ORIGINAL RESEARCH PAPER
Volume - 12 | Issue - 06 | June - 2023 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

AN OBSERVATIONAL STUDY OF IMMUNOPHENOTYPIC PATTERN IN MORPHOLOGICALLY DIAGNOSED CASES OF ACUTE MYELOID LEUKEMIA AT SMS MEDICAL COLLEGE, JAIPUR.

Pathology

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ABSTRACT

Background : The diagnosis and classification of AML were based on the French–American–British (FAB) system for nearly three decades. Morphological examinations of peripheral blood and bone marrow have consistently added value to this classification. Multiparameter flow cytometry is the preferred method of immunophenotypic analysis in AML due to the ability to analyze large numbers of cells in a relatively short period with simultaneous recording of information about several antigens for each cell. **Methods and Material :** This observational type of cross sectional study was carried out in the Pathology department, S.M.S. Medical College, Jaipur, during the period of 2021-2022. A total of 100 patients of all ages and both genders who were newly diagnosed with acute myeloid leukemia were included. Demographic data was noted. The samples were analysed for blast percentage and morphology on leishman stained peripheral blood and bone marrow smears. Immunophenotyping was done by flow cytometry. This included samples of peripheral blood or bone marrow aspirate from patients of morphologically diagnosed AML patients. **Results :** The most common subtype was AML- M2 and most commonly expressed antigen was CD33 followed by CD13.The mean positivity for CD33 among all AML subtypes was 98.94%. Aberrant expression of CD7 and CD19 were expressed in 5.32% and 4.26% of all cases respectively. There was concordance rate of 94% between morphology and FCM in our study. **Conclusion :** A combined evaluation of peripheral blood film, bone marrow aspiration and flow cytometry were done. Flow cytometric analysis must always be performed in conjunction with cytomorphology for definitive diagnosis of acute myeloid leukemia.

KEYWORDS

Immunophenotyping, Acute myeloid leukemia (AML), Aberrant phenotype in AML, Flowcytometry.

INTRODUCTION

Acute myeloid leukemia (AML) refers to a heterogeneous group of malignant diseases characterized by the accumulation of aberrant hematopoietic progenitor cells, known as AML blasts, that cannot progress beyond various stages of maturation and are unable to develop into mature blood cells.[1] It is a common hematological malignancy and accounts for approximately 80% of acute leukemia in adults and 20% of acute leukemia in children.[2] The most commonly used classification was proposed by the FAB cooperative group in 1976. The latest WHO classification of acute leukemias differs from the FAB classification in that greater than or equal to 20% of blasts are used for diagnosis of acute leukemias.[3]The diagnosis and classification of AML were based on the French–American–British (FAB) system for nearly three decades. It was based on morphological and cytochemical findings.[4]

Multiparameter flow cytometry is the preferred method of immunophenotypic analysis in AML due to the ability to analyze large numbers of cells in a relatively short period with simultaneous recording of information about several antigens for each cell.[5] Flow cytometry immunophenotyping is a powerful ancillary tool in the assessment of cell surface and cytoplasmic markers in acute myeloid leukemia. Characteristic myeloid lineage markers MPO, CD13, CD33 and CD117 expressions enable the distinction of acute myeloid leukemia from other types of leukemia.[6]

AIM AND OBJECTIVE

To study the immunophenotypic pattern in morphologically diagnosed cases of acute myeloid leukemia by flowcytometry. Sub-typing of acute myeloid leukemia according to FAB classification by flow cytometry.

METHODS AND MATERIAL

This observational type of cross sectional study was carried out in the Pathology department, S.M.S. Medical College, Jaipur, during the period of 2021-2022. Approval was taken from ethical review board and institutional review board. A total of 100 patients of all ages and both genders who were newly diagnosed with acute myeloid leukemia were included. Treated cases of acute myeloid leukemia ,relapsed cases of acute myeloid leukemia and blast crisis in chronic myeloid leukemia were excluded.

The samples were analysed for blast percentage and morphology on leishman stained peripheral blood and bone marrow smears. Immunophenotyping was done by flow cytometry. This included samples of peripheral blood or bone marrow aspirate from patients of morphologically diagnosed AML patients.

RESULTS

Total 100 cases of acute myeloid leukemia (AML) were studied. Following results were recorded and analyzed.

1. Age And Gender Distribution

The age of the patients at the time of diagnosis ranged from one year to 60 years and above. Mean and standard deviation of age groups was 29.76±16.69. Males constituted 62% and females were 38% with male to female ratio 1.63:1.

