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Original Article

Correlation of cytomorphology with flowcytometric immunophenotyping in patients of acute leukemia in tertiary care hospital

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ABSTRACT

Background: Morphological diagnosis of leukemias may sometimes not correlate with flow cytometry diagnosis. Classification of hematological neoplasm by the World Health Organization gives priority to cytogenetic, molecular biology, and even patient history, in an attempt to classify patients primarily regarding prognosis. Cytomorphology and immunophenotyping complement each other. Morphologyis burdened by a high degree of subjectivity. That is why correlation of information provided by the two techniques is still absolutely necessary. The aim of our study was to correlate between cytomorphological findings and immunophenotyping results on a group of patients investigated for acute leukemia.

Materials and Methods: Study was conducted in department of pathology in Chirayu Medical College and Hospital, Bhopalfrom December 2018 to September 2019. Cases were diagnosed as acute leukemia based on complete blood count and bone marrow aspirate/peripheral smear.These cases were sent to flowcytometry for immunophenotyping for further confirmation.

Results: Total 74 cases of acute leukemias were diagnosed, out of which 30 were Acute myeloid leukemia and 44 were Acute lymphoblastic leukemia. There was 95.95% correlation between diagnosis on cytomorphology and flowcytometry. Two cases remain unclassified on cytomorphology which turn out to be Acute myeloid leukemia on flowcytometry. One case of Acute myeloid leukemia on cytomorphology was diagnosed as Acute lymphoblastic leukemia on flowcytometry.

Conclusions: Inclusion of flowcytometry in routine diagnostic workup of acute leukemia ensures proper characterization as well as management.Major challenges for the near future are the standardization of technical procedures, data interpretation, and reporting.

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INTRODUCTION

Leukemias form a major proportion of hematopoietic neoplasms that are diagnosed worldwide.[1] Flow cytometric (FC) immunophenotyping and morphological analysis of peripheral blood are mandatory investigations in the diagnosis of acute leukemia.They allow classification of acute leukemia by establishing the proliferating cell line and the degree of maturation of the neoplastic cells.In 2016 a revision of the World Health Organization (WHO) classification of leukemia resulted in disease categories that are defined by a combination of clinical, morphologic, immunophenotypic, and genetic features in an attempt to define clinically relevant, biologic entities.[2]

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It should be noted that this hierarchy is operative for clinical practice, giving the most useful data for therapeutic decision making. In terms of the diagnostic algorithm however, the first investigations remain cytomorphology and immunophenotyping. Cytomorphology and immunophenotyping complement each other primarily because they have as common object malignant cell phenotype as awhole (morphology, i.e. surface and intracellular marker expression). On the other hand, morphology is burdened by a high degree of subjectivity. That is why correlation of information provided by the two techniques is still absolutely necessary.[3]

Multiparameter flowcytometry is the preferred method of immunophenotypic analysis due to the ability to analyze large numbersof cells in a relatively short period of time with simultaneousrecording of information about several antigens for eachindividual cell.[4]

MATERIALS AND METHODS

It is a descriptivecomparative study done on 74 patients of suspected acute leukemia in the Department of pathology in Chirayu Medical College and hospital,Bhopal between December 2018 to September 2019. All cases were reviewed for morphologic features by light microscope. On cytomorphological examination,we found 74 cases of acute leukemia and these cases were then analyzed on immunophenotyping by 6 color flowcytometerusing a predefined panel of antibody. All patients who were diagnosed to have acute leukemia on bone marrow aspirate/ peripheral blood examinationwere included in study population. Informed consent was taken from the patients. Study has been approved by institutional ethical committee. Statistical analysis was done on basis of percentage correlation(concordance).

The immunophenotyping by flow cytometry with a panel of Monoclonal Antibodies specific to acute leukemias5,6usually used as: CD3, CD5, CD7, CD10,CD13, CD 14, CD19, CD20, CD33, CD34, CD117, CD 45, CD 46, HLA-DR,cytoplasmic myeloperoxidase (Cy-MPO) andTdT.

T-cell ALL: cytoplasmic (cy) CD3, CD5, CD7 For B-cell ALL: CD19, CD10 and CD 20.

Myeloid cells: CD13, CD33, CD117, CD14, CD64 and Cy-MPO.

Panleukocyte marker: CD45.

Precursor markers: CD34, TdT, HLA-DR. Any antigenic marker was considered positive if 20% or more of the blast cells reacted with a particular antibody.

Inclusioncriteria: All cases of acute leukemia diagnosed on cytomorphology of all age groups during study period.

