Original Research Article

Flowcytometry Immunophenotyping: Role in Acute Leukemia and detection of aberrant expressions in the Indian population

ABSTRACT:

Aim: To study the distribution of Acute Leukemia cases, and the expression of commonly used Cluster of Differentiation (CD markers) in sub-classification of Acute Leukemia and aberrant expression of these markers.

Method: The retrospective study was conducted at a Global Reference Lab in Mumbai from January 2017 to December 2021 on 1538 Acute Leukemia cases diagnosed based on20%& more blasts on morphology and Flowcytometry immunophenotyping.

Results: Out of 1538 Acute Leukemia(AL) cases, Acute Myeloid Leukemia (AML) was found to be more common (59.49%) than Acute Lymphoid Leukemia (ALL) (39.27%). Age distribution showed that ALL was more common in Paediatric age group whereas AML was seen more common in adults and old age. In further analysis of ALL, B-ALL was found higher as compared to T-ALL. CD33 (92.74%), CD19 (100%) and CD7 (97.79%) were most sensitive markers for AML, B-cell and T-cell respectively. CD7, CD33 and CD13 were most common expressed aberrant markers found in them respectively.

Conclusion: Flowcytometry immunophenotyping is indispensable, fastest and very precise tool in defining lineage of Acute Leukemia and identification of Mixed Phenotypic Acute Leukemia (MPAL) and Undifferentiated Leukemia. Aberrant expression of markers guides for further cytogenetic and molecular studies to great extent and identification of blasts during assessment of minimal residual disease.

Keywords: Acute Leukemia (AL), Acute Myeloid Leukemia (AML), Acute Lymphocytic Leukemia (ALL), Flowcytometry, Aberrant Markers, Cluster of Differentiation (CD), Mixed Phenotypic Acute Leukemia (MPAL), Minimal Residual Disease (MRD)

INTRODUCTION:

Acute Leukemia (AL) is one of the malignant haematological disorders, which is caused by uncontrolled proliferation of abnormal hematopoietic cells. On the basis of different methods of diagnosis Acute Leukemia has been divided into different types based on morphology, antigen presentation, genetic abnormalities. In today's era with advanced targeted therapy and study of minimal residual disease, just morphological differentiation is not enough and there is a need of additional information for better prediction of prognosis

and treatment (1,2). WHO has broadly classified acute Leukemia into AML, ALL and MPAL on the basis of flowcytometric presentation of Cluster of differentiation (CD) on hematopoietic cells and or immature cells and presence of 20% or more blasts except those with specific recurrent genetic abnormalities (3,4). Other than above mentioned categories, acute Leukemia of ambiguous or undifferentiated lineage are also described (4). Flow cytometry immunophenotyping is a technique, which measures size, granularity and antigen presentation of every single cell present in suspension (5). Continuous improvement and development in multi-colour cytometers, various sample processing techniques, wide range of antibodies, soft wares, gating strategies has made flow cytometry immunophenotyping a method of choice for analysis of case of Acute Leukemia (6). Flowcytometric immunophenotyping not only gives timely and accurate diagnosis by assigning their lineage, which helps in management of Acute Leukemia but also tells about presence or absence of aberrancy. Aberrancy in acute Leukemia could be presence or absence of antigen different from that seen in normal maturation or of different lineage or expression of both mature and immature markers together. (3) These aberrancies are of great help in identification of abnormal blast in treated cases and assessment of Minimal / Measurable Residual Disease (MRD). It also guides to recurrent genetic abnormality and molecular studies, initiating targeted therapies and assessing prognosis (1,6). The current study studied the data of cases diagnosed as Acute Leukemia, the expression of some common markers in these acute Leukemia cases and some common aberrancies observed.

