IMMUNOPHENOTYPING IN ACUTE LEUKAEMIA- AN INSTITUTIONAL STUDY

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ABSTRACT

BACKGROUND

Leukaemias are biologically a diverse group of disorders with differences in their morphology, antigen expression, chromosomal and molecular abnormalities, response to treatment, and prognosis. The main objective of the study was to compare morphological and flowcytometric diagnosis in patients diagnosed with acute leukaemia.

MATERIALS AND METHODS

The prospective study was carried out at S.C.B. Medical College and hospital, Cuttack, in department of Pathology and Clinical haematology, for the period of November 2015- November 2017. The findings were based on 100 patients who underwent both flow cytometry and peripheral smear/bone marrow morphology tests for diagnosis of acute leukaemia.

RESULTS

Using the peripheral smear/bone marrow morphology, 27% patients had ALL-L1, 38% had ALL-L2, 05% had AML-M1, 21% had AML-M2, 06% had AML-M3, 02% had AML-M4, and 01% had AML-M5. Immunophenotyping by flow cytometry confirms 50% patients to be B-ALL, 07% to be T-ALL, 32% AML, 08% APML/AML-M3, and 03% to be MPAL. There was a concordance between the morphological and flowcytometry of 88% in ALL, 91% in AML, 75% in APML, but, no concordance at all for MPAL.

CONCLUSION

Hence, flowcytometry is mandatory in all cases of acute leukemia, to confirm a definite diagnosis, as treatment nowadays is target oriented.

KEYWORDS

Acute Leukaemia, Flowcytometry, Immunophenotyping, ALL, AML, APML, MPAL.

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BACKGROUND

Leukaemias are the most common hematopoietic malignancies, and these disease categories represent various heterogeneous disease groups that include a large number of distinct biologic entities. While the diagnosis and classification of these malignancies were originally based primarily on morphologic features, at times supplemented by cytochemical studies, the diagnosis of hematopoietic malignancies now requires a complex battery of specialized tools that include immunophenotyping and cytogenetics.[1]

The acute leukaemia (AL) are divided into acute myeloid leukaemia (AML) and acute lymphoid leukaemia (ALL). AML and ALL differ substantially in response to therapy and course, and accurate differentiation of the two is fundamental to therapeutic decisions. Sub classification of each group is also of increasing importance, as treatment continues to evolve for Specific genetic and pathogenic subgroups of disease. Myeloid and lymphoid lineages may be distinguished on the basis of cellular morphology, cytochemical staining, and expression of lineage specific antigens.[2] However, cytochemistry alone failed to complement morphology in vast majority of acute leukaemias.

Morphological diagnosis of acute leukaemia carries a lot of limitations like differentiation between AMLM0 and M1, subtyping BALL/TALL, to detect mixed phenotypic leukaemia, to detect aberrant antigen expression and minimal residual disease (MRD).

Hence, paving the way for the use of flowcytometry for better characterization of these leukaemias.[3] Flowcytometric immunophenotyping for acute leukaemia is important for the distinction between ALL & AML, identification of B-cell or T-cell Phenotype, detect expression of aberrant markers, assessing the response to treatment, including the identification of early responders and detection of minimal residual disease.

The present study is designed to undertake immunophenotyping by flowcytometry, of cases of acute leukaemia diagnosed morphologically, attending the Department of Pathology and Department of Clinical

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Haematology of S.C.B. Medical College and Hospital, Cuttack. The study is aimed at comparing the morphological diagnosis in acute leukaemia with the immunophenotyping of the same cases by using flowcytometry. Thereby, highlighting the pivotal role played by immunophenotyping by flowcytometry in acute leukaemias for specific lineage determination before the onset of therapy, and thereby, decrease the morbidity and mortality in patients of leukaemia.

MATERIALS AND METHODS

The study was carried out in the departments of Pathology and Clinical haematology, S.C.B. Medical College and Hospital, Cuttack, within a period from November 2015-November 2017. A total of 100 cases were taken. All diagnosed cases of acute leukaemia detected on morphological basis in the department of Pathology and Clinical Haematology were included in the study. While, chronic leukaemia in blast crisis, and, MDS transformed to acute leukaemia were being excluded from the study. It was a Prospective Study.