Table 1: Age And Gender Distribution

Age (years)	No. of cases	Percentage	Male	Female
1-10	12	12%	9	3
11-20	21	21%	15	6
21-30	22	22%	11	11
31-40	20	20%	11	9
41-50	13	13%	9	4
51-60	9	9%	6	3
>60	3	3%	1	2
Total	100	100%	62	38

2. Clinical Findings Distribution

It was observed that pallor in 99% cases was the most common clinical sign followed by hepatosplenomegaly 22 (22%), lymphadenopathy 12 (12%), and Bleeding Manifestation had 8 (8%).

3. Comparison Of Cases Of Flow Cytometry And Cytomorphology

All AML cases were then subjected to flow cytometric immunophenotyping for confirmation and subtyping based on FAB classification. Out of 100 cases , 94 cases were diagnosed as acute myeloid leukemia by flow cytometry and 6 cases were turned out to be ALL on flow cytometry. The concordance rate between morphology

and flow cytometry is seen in 94% cases.

Table 2 : Comparison Of Cases Of Flow Cytometry And Cytomorphology :

S. No.	Classification	Morphology	Flowcytometry	Correlation
1	AML	100	94	94%
2	ALL	0	6	-

4. Classification Of Aml Cases Based On Flow Cytometry

Out of 94 cases, AML-M2 was the most frequent subtype constituting 55.32% followed by AML-M4 (22.34%). The least common subtype was AML-M1 (2.13%). There were no cases of AML-M0, AML-M6 and AML-M7.

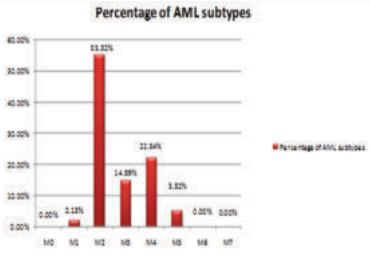

Figure 1 : Classification Of AML Cases Based On Flow Cytometry

5. Distribution Of Positive Results Of Different Markers Among Different AML Subtypes

According to positive markers distribution , CD33 showed positivity in 98.94% cases and CD13 in 91 (96.81%) cases. The aberrant expression of CD7 showed positivity in 5.32% cases, expressed in AML-M2 and CD19 was expressed in 4.26% cases.

Table 3 : Distribution Of Results Of Different Markers Among Different AML Subtypes

AML Subtypes	No. of cases	MPO (%)	CD 13 (%)	CD 33 (%)	CD 117 (%)	CD 34 (%)	HL ADR (%)	Cd 14 (%)	CD 64 (%)	CD 19 (%)	Cd 7 (%)
AML- M1	2	50.00	50.00	100.00	100.00	100.00	100.00	0.00	0.00	0.00	0.00
AML- M2	52	94.23	96.15	98.08	96.15	84.62	88.46	0.00	0.00	7.69	9.62
AML- M3	14	92.86	100.00	100.00	14.29	7.14	7.14	7.14	0.00	0.00	0.00
AML- M4	21	95.24	100.00	100.00	90.48	85.71	90.48	61.90	28.57	0.00	0.00
AML- M5	5	80.00	100.00	100.00	100.00	100.00	100.00	40.00	80.00	0.00	0.00
Total	94	92.55	96.81	98.94	82.98	74.47	77.66	17.02	10.64	4.26	5.32

DISCUSSION

Acute myeloid leukemia is a clonal disorder of hemopoietic stem cells characterized by the inhibition of differentiation and the subsequent accumulation of cells at various stages of incomplete maturation and by reduced production of healthy hemopoietic elements.[7]

Morphological examinations of peripheral blood and bone marrow have consistently added the value to this classification especially in the identification of specific features of blast and non-blast cells. Flow cytometric analysis of acute leukemia is interpretive, combining the patterns and intensity of antigen expression to reach a definitive diagnosis. Flow cytometry immunophenotyping is a powerful ancillary tool in assessment of cell surface and cytoplasmic markers in acute myeloid leukemia.[4] The age-adjusted incidence rate is 3.6 per 100,000 men and 2.8 per 100,000 women per year.[8]

In the present study patients were predominantly in the age group of 21-30 years (22%). Our study showed that the cases were common amongst adult age group. Similar age groups were found in other studies in different parts of world.[5,9,10,11]

In our study the male to female ratio was 1.63 : 1. Thus males formed the majority of patients, similar to some other international studies.[5,10,12,13]

