Section: Pathology

Original Research Article

A RETROSPECTIVE STUDY OF FREQUENCY OF GENETIC ABNORMALITIES IN AML WITH ITS MORPHOLOGIC CORRELATION

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ABSTRACT

Background: To study the frequency of genetic abnormalities and their correlation with morphology in cases of AML in western Maharashtra.
Materials and Methods: Total 90 cases of AML diagnosed by morphology and immunophenotyping were included. Molecular study was carried out by AML- Multiplex RT-PCR to detect genetic abnormalities.
Results: Out of 90 cases, 27 cases showed genetic abnormalities (30%), while 63 cases did not show any abnormality (70%). PML: RARA fusion was the most common abnormality (21.1%) followed by RUNX1::RUNX1T1 fusion (6.7%) and NPM1 mutation (2.2%). Concordance between the cytomorphology and specific genetic abnormality was found in 92.3 % cases. The cytomorphological accuracy in predicting the associated genetic abnormality for AML with PML::RARA fusion was 100% (19/19 cases), for AML with RUNX1::RUNX1T1 fusion 50% (3/6 cases) and 100% (2/2 cases) for AML with NPM1 mutation.
Conclusion: Specific genetic abnormalities in AML form a distinct subgroup and are important in diagnosis and management of patients. In our study 30% cases showed genetic abnormalities. High concordance rate of 92.3% between the cytomorphology and specific genetic abnormality stresses the role of cytomorphology as an important tool in the predicting the genetic abnormalities in AML in resource poor settings providing fast and accurate diagnosis, till the cytogenetic and molecular study reports are awaited. This will help the clinical hematologist to start immediate treatment especially for cases of APML which is a medical emergency.
Keywords: AML- Acute myeloid leukemia, APML - Acute promyelocytic leukemia, genetic abnormalities, RCA - Recurrent cytogenetic abnormalities.

INTRODUCTION

Acute myeloid leukemia (AML) is clinically and biologically heterogeneous clonal hematopoietic stem cell malignant neoplasm characterized by uncontrolled proliferation of hematopoietic progenitors in the bone marrow and peripheral blood.[1] It's an aggressive type of malignancy that is characterized by having heterogenous genetic makeup and a complex clonal evolution. Morphology, immunophenotyping, cytogenetic and molecular genetic features are used to subtype them.[2] The earliest is the FAB classification which uses morphological system based on the evaluation of blast population in bone marrow by qualitative and quantitative analysis. It has a poor reproducibility and prognostic significance. Furthermore, the developments into the field of immunophenotyping, cytogenetic and molecular genetic analysis have made way for the integrated diagnosis of leukaemia based on morphology, immunophenotyping and cytogenetic analysis.[3,4]

The World Health Organization (WHO) classification of haematolymphoid neoplasms was introduced in 2001 and updated in 2008, 2016 and 2022. It requires integration of clinical history, morphology and cytogenetic analysis along with immunophenotyping. The results of cytogenetic and molecular analyses may not become available immediately, so, the role of morphology plus immunophenotyping is important to rapidly reach a likely diagnosis.[5]

The 2022 WHO classification has separated AML into two categories: AML with defining genetic abnormalities and AML defined by differentiation. Another important change is elimination of 20% blast criteria for AML types with defining genetic abnormalities.[6]

This study aims at study of types and frequency of genetic abnormalities in Acute Myeloid Leukaemia and their correlation with the morphologic findings which will help in providing rapid complete diagnosis to the clinicians to initiate definitive treatment at the earliest.

MATERIAL AND METHODS

Patient Samples

90 cases of AML diagnosed by morphology, immunophenotyping and molecular study were retrospectively evaluated. The bone marrow aspiration slides were retrieved. The data on immunophenotyping and molecular study was collected from the patients medical records.

Cytomorphologic Analysis

Leishman stained bone marrow aspiration smears were used for morphological study. All the cases were independently reviewed by two pathologists to ascertain the morphological features to predict the associated cytogenetic abnormalities.

