ORIGINAL RESEARCH PAPER
Volume - 9 | Issue - 7 | July - 2020 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

FLOW CYTOMETRY EVALUTION AND CYTOGENETICS OF ACUTE PROMYELOCYTIC LEUKEMIA

Pathology

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ABSTRACT

Acute promyelocytic leukemia (APL, AML-M3) is a distinct subtype of AML with characteristic cytomorphology,immunophenotyping , maturation arrest at the promyelocytic stage of granulocytic differentiation and t(15;17)/PML-RARA that responses to maturation inducing treatment with all trans-retinoic acid (ATRA).

Method-A total of 39 APL patients evaluated between June 2015 And October 2015 with adequate flow cytometry (FC) data, bone marrow aspirates and presence of t(15;17)/PML-RARA by conventional cytogenetics and/or fluorescence in situ hybridization (FISH) studies were included in this study.

Result- Neoplastic cells are positive for MPO (97%), CD13 (~97%), CD33 (94%), CD117 (51%), and negative for CD10, CD11b, CD11c.A subset of cases was positive for HLADR (~0.1%), and CD34 (~0.4%),The expression of CD13 was strong to moderate, expression of CD33 was strong and expression of CD19, if present, was moderate .

Conclusion- Morphology ,flow cytometry and cytogenetic evalution either by FISH or Conventional method helps in Accurate diagnosis of APL and it helps in differential diagnosis from other leukemia.

KEYWORDS

INTRODUCTION -

Acute myeloid leukemia (AML) represents a heterogeneous group of disorders with variable clinical presentation, cellular morphology, immunophenotype, therapeutic response and overall prognosis. Acute promyelocytic leukemia (APL, AML-M3) is a distinct subtype of AML with characteristic cytomorphology, maturation arrest at the promyelocytic stage of granulocytic differentiation and t(15;17)/*PML-RARA* that responses to maturation inducing treatment with all trans-retinoic acid (ATRA) . Because of tendency for disseminated coagulopathy, a typical and life-threatening complication of APL, establishing correct diagnosis of APL in a timely fashion is critical in management of patients with APL. Characteristic translocation between the long arms of chromosomes 15 and 17, which fuses the promyelocytic leukemia gene (PML) on chromosome 15 to the retinoic acid receptor- α (RARA) gene on chromosome 17 resulting in the chimeric gene encoding PML/RARA fusion protein (1-3). The characteristic cytomorphology and immunophenotype allow correct identification of cases suggestive of APL, leading to mandatory chromosomal/molecular testing, either by fluorescence in situ hybridization (FISH) or by reverse transcriptase polymerase chain reaction (RT-PCR) for definite confirmation of APL diagnosis .(4-7)

MATERIALAND METHODS -

A total of 39 APL patients evaluated between June 2015 And October 2015 with adequate flow cytometry (FC) data, bone marrow aspirates and presence of t(15;17)/*PML-RARA* by conventional cytogenetics and/or fluorescence in situ hybridization (FISH) studies were included in this study. Cases negative for t(15;17)/*PML-RARA* or cases in which the cytogenetics/FISH results were not available were excluded. Only cases with a new diagnosis of APL from untreated patients were included. At the time of original sample processing and analysis, we used 28 heparinized bone marrow (BM) aspirate and 11peripheral blood, and processed the specimens within 24 hours of collection.

For flow cytometry study, 100µl of whole blood or bone marrow samples were collected and treated with the respective panel of antibodies for APML (CD45, CD117, CD33, CD13, CD15, HLADR, CD14 and CD34) and incubated for 10 minutes at room temperature. After incubation, the cells were treated with BD Erythrocyte lysing solution (1:10 dilution) and incubated for 10min at RT. Later centrifuged at 1000 rpm for 5min to pellet down the WBCs and later washed twice with 2ml of sheath fluid. The final pellet is resuspended in 500 µl of Phosphate buffer saline (PBS) solution, and proceeded for acquisition. Acquisition and analysis was performed in BD Facs Canto.

Fluorescent In Situ Hybridization (FISH) PML-RARA Translocation Using Locus Specific Identifier Probe

Requirements:

Sample slide, Water bath, Diamond marker, Phase contrast microscope

Reagents:

20X SSC, 2X SSC, NP-40 (Nonidet-40/2X SSC) 0.1% NP-40 (Nonidet-40/2X SSC), Nucleic acid stain (Counter Stain), Rubber cement: This FISH probe is intended to detect the t (15;17) The probe is used with metaphase chromosomes or interphase nuclei.

Protocol:

Denaturation and hybridization of the probe

· Slide is prepared .
· Gently outline the area containing the interphase nuclei on the back of the slide with a diamond tipped scribe.
· Required amount of probe mixture is applied to the target area.
· Coverslip is applied immediately and sealed with rubber cement.
· Slide is placed in humidified chamber.
· Co-Denaturation of target DNA and probe DNA is carried out at 750C which is followed by hybridization at RT for 17 hrs.
· Rubber cement and coverslip are removed
· Couplin jar with 0.4XSSC NP-40 is placed in a 730C water bath
· Slide is placed in 2XSSC NP-40 at room temperature for 1 minute.
· Air-dried in dark.
· DAPI- counter stain is applied to the target area and coverslip is applied.
· Observe slide under the fluorescence microscope.