The classification scheme proposed by the French-American-British (FAB) Cooperative Group divides ALL into 3 subtypes (L1 to L3) and AML into 7 subtypes (M0 to M7).[4]

For Morphological diagnosis, bone marrow aspirate smear/ direct PBS were taken and, Leishman stain, Myeloperoxidase (MPO) stain were used. For Immunophenotyping by Flowcytometry, six-colour and two laser computerized BD FACS Cantoll Flow cytometer was used. 2 ml blood/bone marrow aspirate with EDTA were taken as samples.

Fluorochromes and Antibodies used Acute Leukaemia Basic Panel 6-colour

Tube	Lineage	FITC	PE	PerCP-Cy5.5	PE-Cy7	APC	APC H7
Tube 1	B-Tube	CD 20	CD 10	CD 38	CD19	CD 34	CD 45
Tube 2	T-Tube	CD 8	CD 56	CD 3	CD 4	CD 7	CD 45
Tube 3	Myeloid Tube	CD 64	CD 33	HLA-DR	CD 13	CD 117	CD 45
Tube 4	Cytoplasmic Tube	MPO	CD 79a	CyCD 3		CD 34 (Optional)	CD 45

RESULTS

A total number of 100 cases of acute leukaemia were studied during the period of 2 years (Nov 2015- Nov 2017). The spectrum of cases studied were divided on the basis of cytomorphology. All acute leukaemia cases were then subjected to flowcytometric immunophenotyping for confirmation of lineage. In our study, cytomorphologically, ALL-L2 accounted for 38% cases, followed by ALL-L1 for 27% cases. While, AML-M2 accounted for 21% cases, followed by AML-M3 for 06% cases, AML-M1 for 05% cases, AML-M4 for 02% cases, and, AML-M5 for 01% cases, according to the FAB classification. (Figure 1). Immunophenotypic results revealed B-ALL to be 50% cases, T-ALL to be 07%, AML to be 32%, APML to be 08% and, MPAL to be 03% cases. (Figure – 2). Now, among 65 cases of ALL, based on morphology and cytochemistry, immunophenotyping demonstrated lymphoid lineage in 56 cases, while rest 9 cases demonstrated myeloid lineage, with AML to be 5 cases, APML 2 cases and MPAL 02 cases. Lineage correction thus done in these 09 cases (Table 1). Similarly, among 29 AML cases based on morphology and cytochemistry, immunophenotyping. Demonstrated myeloid lineage (AML) in 25 cases. Thus, lineage correction done in 04 cases, which immunophenotypically came out to be ALL 01 case, APML 02 cases, and MPAL 01 case. (Table 2). And, out of 06 APML cases, 04 cases immunophenotypically came out to be APML, while 02 cases needed lineage correction from APML to AML. (Table 3). Lastly, in this study, the correlation between the morphological and immunophenotypic diagnosis showed, concordance of 88% in ALL, 91% in AML, 75% in APML and, no concordance a tall, in MPAL. (Table IV).

Methods Used				
Cytomorphology	**65 ALL Cases**			
Immunophenotyping	ALL 56	AML 05	APML 02	MPAL 02

Table 1. Cases Showing Lineage Correction (ALL to AML, APML, MPAL) after Flowcytometric Analysis

Methods Used				
Cytomorphology	**29 AML Cases**			
Immunophenotyping	AML 25	ALL 01	APML 02	MPAL 01

Table 2. Cases Showing Lineage Correction (AML to ALL, APML, MPAL) after Flowcytometric Analysis

Methods Used		
Cytomorphology	**6 APML Cases**	
Immunophenotyping	APML 04	AML 02

Table 3. Cases Showing Lineage Correction (APML to AML) after Flowcytometric Analysis