MATERIALS AND METHODOLOGY

A retrospective study of 1538 cases from January 2017 to December 2021 was conducted at Global Reference Laboratory on acute leukemia cases diagnosed on the basis of morphology (20% blasts) registered for flowcytometry immunophenotyping. All the cases included in this study fulfill the criteria >=20% blast cells on morphology. For all the cases registered, sample type was either blood or bone marrow EDTA or Sodium heparin.Flowcytometric immunophenotypic analysis was done on 8-color, BD Fasc Canto II. In all the cases 50000 cell events were achieved. Stain-lyse-wash method was used for sample processing.CD Markers showing positivity of >20% were considered to be positive.

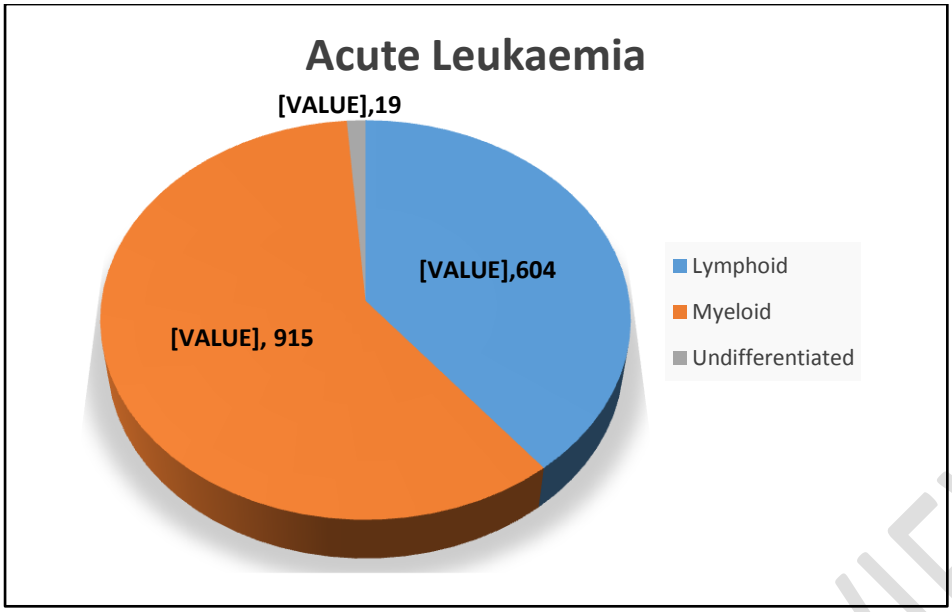
STATISTICAL ANALYSIS

The data were analyzed using “R Studio version 1.4.1103”. Descriptive analyses were made to obtain the frequency and percentage of Acute Leukemia classification in this given population, in addition to the characteristics of the sample Age and Gender. Comparison of Acute Leukemia classification with Age and gender was done by Chi square test. Two sided p value of less than 0.05 was considered to be statistically significant.

RESULTS

Out of total 1538 cases with Acute Leukemia (AL), 915 (59.49%) were Acute Myeloid Leukamia (AML) and 604 (39.27%) were Acute Lymphoid Leukamia (ALL) whereas remaining 19 (1.24%) were undifferentiated. (Figure 1)

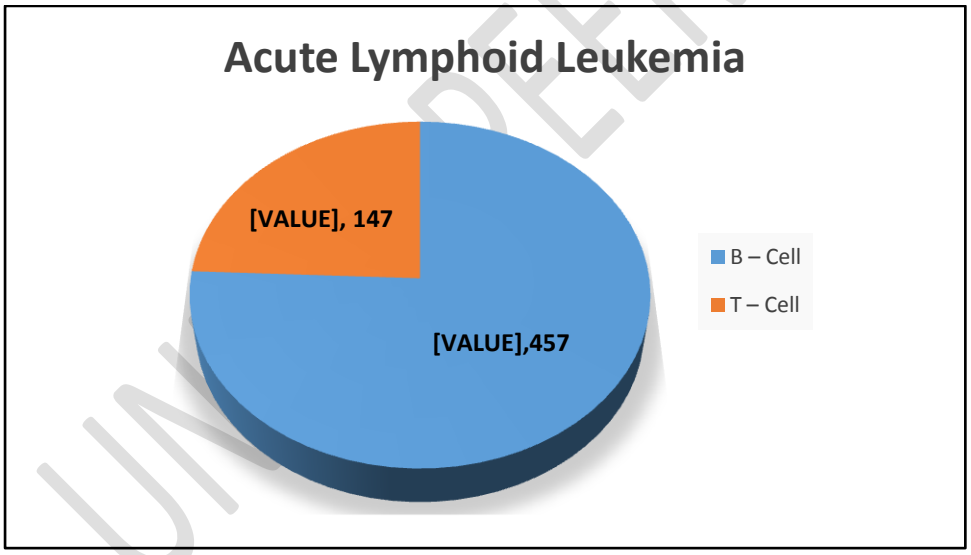
Figure 1: Frequency distribution of Acute Leukemia classification.