Interpretation:

The labeled DNA probe that is bound to the chromosomes fluoresces when the chromosomes are exposed to ultraviolet light, showing the presence and location of the probe-bound DNA sequences.

RESULTS –

Thirty nine cases of APL with t(15;17)/ *PML-RARA* confirmed by conventional cytogenetics and/or FISH studies were analyzed for FC immunophenotypic features. The age of patients ranged from 3 to 55 years (average 33.1). There were 17 men and 22 women.

Morphological diagnosis was thought of Acute promyelocytic leukemia , in cytochemistry of 37 cases are Sudan positive two cases were Sudan negative.

On flow cytometry Predominant population of atypical promyelocytes shows markedly increased SSC (leukemic cells distributed in “granulocytic” gate on CD45 versus SSC dot plot display).

These cells were positive for MPO (97%), CD13 (~97%), CD33 (94%), CD117 (51%), and negative for CD10, CD11b, CD11c, (see Table I for details). A subset of cases was positive for HLADR (~0.1%), and CD34 (~0.4%),The expression of CD13was strong to moderate, expression of CD33 was strong and expression of CD19, if present, was moderate . Figure 1 presents a typical example of classic APL.

Table -1 IPT profile of APML

Marker	Frequency (%)	Expression
MPO	97	Week to Moderate
CD 34	0.4	Week to Moderate
HLADR	0.1	Week
CD 13	97	Strong to Moderate
CD 33	94	Strong to Moderate
CD 117	51	Week
CD 5	0.2	Week
CD 19	10	Moderate
CD 79A	0.1	Moderate

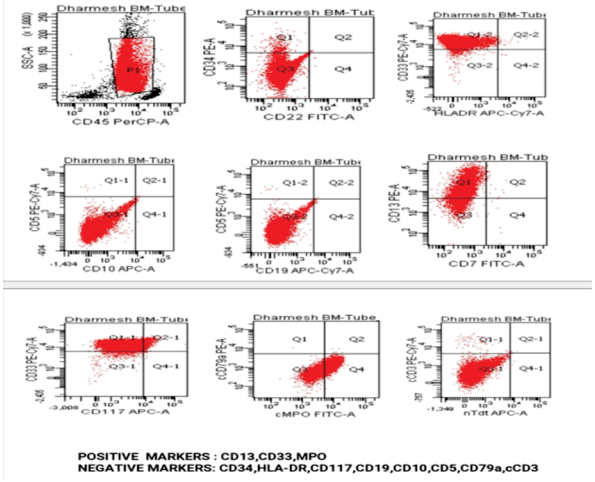
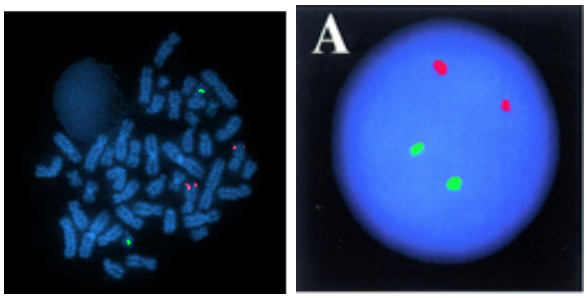


Figure 1- Flow Cytometry Of Classic APML

Fluorescent In Situ Hybridization (FISH) PML-RARA translocation using Locus Specific Identifier Probe identified translocation (15:17) positive in all 39 cases.(Figure 2)



DISCUSSION

Flow cytometry and cytogenetics plays an important role in the diagnosis and subclassification of acute leukemias. Based on side scatter and phenotypic characteristics of blasts FC allows for differentiating between major types of acute leukemias (e.g. minimally differentiated AML versus ALL, acute monoblastic leukemia versus NK-cell lymphoma/leukemia or B-ALL versus T-ALL).

It suggests specific diagnoses such as acute promyelocytic leukemia or acute monoblastic leukemia, and helps to monitor patients after treatment.(10)

Classic APL has a well-recognized flow cytometric pattern with increased side scatter, lack of expression of HLA-DR,CD34, CD11a, CD11b, positive CD117, often positive CD19, variable (heterogeneous)positive for CD13 and bright positive for CD33 and often positive for CD (19) .This current studies show 0.1% cases positive for HLADR and 0.4% cases positive for CD34 .

Flow cytometry plays an important role in identifying cases highly suggestive of APL, which allows for immediate reflex testing by FISH and/or PCR for t(15;17)/*PML-RARA* for final confirmation of the diagnosis.(10)

Awareness of the flow cytometric pattern of APL may help to identify an additional group of patients who would benefit from fast confirmatory FISH and/or PCR testing. FISH is thus more sensitive for diagnosis of APML; however karyotype is more useful for diagnosis of other additional quantitative and qualitative chromosomal abnormality.

CONCLUSION –

Morphology ,flow cytometry and cytogenetic evalution either by FISH or Conventional method helps in Accurate diagnosis of APL and it helps in differential diagnosis from other leukemia.

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