In the present study , out of 94 cases diagnosed as AML in flow cytometry, AML-M2 was the most frequent subtype. Similar findings were observed in other studies which were conducted in different parts of world.[5,11,14]

The most common myeloid markers expressed in AML cases in the present study were CD13, CD33, MPO . Among them CD 33 was the most common expressed marker. The results were consistent with the previous studies.[14,15,16,17]

CD7, a T-cell antigen known to show aberrant expression was most commonly expressed in our study (5.32%) followed by CD19 (4.26%). Same finding was observed in most of the international studies where CD7 was most commonly expressed aberrant antigen followed by CD19.[14,16,18]

In this study, correlation between morphological diagnosis with that of immunophenotyping by flow cytometry showed concordance of 94% cases. Our study showed a high concordance rate as compared to studies.

Table 4: Comparison Of Concordance Rate Of Different Studies

Study	Year	Concordance rate (%)
Belurkar et al19	2013	84%
Murmu et al20	2016	89%
Aparajita D et al21	2018	91%
Shailendra Jambhulkar et al5	2019	88%
Basharat M et al14	2019	90%
Present study	2022	94%

CONCLUSION

To conclude, the accurate diagnosis of acute myeloid leukemia is effectively accomplished by multifaceted approach as clinical, peripheral blood film, bone marrow aspiration and immunophenotyping using flow cytometry.

Although considered superior, flow cytometric analysis must always be performed in conjunction with cytomorphology for definitive diagnosis of acute myeloid leukemia.

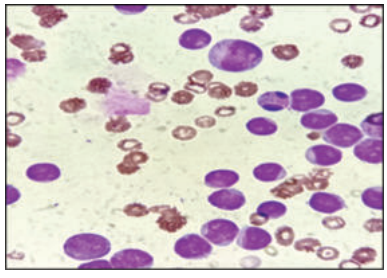

Figure 2 :pbf Of Aml-m2 Under High (1000x) Power Showing Myeloblasts

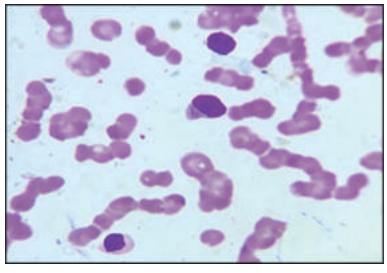

Figure 3 : Bma Of Aml-m2 Under High Power Showing Blast Cell Having Single Auer Rod.

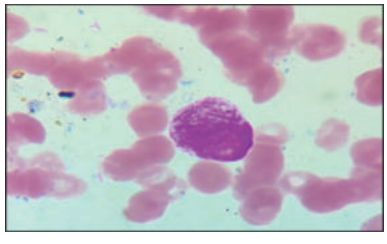

Figure 4 : Faggot Cell Having Multiple Auer Rods Are Characteristic Of Aml-m3.

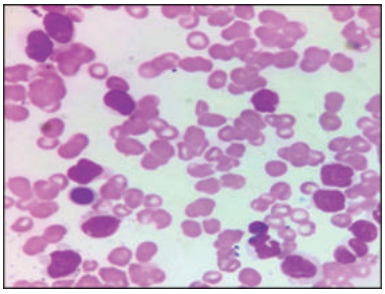

Figure 5 : Bma Of Aml-m4 Under High- Power Showing Blast Cells, Few Normoblast

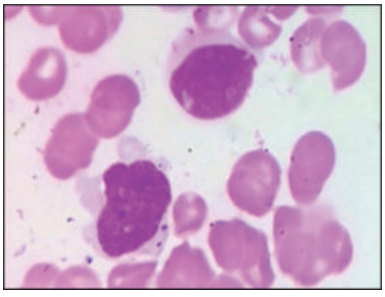

Figure 6 : Blast Cells In Aml-m4 Having Under Vacuolated Cytoplasm.

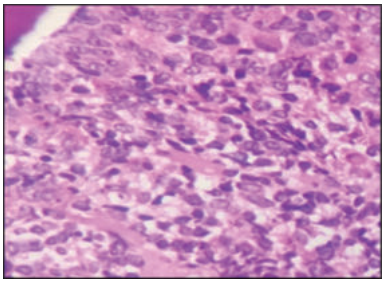

Figure 7 : Bone Marrow Biopsy Of Aml- High Power Showing Sheets Of Blast

Financial Support And Sponsorship: Nil
Conflict Of Interest: Nil

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