Types of Acute Leukaemia	Morphological Diagnosis (%)	Flowcytometric Diagnosis (%)	Final Diagnosis (%)	Concordance of My Study (%)
ALL	65	57	57	88
AML	29	32	32	91
APML	06	08	08	75
MPAL	00	03	03	00

Table 4. Table Showing Correlation between Morphological Diagnosis and Flowcytometric Diagnosis

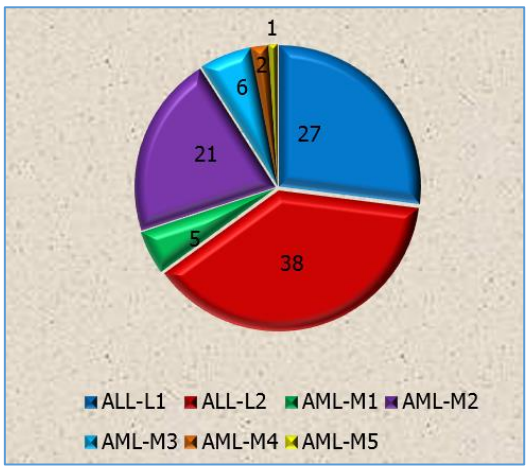

Figure 1. Spectrum of Cases Studied According to Morphology

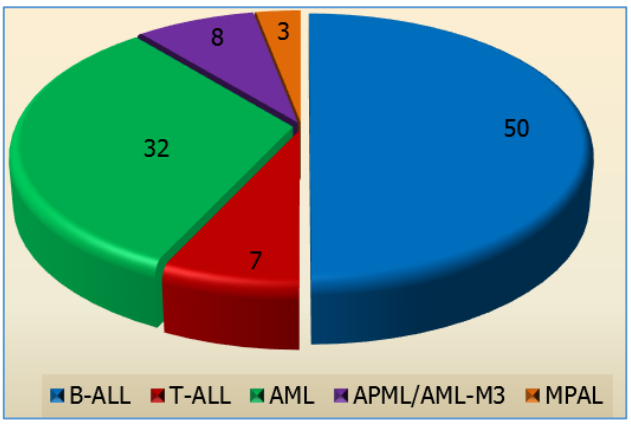

Figure 2. Immunophenotypic Results of Acute Leukemia Cases

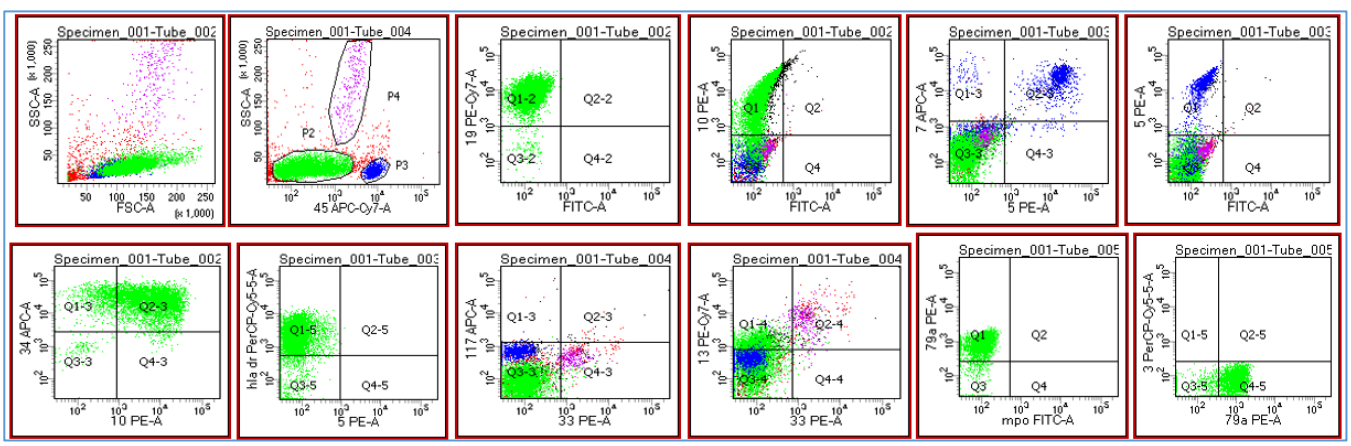

Figure 3. Immunophenotype of B-ALL

(CD 45 = dim+ ve, HLA DR= +ve, CD 34= +ve, CD 10= +ve, CD 19= +ve, cCD79a = +ve)