Data is represented as Percentage (%), Frequency(N)

ALL were further subclassified into B-cell ALL and T-cell ALL. Maximum cases were of B-ALL 457(75.6%) and T-ALL were 147(24.3%). (Figure 2)

Figure 2: Sub classification of Acute Lymphoid Leukamia



Data is represented as Percentage (%), Frequency(N)

Overall] distribution of acute leukemia showed that proportion of males were higher than females (57.8% vs 42.42%) and maximum cases (40.51%) belonged to patients >45 years of age. (Table 1)

However, in further classification of AL it was observed that ALL were seen maximum in age group of 1 to 12 years while AML were seen maximum in age group of >60 years. Frequency

of ALL was higher in males 43.44% while AML was commoner among females 65.44%(Table 2).

Table 1: Overall distribution Age and Gender

	Frequency	Percentage
Age Group(in Years)		
1 – 12	244	15.86%
13 – 18	141	9.17%
19 – 30	246	15.99%
31 – 45	284	18.47%
46 – 60	296	19.25%
> 60	327	21.26%
Gender		
Female	654	42.52%
Male	884	57.48%

Table 2: Comparison of Acute Leukemia classification with age group and gender

	Acute Leukemia						
	Lymphoid		Myeloid		Undifferentiated		P value
	Frequency	Percentage	Frequency	Percentage	Frequency	Percentage	
Age Group							
1 – 12	196	80.33%	48	19.67%	0	0.00%	<0.0001
13 – 18	88	62.41%	53	37.59%	0	0.00%	
19 – 30	119	48.37%	124	50.41%	3	1.22%	
31 – 45	88	30.99%	193	67.96%	3	1.06%	
46 – 60	65	21.96%	224	75.68%	7	2.36%	
>60	48	14.68%	273	83.49%	6	1.83%	
Gender							
Female	220	33.64%	428	65.44%	6	0.92%	0.0002
Male	384	43.44%	487	55.09%	13	1.47%	

CD Marker analysis for AML showed that CD33(92.74%) was the most commonly expressed marker followed by CD13(87.68%) and CD117(77.12 %).In B-ALL, CD19 expression is seen in all cases and CD10 (CALLA) and CD20 were expressed in 93.5% and 44.6% respectively. In T-ALL CD7 was the most frequent expression followed by CD5 and CD3. FurtherCD10 were seen expressed in 47.0% cases. (Figure 3)