For AML with t(15;17) i.e. PML::RARA fusion, features of interest considered were as:

1. For hypergranular APML - abnormal hypergranular promyelocytes, bilobed nucleus, Auer rods and faggot cells.
2. For hypogranular type APML - abnormal hypogranular promyelocytes, bilobed nucleus, Auer rods, and faggot cells.

For AML with t(8:21) i.e. RUNX1::RUNX1T1 fusion - features taken into consideration were long thin Auer rods in blasts or maturing myeloid cells, myeloid dyspoiesis (in the form of hypo-granularity, basophilic cytoplasm with salmon coloured granules or pseudo- Pelger- Huet anomaly), eosinophilia (>3% of all nucleated cells), cytoplasmic vacuoles and perinuclear clearing/hoff in the Golgi zone.[3,15]

For AML with NPM1 mutation, cytomorphological feature considered was blasts with cup shaped nuclei.[3]

For AML with inv(16) i.e. CBFB::MYH11 fusion, increase in eosinophils and their precursors with basophilic staining characteristics was considered.

Molecular Analysis

Molecular cytogenetic analysis was carried out using Real Time Polymerase Chain Reaction with Gel electrophoresis. Heparinized bone marrow samples were used for the test. The samples were tested for t(15:17) i.e. PML::RARA, t(8;21) i.e. AML1-ETO, Inv(16) i.e. (CBFB::MYH11), NPM1 mutation, FLT3 and c- KIT mutations.

RESULTS

Total 90 cases of AML diagnosed by using morphological findings and immunophenotyping were included in the study. Morphologic analysis revealed 20 cases with morphologic features indicative of AML with PML::RARA fusion, 4 cases with AML with RUNX1::RUNX1T1 fusion and 2 cases with NPM1 mutation. Thus total 26 cases were predicted to have genetic abnormalities.

Molecular analysis was done in all the cases. 27 out of 90 cases (30%) showed genetic abnormalities. The results revealed highest frequency for AML with PML::RARA fusion which was seen in 19 cases (21.1%) followed by AML with RUNX1::RUNX1T1 fusion in 6 cases (6.7%) and AML with NPM1 mutation in 2 cases (2.2%).

Morphologic findings observed with specific cytogenetic abnormalities were as follows:

Out of 19 cases with AML with PML::RARA fusion, 13 cases (67%) were classical hypergranular type and 6 cases (33%) were hypogranular variant.

Of these 13 cases of classical APML, all (100%) cases showed abnormal hypergranular promyelocytes, bilobed nucleus and Auer rods while multiple Auer rods (faggot cells) were seen in 8 cases (67%).

Out of 6 cases of hypogranular variant of APML, all cases showed bilobed nucleus and Auer rods (100%) while few hypergranular promyelocytes and Faggot cells were observed in only 2 cases (34%).

For AML with RUNX1::RUNX1T1 fusion, all 6 cases showed blast percentage more than 20% in the bone marrow. 3 out of 6 cases showed characteristic morphology. Myeloid dyspoiesis was seen in 3 cases (100%), long thin Auer rods, cytoplasmic vacuolations and blasts having basophilic cytoplasm with perinuclear Golgi hoff were seen in 2 cases (67%), and eosinophilia was seen in 1 case (33%). Other 3 cases showed morphology of AML -M1.

Both cases (100%) of AML with NPM1 mutation showed AML with monocytic maturation with blasts showing cup shaped nuclei.

We did not encounter any case with inv(16) in our study.

Concordance between specific morphology and genetic abnormality was observed 92.3% (24/26) cases.

For AML with PML::RARA fusion, 19/20 cases (95%) show concordance. One case showing morphology of hypogranular variant of APML was

tested negative for PML::RARA fusion. Immunophenotyping in this case showed AML with monocytic differentiation.

For AML with RUNX1::RUNX1T1 fusion (N=6), only three out of four cases which were predicted to have genetic abnormality on morphology tested positive. Other 3 cases were labelled AML without maturation (AML-M1) on initial morphologic evaluation but were positive for RUNX1::RUNX1T1 fusion on molecular study.

