromes that absorbed and emitted light, allowing simultaneous multicolour flow cytometric analysis of two or more cell-associated antigens Bone marrow aspirated blood or peripheral blood samples were processed within 6 hours of collection. Complete blood count from peripheral blood sample was done by six-part SYSMEX XN-2000 haematology analyser. Immunophenotyping was done by BD FACSlyric flow cytometry machine using BD FACSuite software.

Highest level of confidentiality and ethical standard was maintained during storage and analysis of the data. Statistical analysis was done by statistical software, SPSS 24.

Result

A total of 100 newly diagnosed Acute Myeloid Leukaemia patients were included in this study. Among them, more than half (56%) had expression of aberrant markers in their immunophenotyping profile (Figure 1). CD7 (23%) is the commonest aberrant marker in AML patients, followed in decreasing order CD10, CD19, CD4, CD2, CD20 (Figure 2)

Figure 1: Prevalence of aberrant immunophenotype in AML patients (n=100)

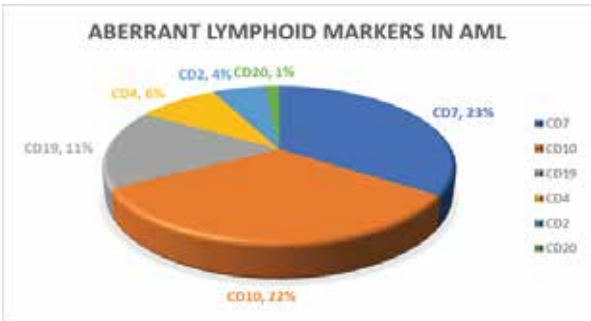

Figure 2: Frequency of aberrant lymphoid markers in AML (n=100)

Expression of aberrant marker was relatively more common among males and presence of aberrant immunophenotype was relatively more in age group between 40-59 years. But these were not statistically significant (p> 0.05). (Table I)

Table I: Distribution of age and sex according to presence of aberrant immunophenotype in AML patients (n=100)

Variables	Aberrant immunophenotype		p-value
	Present (n=56)	Absent (n=44)	
Age (in years)			
<20	5 (8.9)	6 (13.6)	0.455*
20-39	17 (30.4)	18 (40.9)	0.27*
40-59	24 (42.9)	13 (29.5)	0.171*
≥60	10 (17.9)	7 (15.9)	0.797*
Mean ±SD	42.36±16.27	39.02±15.91	0.307**
Sex			0.562*
Male	33 (58.9)	23 (52.3)	
Female	23 (41.1)	21 (47.7)	

Study participants had a median peripheral blood blast percentage of 60%. They had a median haemoglobin level of 8.2 gm/dL, total WBC count of 14250/mm^3. (Table II)

Table II: Haematological (Peripheral blood film) parameters at diagnosis of the study patients (n=100)

Parameters	Mean±SD	Median (Minimum-maximum)
Haemoglobin (g/dL)	8.33±1.84	8.2 (4.60-13.50)
Total count WBC (x10^9/µL)	25.90±29.06	14.25 (0.90-180)
Platelet (x10^3/µL)	57.67±47.82	47.5 (8.0-305)
Blast percent (%) in PB	58.97±24.52	60 (0-90)

Among 100 study participants, 23 patients (23%) had aberrant T-cell markers only, 26 patients (26%) had aberrant B-cell markers only and 7 patients (7%) had had presence of both T-cell and B-cell aberrant markers. Overall, maximum had B-cell markers (33%). Thirty patients had T-cell markers (30%). Three patients had multiple T-cell markers while only one patient had multiple B-cell markers. (Table III)

Table III: Distribution of aberrant lymphoid markers in AML patients (n=100)

Markers	Frequency (n)	Percentage (%)
Type of aberrant markers (n=56)		
T-cell markers only	23	23
B-cell markers only	26	26
Both T and B cell markers (Single B + Single T)	7	7
Number of T-cell markers (n=30)		
Single T-cell markers	27	27
Multiple T-cell markers	3	3
Number of B-cell markers (n=33)		
Single B-cell markers	32	96.67
Multiple B-cell markers	1	3.33

Among 30 patients with aberrant T-cell markers, CD7 (70%) was the commonest isolated T-cell marker, followed by CD4 (13.33%) and CD2 (6.67%) and few (10%) patients had simultaneous expression of two aberrant T-cell markers. (Table IV)