Figure 3: CD Marker Analysis showing common positive markers

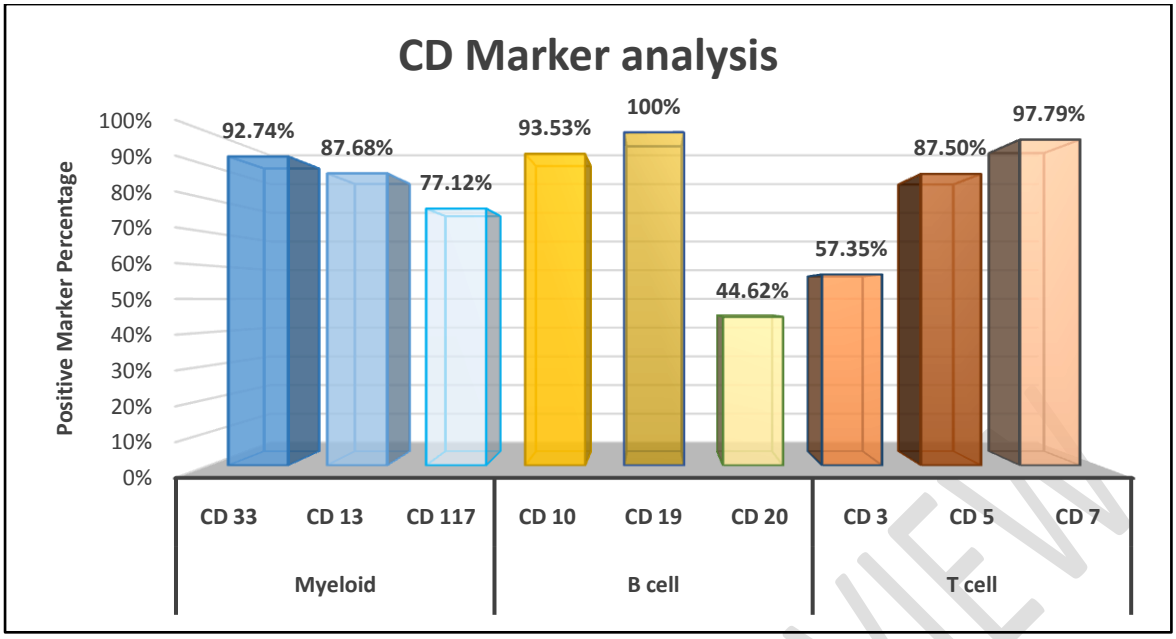