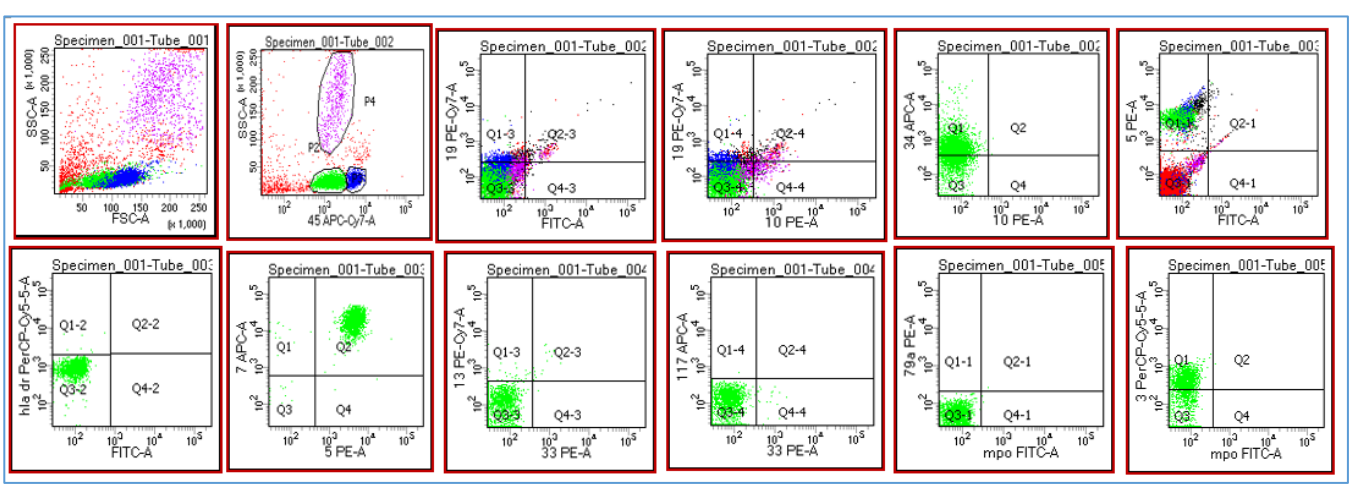

Figure 4. Immunophenotype of T-ALL

(CD 45= 93% (Blasts), HLA DR= +ve, CD 34= +ve, CD 5= +ve, CD 7= +ve, cCD3= +ve).