Table IV: Distribution of aberrant T-cell markers in AML patients (n=30)

Markers	Frequency (n)	Percentage (%)
Isolated T-cell markers		
CD7	21	70
CD4	4	13.33
CD2	2	6.67
CD3	0	0.0
CD5	0	0.0
CD8	0	0.0
Multiple T-cell markers		
CD4+CD7	1	3.33
CD2+CD7	1	3.33
CD2+CD4	1	3.33

Among 33 patients with aberrant B-cell markers, CD10 (66.67%) was the most common isolated B-cell marker followed by CD19 (30%). (Table V)

Table V: Distribution of aberrant B-cell markers in AML patients (n=33)

Markers	Frequency (n)	Percentage (%)
Isolated B-cell markers		
CD10	22	66.67
CD19	10	30.30
CD20	0	0
CD79a	0	0
Multiple B-cell markers		
CD19+CD20	1	3.03

Among 7 patients with both B-cell and T-cell markers, CD10+CD7 (57.14%) was the most common combined aberrant markers (Table VI)

Table VI: Distribution of combined aberrant B-cell and T-cell markers in AML patients (n=7)

Markers	Frequency (n)	Percentage (%)
Both B-cell and T-cell markers		
CD10+CD7	4	57.14
CD19+CD7	2	28.57
CD10+CD2	1	14.29

Discussion

The detection of aberrancies in newly originating blood cells by flow cytometry is of diagnostic and prognostic importance in a patient with Acute Myeloid leukaemia. Aberrant expression of antigens is thought to be associated with adverse outcomes.[9] This Observational study was performed to assess the frequency of expression of aberrant Immunophenotype markers in 100 newly diagnosed AML patients. In this study, maximum AML patients had presence of aberrant lymphoid markers (56%). Similarly, in a previous Bangladeshi study published on 2018, also found aberrant CD expression in 58% AML patients.[3] In the same year an Indian study found aberrant lymphoid markers in 49% AML cases.[1] However, the incidence of the aberrant phenotypes in AML is still controversial and different results have been found by different groups.[5] The mean age of the study patients was 40.89±16.12 years with male predominance. Several previous also reported similar age and gender distribution.[2] However, this study found no significant association of age and sex with presence of aberrant cell markers, which was also supported by previous study. In current study, Haematological (CBC) analysis demonstrated a wide range of variation. Aberrance present similarly among patient with ≥50% and <50% of blast percentage. Presence of aberrant cell markers had no significant association with any haematological markers and bone marrow examination findings. Similarly, in an Indian study they found no significant association between haematological parameters and aberrant lymphoid phenotype in AML.[1] On the contrary, another Indian study observed a higher total leucocyte count and increased percentage of blasts in peripheral blood among aberrant lymphoid phenotype in AML.[10]

This study also shows aberrant expression of T-lymphoid cell markers are 23%, B-lymphoid cell markers are 26% and presence of both B and T lymphoid cell markers are 7% in AML patient. Among the T-lymphoid cell markers CD7 is the most common aberrant marker and among B-lymphoid cell markers CD10 is the most common aberrant marker. In present study, CD7 was the commonest aberrant marker, followed in decreasing order CD10, CD19, CD4, CD2 and CD20. These findings are in agreement to the previous Bangladeshi study held on 2018, who also reported CD7 as the most common aberrant lymphoid antigen.[3]

Conclusion

This study found that more than half of AML patients expressed one or more of aberrant lymphoid immunophenotypic markers. Aberrant expression of B-lymphocytic and T-lymphocytic immunophenotype

were similar in frequency. Some patients had aberrant expression of multiple B-cell immunophenotype, multiple T-cell immunophenotype or expression of concomitant B-cell and T-cell immunophenotype. CD7 was the commonest aberrant marker, followed, in decreasing order, by CD10, CD19, CD4, CD2 and CD20. However, presence of aberrant immunophenotype had no significant association with age, sex and any haematological parameters. With a few minor differences, these results are consistent with those of earlier studies in different countries. However, further study should be done for evaluation of association of aberrant immunophenotype with different cytogenetic abnormalities, therapeutic response and overall prognosis of the patients.

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