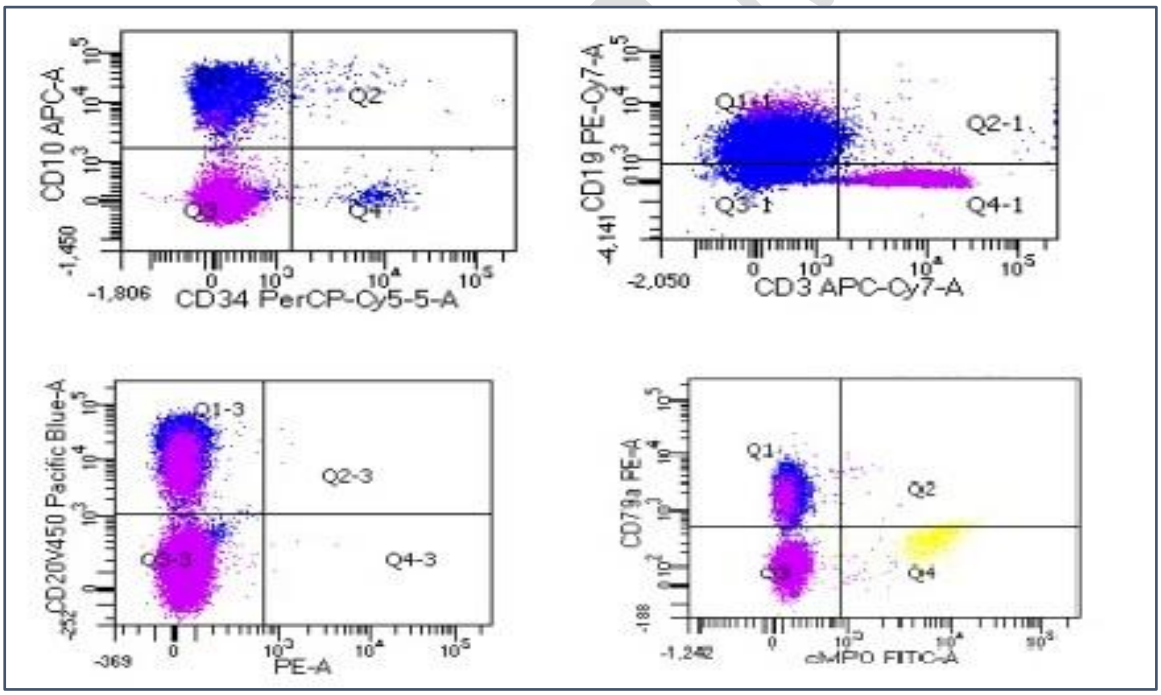
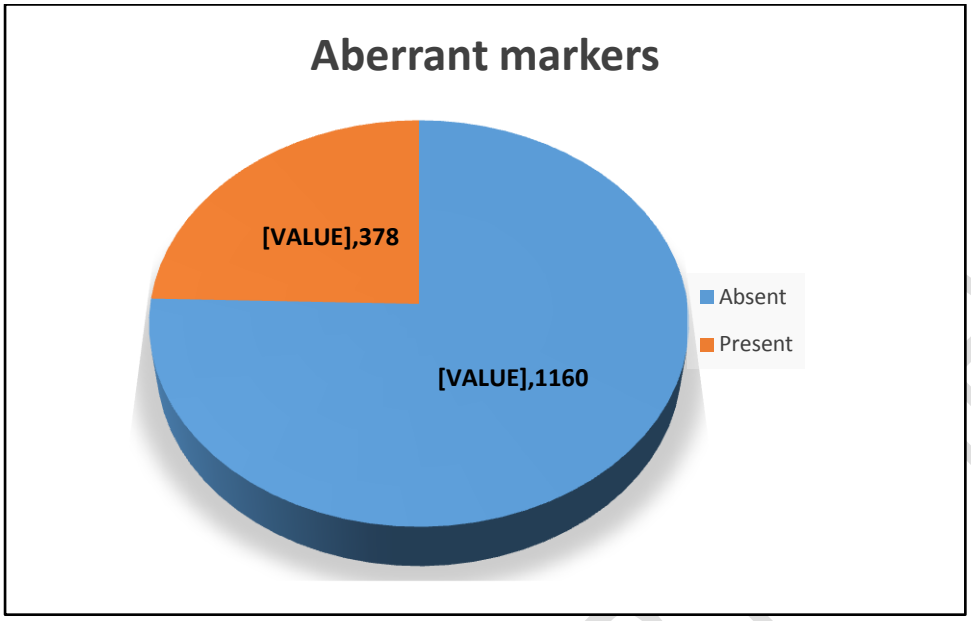