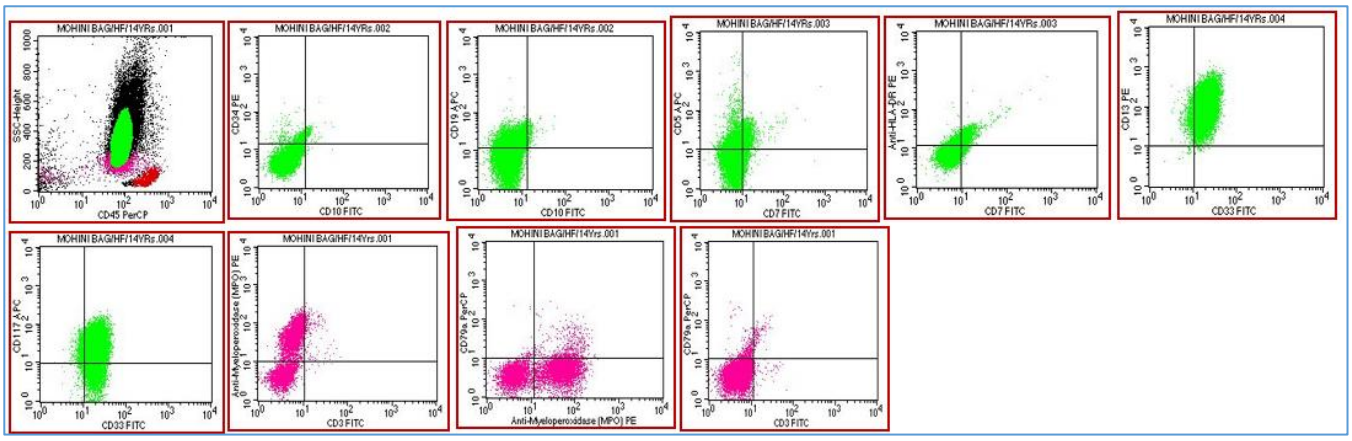

Figure 5. Immunophenotype of AML

(CD 45= 93% (Blasts), HLA DR= +ve, CD 34= +ve, CD 13= +ve, CD33= +ve, CD 117= +ve, cantiMPO= +ve)

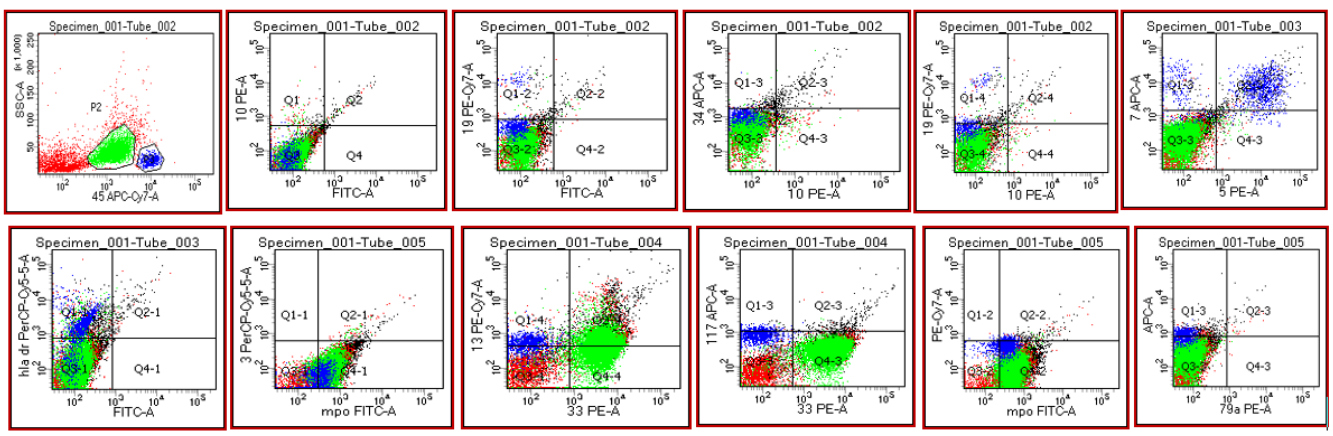

Figure 6. Immunophenotype of APML (AML M3)

(CD 45= +ve, CD 13= +ve, CD33= +ve, cantiMPO= +ve, CD34= -ve, HLA DR=-ve)