Exclusion criteria:Patients not consenting for study, patients withhaematological malignancies like lymphoma and multiple myeloma, leukemia associated with other malignancies, patients on adjuvant chemotherapy and patients of chronic leukemia were excluded from the study.

2 ml of peripheral blood were collected mostly from antecubital vein with aseptic precautions. All the samples were collected in EDTA tubes. All specimens were obtained and peripheral blood smears were prepared for morphologic examination using standard technique of Leishman stainingfollowed by light microscopy.4Complete blood count was performed on automated analyzer. Sample collected in EDTA tube were sent for immunophenotyping by flowcytometry.[5] Immunophenotyping was performed on 6 color flowcytometer BD FACSCANTO II by using monoclonal antibodies.For reporting and quality control, Westgard rules are followed to validate the result.

RESULTS

In our present study,we found 74 cases of acute leukemia diagnosed by cytomorphology and these cases were then analyzed by flowcytometry for confirmation.According to analysis based on cytomorphology and immunophenotyping the acute leukemia cases were classified as Acute lymphoblastic leukemia (ALL) and Acute myeloid leukemia (AML). In our study AML comprised of 29 (39%) and ALL 45(60.81%) patients.In ALL cases B-ALL 37(50%) contributed more than T-ALL 8(11%).Among pediatric age group B-ALL was predominant (75%) while AML and T-ALL was seen in 12.5% cases each.Among adults (age>14 yrs) AML was predominant (60%) while B-ALL constituted 31%cases and T-ALL constituted 9% cases. Among all cases, 55.4 % were males and 44.6% were females.

Table 1: Age wise distribution of acute leukemia

Cases	Paedriatic(<14yrs)	Adult(>14yrs)	Total(no.)	%	
AML	4	25	29	39%	
T ALL	4	4	8	11%	61%
B ALL	24	13	37	50%	
TOTAL	**32**	**42**	**74**	**100%**	

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Table 2: Comparision between cytomorphology and flowcytometry results

Case on cytomorphology (No.)		Cases on flowcytometry (No.)	
AML	28	27	AML
		1	TALL
8.0	100	37	BALL
6.6	190	7	TALL
UNCLASSFIED	2	2	AML

Table 3:Concordance and disconcordance between results on cytomorphology andflowcytometry

Concordance/disconcordance	No.	%
Complete concordance	71	95.95%
Partial concordance	2	2.70%
Disconcordance	1	1.35%
Total	74	100%

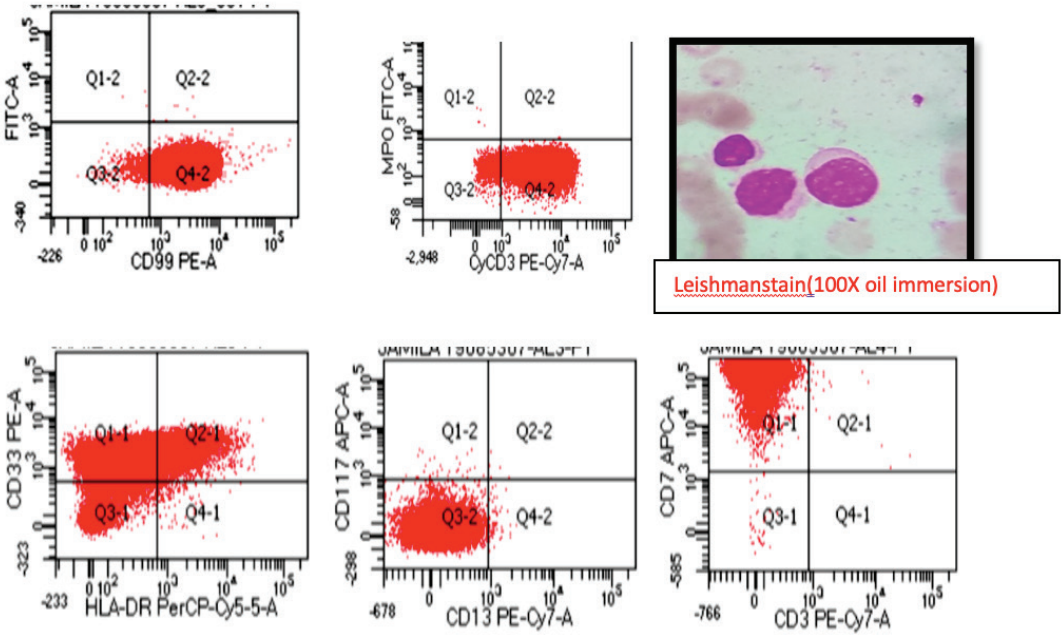

Figure 1: Discrepancy of one case which on light microscopy was reported as AML but on flowcytometry, immunophenotypic features were consistent with T ALL