Figure 4:Positive expression of CD19, CD10, CD20 and cytoplasmic CD79alpha in a case of B-ALL. Blasts are represented with blue colour and lymphocytes with pink.



Of the total AL cases, 378 cases (24.58%) has shown aberrant expression of markers. (Figure 5). With most commonly found aberrant marker in acute Leukemia was CD7 (47.73%) followed by CD33(21.36%), CD13 (17.05%) and CD19(8.86%). (Table 3)

Figure 5: Aberrant Markers in AL



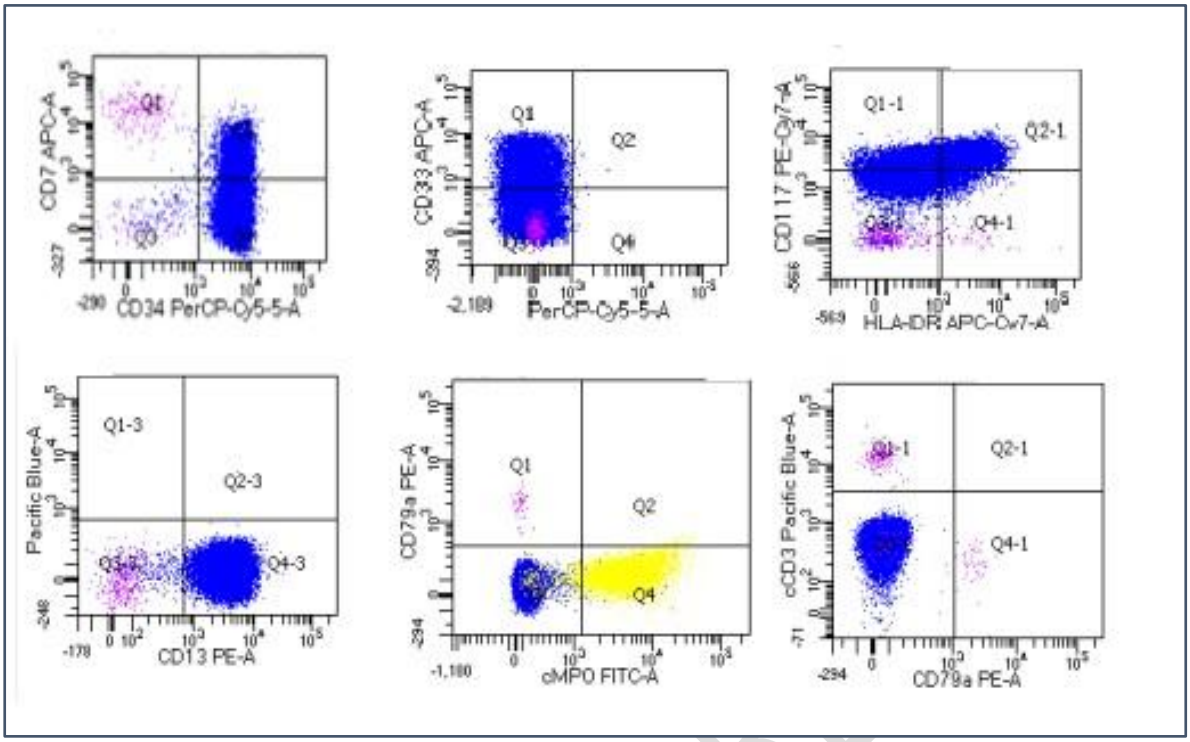
Data is represented as Percentage (%), Frequency (N)

Table 3:Common aberrant markers seen

Aberrant Marker	Frequency	Percentage
CD 2	1	0.23%
CD 3	1	0.23%
CD 4	3	0.68%
CD 5	4	0.91%
CD 7	210	47.73%
CD 8	1	0.23%
CD 10	7	1.59%
CD 13	75	17.05%
CD 19	39	8.86%
CD 33	94	21.36%
CD 117	5	1.14%

Figure 6:Blasts (blue) showing positive expression of CD34, CD33, CD117, HLADR and CD13 and are negative for cytoplasmic MPO, cytoplasmic CD79alpha and cytoplasmic CD3 in a case of Acute Myeloid Leukemia. There myeloid blasts show aberrant expression of CD7 (CD34/CD7 plot).

Lymphocytes are represented with pink colour.



In AML, CD7 (81.86%) was most aberrant marker followed by CD19 (14.77%). (Table 5)

Table 4: Aberrant Markers in Acute Myeloid Leukemia (AML)

Aberrant markers	In Myeloid (AML)	
	Frequency	Percentage
CD 2	1	0.42%
CD 3	1	0.42%
CD 4	3	1.27%
CD 5	1	0.42%
CD 7	194	81.86%
CD 8	0	0.00%
CD 10	2	0.84%
CD 19	35	14.77%

In ALL sub classification, CD33(52.83%) was most frequent aberrant marker in B-ALL followed by CD13(34.59%) and CD7(10.06%) were as in T-ALL, CD13 (50%) was most frequent aberrant marker followed by CD33(25%) and CD19, CD117 both 12.50%. (Table 5,6)

Table 5: Aberrant Markers in T-ALL

Aberrant markers	In T-ALL	
	Frequency	Percentage
CD 13	20	50.00%
CD 19	5	12.50%
CD 33	10	25.00%
CD 117	5	12.50%