Both the cases (100%) of AML with NPM1 mutation show concordance with the morphology.

Morphologic and cytogenetic discordance was observed in two cases (7.7%), one case each of PML::RARA fusion and RUNX1::RUNX1T1 fusion.

The cytomorphological accuracy for AML with PML::RARA fusion was 100% (19/19 cases), for AML with RUNX1::RUNX1T1 fusion 50% (3/6 cases) and 100% (2/2 cases) for AML with NPM1 mutation.

The positive predictive value was 95% for AML with PML::RARA fusion (19/20), 75% for AML with RUNX1::RUNX1T1 fusion (3/4) and 100% for AML with NPM1 mutation (2/2).

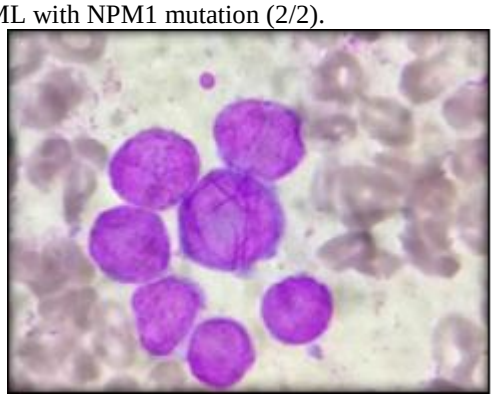

Figure 1: Bone marrow aspiration smear of APML showing hypergranular promyelocytes with bilobed nuclei and faggot cells.

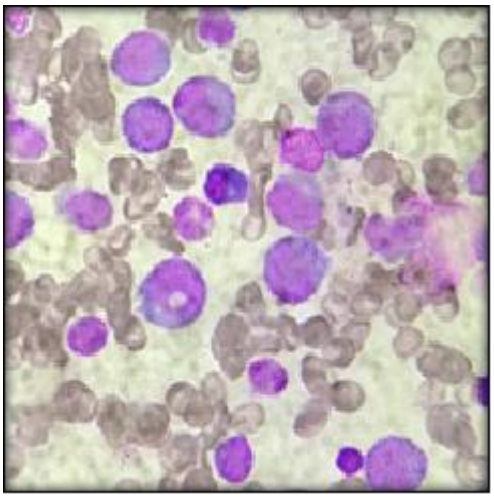

Figure 2.1: Bone marrow aspiration smear of AML with t(8;21) showing blasts with blue cytoplasm, large granules and cytoplasmic vacuoles.

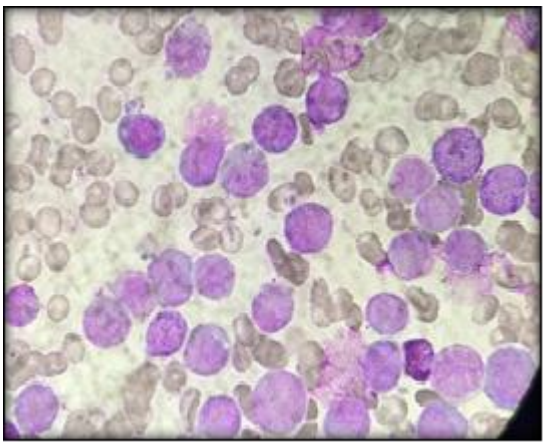

Figure 2.2: Bone marrow aspiration smear of AML with t(8;21) showing blasts with blue cytoplasm, large granules, cytoplasmic vacuoles and long thin Auer rod.