Among all cases, 28 were cytomorphologically reported as AML, 27 cases were confirmed as AML on flowcytometry and onecase was confirmed as TALL. Two cases that were cytomorphologically unclear, were confirmed as AML on flowcytometry. 44 cases of ALL were diagnosed cytomorphologically, out of which 37 were B ALL and sevenwere T ALL on flowcytometry.Out of 74 cases, 71 cases were diagnosed correctly on cytomorphology and results were concordant on flowcytometry. So, overall concordance was (71/74) 95.95 %.Two cases were cytomorphologically unclear, and were confirmed as AML on flowcytometry. So,there was partial concordance of (2/74) 2.70%. One case was cytomorphologically reported as AML but when subjected to flowcytometry came out to be T ALL (fig. 1). Thus, there was complete disconcordance of 1.35%(1/74).

DISCUSSION

The acute leukemias are a heterogeneous group of diseases characterized by the rapid expansion of a malignant clone of early hematopoietic progenitors that ultimately replace normal bone marrow tissue resulting in marrow failure.[8,9] Timely intervention can minimize morbidity in acute leukemia andthey are usually highly responsive to chemotherapy in the initial phase.[8-10] Earlier classification system for acute leukemias was based solely on cytomorphology and cytochemical examination. World Health Organization's in its Classification of Tumours of Haematopoietic and Lymphoid Tissues incorporated immunophenotyping along with other parameters for best patient outcome.[8] For immunophenotyping of leukemia flowcytometry is the most preferred and convenient method as large number of cells can be assessed accurately within a very short period of time. It can type acute leukemia into AML & ALL and ALL is further subclassified into B-ALL and T-ALL. AML is the second most common type of acute leukemia diagnosed in both adults and children, commoner in adults that are also confirmed in this study which is similar to study done by Subhashjha et al.[11] Various studies reported ALL to be the predominant type of acute leukemia in early years of life and this trend continues up to 20 years, incidence of AML rises with age that mainly affects the adult

Table 4: Cytomorphological correlation with immunophenotyping in various other studies compared with present study

Studies	Concordance (%)
Kheiri et al[16]	77.4%
Mhawech et al[17]	80.32%
Belurkar et al[18]	58%
Monika Gupta et al[19]	96.5%
Aparajita das et al[20]	85%
Dr. Ravi murmu et al[21]	81%
AsifRashed et al[4]	81.2%
Present study	**95.95%**

and elderly population.[13] This study included 74 patients with acute leukemia diagnosed by cytomorphology.

Immunophenotyping was carried out by flow cytometry. In this study, 74 acute leukemia (AL) cases were diagnosed by flow cytometry of which 29 (39%) were AML, 45 (61%were ALL ,similar to study done by ManjuSengar et al.14But different studies reported the incidence of AML and ALL were 70% and 30%15, 53% and 47%16; and 60% and 40%17 respectively. So this study differs from other studies. In this study 32/74 (43.24%) cases were in <14 years age group where ALL was predominant 87.5% (28/32). But in the rest 42 cases aged >14 years AML were found in 29/42 (69.04%) cases and ALL in 17/42 (40.47%) cases. Thus ALL was more common in children while AML was predominant among adults.In our study,age ranged from 1 yr to 64 yrs with median age of 24.5 yrs.55.4% were males and 44.6% were females with male to female ratio of 1.24:1.There was 95.95 % concordance between cytomorphology and flowcytometry results which is much higher as compared to study done by Asifrashedet al (81%).7Other studies showed similar concordance percentage (90%).[18] (Table 4)

Lineage correction was done for one case which were diagnosed as AML on morphology expressed different lineage on FCA. The AML on morphology, was diagnosed as T ALL on immunophenotyping. Two cases that remain unclear on cytomorphology were diagnosed as AML on immunophenotyping. These three cases would have been misdiagnosed or remained unclassified without the aid of flowcytometry. Belurkar et al reported a case which was diagnosed as M0/ALL L2 on morphology and MPAL on FCA.[21] Qadir et al also reported lineage correction in 2% cases.[25] Therefore, immunophenotyping with a limited panel must be routinely performed for the correct diagnosis of acute leukemia.

CONCLUSIONS

Flowcytometry was helpful in assigning correct lineage to leukemia cells and support the use of particular panel of CD markers as a mandatory diagnostic tool after preliminary investigations. Flowcytometry 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 is 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.

Conflict of interest: None

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