Table 6: Aberrant Markers in B-ALL

Aberrant markers	In B-ALL	
	Frequency	Percentage

CD 2	0	0.00%
CD 3	0	0.00%
CD 4	0	0.00%
CD 5	3	1.89%
CD 7	16	10.06%
CD 8	1	0.63%
CD 13	55	34.59%
CD 33	84	52.83%
CD 117	0	0.00%

DISCUSSION

The immunophenotyping of acute leukemia not only differentiates leukemia into two different types, AML or ALL but further subcategories as well. ALL into B-ALL or T-ALL. AML can also be sub-categorized into specific sub-type such as M0, M1, M2, M3, AML with monocytic differentiation or Megakaryocytic Leukemia in conjunction with morphology and availability of markers. Flowcytometry also plays an important role in diagnosing cases of acute leukemia with mixed phenotypes.

We studied distribution of acute leukemia in different age group, gender, and role of CD markers in AL. It was found that acute leukemia is seen in all age groups; however, its distribution varies with age. In our study we found that acute leukemia cases increase with increase in age with maximum cases seen in age group of >60 years followed by 46 to 60 years. On further sub-classification analysis of AL it was observed that AML had increasing trend with age group, with highest trend observed in age group of >60 years, similar to a study by Raza H et al where maximum cases were seen in patients >50 years of age (7). Contrary to this, ALL showed a decreasing trend with respect to age, with age group 1 to 12 year showing maximum frequency of cases. In our study B-ALL was seen more in age group 1 to 12 years, a similar observation was found in study done by Seegmiller AC et alt (8). In our study, the proportion of AML cases were higher compared to ALL 57.48% vs 42.52%. This coincided with the findings by Monika Gupta et al where 43% of ALL and 53% of AML cases were observed (3). However, Gujral S et al found higher ALL cases as compared to AML, with 58% of acute lymphoblastic Leukemia (ALL) cases and 38% of acute myeloid Leukemia (AML) cases (9). However, ALL subtypes were similar to our study. The Study done by Gujral S et al. observed 76% B cell phenotype while 24% T cell phenotype which coincide with our study finding of 75.66% in B-ALL and 24.34% in T-ALL(9).

In our study, males and females are found to be affected equally in either type, AML or ALL. Different results have been obtained in other studies (7). It was observed that in B-ALL, while blasts showed CD10 expression in majority of the cases, CD10 negative B-ALL comprise 6.47% of the cases, which is similar to study conducted by Anja Möricke et al. where it was observed that 5.2% were CD10negative. In another study conducted by D.A Salem et al. also showed around 10% CD10 Negative in B-ALL cases (10,11). A study done by Gujral et al. found CD10 positivity be to 43% in T-ALL cases which was similar to our finding of CD10 positivity (47.10%) in T-ALL. (9,11). CD19 is a Pan-B marker and is present on B cell from very early stage of maturation till completely mature B-cell formation (4,17).

WHO,2008 mentions CD19 as one of the lineages specific markers along with other two lineage specific makers. We found CD19 expression in all (100%) cases (4,12). Expression of CD20 was seen in 44.6% of B-ALL and 55.38% are CD20 negative, which matches with study done by Saghir A. Jafri(13). In contrast to our study, Jeevan Kumar et al. mentioned 62% patients expressed CD20 while 38% patients were negative for CD20 and another study has shown 26.3% CD20 positive B-ALL (14). CD34 is one of the immaturity markers and was seen in major proportion of AML cases (67.04%), B-ALL (73.95%) and some T-ALL (48.53%) which was similar with the study done by Saghir A. Jafri (13). CD34/CD7 co-expression seen in 47.06% of T-ALL, 21.20% of AML and 3.12% of B-ALL. CD7 originate from stem cell and its expression has been seen with immaturity markers such as CD34 and HLADR (3). Gujral S et al observed that HLADR negativity was seen mostly in T-ALL (89.63%), 37.14% of AML and only 2.23% of B-ALL (9). We found CD33 and CD13 positivity in 92.74% and 87.68% AML and CD117 positivity seen in 76.24% of AML, similar pattern was seen in study by D.A Salem et al. which found CD33 as most commonly expressed marker (89.45%) followed by CD13(77.9%) and CD117 (74.3%) (11). D.A. Salem et al. also found that CD7 was most expressed in T-ALL followed by CD5 and CD3, the current observations were also similar, with CD7 expression seen in 97.79%, CD5 in 87.50% and CD3 in 57.35% of T-ALL(11). CD7 is the first T-cell marker to appear during maturation of T-cell and described as the most sensitive markers of T-cell in T-ALL in most studies (15).