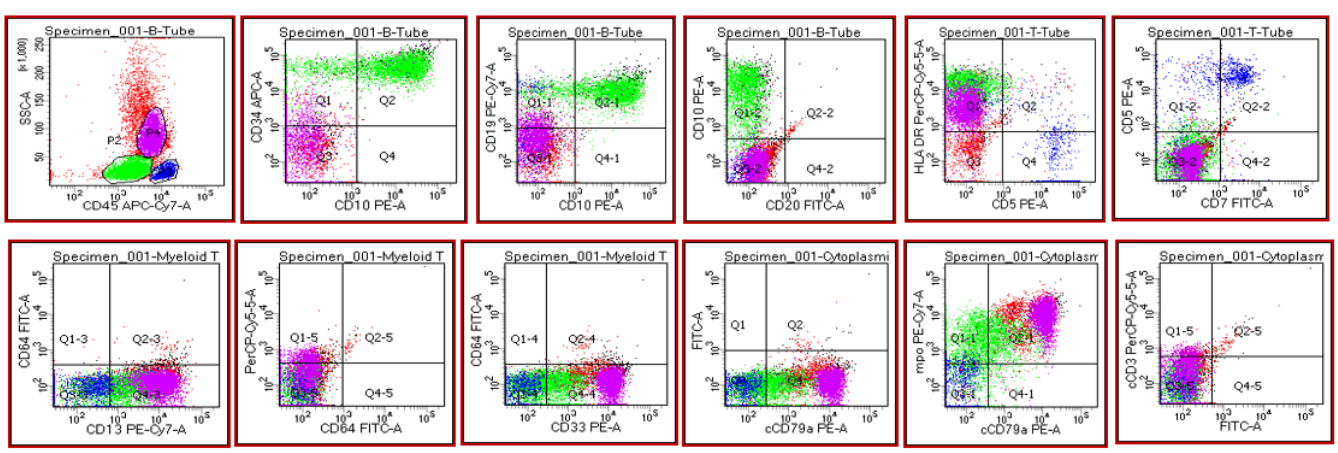

Figure 7. Immunophenotype of MPAL

(CD 45=93% (Blasts), HLA DR= +ve, CD 34= +ve, CD 19= +ve, cCD79a= +ve, CD 13=+ve, CD 33=+ve, CD117= +ve, cantiMPO= +ve)

DISCUSSION

100 cases were evaluated which included detailed history taking, clinical examination, and routine laboratory investigations of the cases. Peripheral smear and bone marrow examination was done to establish the diagnosis, followed by cytochemical staining by Myeloperoxidase staining. The samples were then subjected for immunophenotypic analysis for lineage assessment and subtyping.

In our study, cytomorphologically, ALL-L1 accounted for 27% of cases, ALL-L2 for 38% cases. While, AML-M1 accounted for 05% of cases, AML-M2 for 21% cases, AML-M3 for 06% cases, AML-M4 for 02% cases and AML-M5 for 01% cases according to the FAB classification. (Figure-1) But in contrast to the present study, Choudhary et al[5] report

ALL-L1 (73.3%) to be more common than ALL-L2. According to Sushma Belurkar et al, 2017,[6] AML-M2 is the most common subtype accounting for 14% of cases.

Immunophenotypic results revealed B-ALL to be 50%, T-ALL to be 07%, AML to be 32%, APML to be 08% and MPAL to be 03%. Since our panel of antibodies did not have antibodies for erythroid and megakaryocytic lineage, immunophenotyping could not be used for subtyping acute myeloid leukaemia. (Figure- 2).

According to Metasebia Tegegn et al[7], Addis Ababa, Ethiopia, 2016, AML (including APML) accounts for 52.5% cases and, ALL to be 47.5% cases. With B-ALL accounting for 52.6% cases and, T-ALL for 47.5% cases.

Lineage Correction- Among 65 cases of ALL, based on morphology and cytochemistry, immunophenotyping demonstrated lymphoid lineage in 56 cases, while rest 9 cases demonstrated myeloid lineage. Out of these 9 cases, 05 cases were diagnosed as AML, 02 cases as APML and, 02 cases as MPAL. (Table I). Thus, lineage correction was done in these 09 cases.

Similarly, among 29 AML cases based on morphology and cytochemistry, immunophenotyping demonstrated myeloid lineage (AML) in 25 cases. Rest 4 cases demonstrated immunophenoypically as ALL 01 case, APML 02 case and, MPAL 01 case. (Table II). Thus, lineage correction done in these 04 cases.

And lastly, out of 06 APML cases, 04 cases on immunophenotyping came out to be APML. But, 02 cases had lineage correction from APML to AML. (Table 3)

Here, in our study, out of 100 acute leukaemia cases, 15 cases had lineage correction, thus, accounting for 15% of cases.