DISCUSSION

AML is a heterogenous group of diseases having varied clinical, morphological and cytogenetic features.[5] It is essential to have a precise diagnosis rapidly, particularly in cases of APML as a delay can lead to life threatening bleeding.[3,7,8,9] Also, it is of importance to anticipate the associated cytogenetic abnormalities from morphology as some of them have good prognosis. Further confirmation can be done on immunophenotyping and detailed genetic study.[10]

The frequency of genetic aberrations in AML subgroups is well reported worldwide. The comparison of frequency of genetic abnormalities in AML patients in the present study with various previous studies has been shown in table1 and table 2.[3,5,9,21,22]

In our study, the frequency of genetic abnormality was 30% (27 out of 90 cases). The highest frequency was found for AML with PML::RARA fusion which was seen in 19 cases (21.1%) followed by AML with RUNX1::RUNX1T1 fusion in 6 cases (6.7%), AML with NPM1 mutation in 2 cases (2.2%). These results are comparable with the other studies mentioned above.[3,5,9,21,22]

Few published studies have reviewed the practical significance of distinctive cyto-morphologic features for various types of AML. These researches have revealed that morphology is a crucial component and an effective diagnostic tool in determining the common subtypes of AML even before cytogenetic or molecular tests are carried out.[9,16,17,18]

In the study by Arber et al, on 63 AML-RCA patients, true positive rates of cytomorphological analysis correlating with cytogenetics was 95% for APML i.e. PML::RARA fusion and 87.5% for t(8;21) i.e. RUNX1::RUNX1T1 fusion.[9]

The Foucar et al study on 38 AML-RCA cases, consisting of 12 APML patients and 7 cases of AML with RUNX1::RUNX1T1 fusion, showed significant relevance of cytomorphological analysis with

accuracy of 92% (11 of 12) for APML and 86% (6 of 7) for t (8;21).[18]

In a study by Jakovic et al, findings demonstrated cytomorphological accuracy of 97.56% (40 of 41) for AML with PML::RARA fusion, 57.89% (11 of 19) for RUNX1::RUNX1T1 fusion and 70% (7 of 10) for inv(16)/ t(16;16) i.e. CBFB::MYH11 fusion.[3]

Similar to these studies, in our study the cytomorphological accuracy for AML with PML::RARA fusion was 100% (19/19 cases), for AML with RUNX1::RUNX1T1 fusion 50% (3/6 cases) and 100% (2/2 cases) for AML with NPM1 mutation.

Furthermore, in the available literature there are very few uncommon cases that have typical morphological and immunophenotype findings corresponding to APML without any karyotype abnormalities and molecular evidence of PML::RARA rearrangement on the RT-PCR.[12,13] In our study, we likewise observed a single case with morphological features suggestive of hypogranular type APML but negative t(15;17) i.e. PML::RARA fusion on the cytogenetic study.

The findings of this study are based on thorough morphological examination. These are very useful clinically in resource poor settings where results of molecular studies will not be available immediately. Thus enabling the clinicians to commence the targeted therapy immediately especially in cases of APML which will definitely improve the outcome. However, the definitive diagnosis of these cases will be based upon cytogenetic and molecular tests.

Table 1: Frequency of genetic abnormalities

Name of study	Total No of cases	No of cases with genetic abnormalities
Vundinti,et al[21],	282	145 (51.42 %)
Arber et al[9]	228	74 (32.5%)
Jakovic et al[3]	396	66 (16.66%)
Ranka et al[22]	211	86 (40.47%)
Enjenti et al[5]	454	275 (61%)
Present study	90	27 (30 %)

Table 2: Frequency of PML::RARA and RUNX1::RUNX1T1 fusion

Name of study	AML with PML::RARA fusion	AML with RUNX1::RUNX1T1fusion
Vundinti,et al[21],	38.6%	20.68%
Arber et al[9]	7.9%	6.6%
Jakovic et al[3].	10.10%	4.3%
Ranka et al[22]	11.0%	7.5%
Enjenti et al[5]	11%	7.5%
Present study	21.1%	6.7%

CONCLUSION

Our study affirms that cytomorphology is still a highly pertinent tool in the diagnostic evaluation of various subtypes of AML. It can give rapid and accurate results before the complete cytogenetic and molecular study results become available. This will help the clinicians to start definitive targeted therapy immediately, especially in cases of APML, thus increasing the possibility of better outcome.

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