Aberrant markers can be explained as the expression of markers different from its natural course of maturation for that lineage. This could be expression of marker from different lineage such as expression of myeloid markers on lymphoblastic leukemia or vice versa, or absence of marker which represent its lineage or expression of both mature and immature markers on same neoplastic cell giving asynchronous presentation(3,13,15,16). In our study aberrancy was seen in 24.5%. of the cases. Most of these were frequently observed in AML (59.7%), followed by B-ALL (32.2%) and T-ALL (7.9%). However, variable results have been found in other studies.(13,20) AML associated CD7 aberrancy in our study is 81.86%. Frequencies are seen to vary between 26 % to 37 % in some of the other studies (2,7,11,13,20). CD7 (81.86%) is found to be more frequent aberrant marker, followed by CD19 (14.7%), CD4 (1.27%), CD10(0.84%), CD2(0.42%), CD3(0.42%), CD5(0.42%) and no aberrancy for CD8. CD7 has been seen as the most frequent marker in many other studies but with variable but comparable percentages. (3,7,9).

Second most common aberrancy in AML is of CD19 (14.77%), similar to few studies (7,9,13) and in contrast to other studies (3,11). Aberrancy of CD19 has been found to be associated with t(8;21)AML (13,16). Aberrancy of myeloid antigen in B-ALL is frequently seen, most common myeloid marker observed in our study is CD33(52.83%), followed by CD13(34.59%). CD117 is not seen in any of our cases. Higher percentages showing similar trend have been found in another study with 89% B-ALL showing CD33 expression, 53% expressed CD13 and 5% has shown CD117 (19). In contrast to our study, a study by Saghir A et al has shown very low frequency of expression of CD13 (9.09%) in B-ALL with no case showing aberrancy of CD33 (13). Monika Gupta et al has shown frequency of CD13 (50%) and CD33 (3%) (3). The reason for variable expression of markers could be sample size of

study, age distribution and antibodies used. Different study has shown equal expression of CD13 and CD33 (20.7% each) and 10.3% cases with CD117 as aberrant marker in ALL (13)

In T-ALL, aberrancy of myeloid markers has been observed to be commoner than B-cell markers. Aberrancy of CD13 (50%) is higher thanCD33 (25%), followed by CD117 (12.5%)(13). In other study frequency of expression of aberrant CD13 and CD117 is 5.55% each (15). Another study has shown 1 out of 13 cases i.e. 7.6% showing CD33 expression in T-ALL and none shown positive expression of CD13 (11). Aberrant expression of myeloid markers in ALL has been described to have poor prognosis compared to one that does not show aberrations (21). Expression of CD19 in T-ALL is seen in 10.26% cases in contrast to other studies which has not shown any case with aberrant expression of CD19 in T-ALL (11).

CONCLUSION

In our study we found expression of markers comparable to different studies done in India & other countries with a few variations. We observed CD7 and CD19 were most common aberrant markers in AML, CD33 was common in B-ALL and CD13 in T-ALL. Also, the most sensitive lineage marker for AML is CD33, CD19 for B-ALL and CD7 for T-ALL. Flowcytometry immunophenotyping is indispensable, fastest and very precise tool in defining lineage of acute Leukemia and identification of MPAL and undifferentiated Leukemia. Aberrant expression of markers guides further cytogenetic and molecular studies to great extent and identification of blasts during assessment of minimal residual disease. Our study directs evaluation of aberrant expression with genetic phenotype and prognostic and therapeutic implication.

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