According to Misbah Qadir et al in 2006.[8] in their retrospective analysis of cases of acute leukaemia, shows lineage correction by using flowcytometry in 02% of cases. The higher percentage of lineage correction in our study can be attributed to the difference in the efficacy of staining methods and subjective variations in assessment of morphology.

In this study, the correlation between morphological diagnosis with that of immunophenotyping by flowcytometry, showed Concordance of 88% in ALL, 91% in AML, 75% in APML and, no concordance at all in MPAL, ie, 3 MPAL cases were missed cytomorphologically and, that was diagnosed by flowcytometry. (Table 4)

According to Dr. Ravi Murmu et al, 2016, Concordance was of 84% in ALL, 89% in AML (including APML) and 50% in MPAL.[9]

Limitations of the Study- Due to lack of antibodies for erythroid and megakaryocytic lineage, immunophenotyping could not be used for subtyping acute leukaemia. Thus, AML-M6 (erythroleukaemia) or, AML-M7 (megakaryoblastic leukaemia) were not included and if present may have been missed.

CONCLUSION

In the diagnosis of acute leukaemia Cytomorphological discrepancies warrant the use of immunophenotyping by flowcytometry. It plays a pivotal role in lineage assessment. Subtyping, and therapy, but, aberrant expression also needs a critical judgement for MRD screening. Aberrations- An important role in the prognostication and hence, the intensification of therapy and monitoring.

FCM offers the advantage of efficacy coupled with high degree of sensitivity, especially in- AML/ALL, MPAL, MRD screening.

Immunophenotyping is thus mandatory in all cases of Acute Leukaemia, as treatment nowadays is target oriented.

- Immunophenotyping ambiguity can guide case specific mutational analysis and targeted therapy which can change the prognosis dramatically.
- Experience in interpretation in flowcytometry plays a very important role in making a correct diagnosis.
- Chromosomal rearrangement are used for prognostic indicators but, flowcytometry is more pertinent in Indian scenario presently, as molecular studies are not routinely available in majority of the centres.

REFERENCES

[1] Arber DA. Hematopoietic neoplasms: principles of pathologic diagnosis. In: Greer JP, Foerster J, Rodgers GM, et al, eds. Wintrobe's clinical haematology. 12th edn. Philadelphia: Lippincott Williams & Wilkins 2009:1663-1668.

[2] Jaffe ES, Harris NL, Stein H, et al. WHO classification of tumours: pathology and genetics of tumours of haematopoietic and lymphoid tissues. Lyon, France: IARC Press 2001.

[3] Kheiri SA, MacKerrell T, Bonagura VR. Flow cytometry with or without cytochemistry for the diagnosis of acute leukemia. Cytometry 1998;34(2):82-86.

[4] Singh T. Acute leukemias. In: Singh T, ed. Atlas and textbook of hematology. 3rd edn. Avichal Publishing Company 2014.

[5] Abha C. Acute Lymphoblastic Leukemia in childhood, a study of prognostic factors at presentation. In Annual Conference of Indian Society of Haematology and Blood Transfusion, 1997.

[6] Belurkar S, Mantravadi H, Manohar C, et al. Correlation of morphologic and cytochemical diagnosis with flowcytometric analysis in acute leukemia. J Cancer Res Ther 2013;9(1):71-79.

[7] Tegegn M. Diagnostic utility of immunophenotyping by flow cytometry for diagnosis and classification of acute leukemias in Tikur Anbessa specialized hospital, Addis Ababa, Ethiopia. November, 2016.

[8] Qadir M, Barcos M, Stewart CC. Routine Immunophenotyping in acute leukemia: role in lineage assignment and reassignment. Cytometry Part B (Clinical Cytometry) 2006;70B(5):329-334.

[9] Murmu R, Srivastava RK, Banerjee S, et al. Diagnostic accuracy of acute leukemia by flow cytometry in comparison to morphological diagnosis - a study. IOSR Journal of Dental and Medical Sciences 2016;15(3):52-54.