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Original Research Article

Application of Fluorescence *in situ* Hybridization in Detection of PML/RARA Translocation in Patients with Acute Promyelocytic Leukemia

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ABSTRACT

Background: Acute promyelocytic leukaemia (APML) is a sub-type of acute myeloid leukaemia (AML). APML accounts for 10% of all AML diagnosis. Diagnosis is made by presence of abnormal promyelocytes in circulating blood and bone marrow smear and presence of PML RARA fusion transcript which is responsible for leukemogenesis. Fluorescence in situ Hybridisation (FISH) plays key role in confirming diagnosis by detecting PML/RARA (15;17) translocation which will help in deciding treatment and prognosis in patients diagnosed with APML.

Aims and objectives: To estimate presence of PML/RARA translocation in patients diagnosed with APML using FISH.

Materials and methods: Bone marrow samples of 20 patients diagnosed with APML was collected for standardized lymphocyte culture method followed by standard protocol for FISH using commercially available probes for PML /RARA gene. Ethical clearance was obtained from institutional ethical committee.

Results: Out of 20 patients, 18 patients had shown positive result for PML/RARA translocation. Hence 90% of patients showed positive results for fusion gene.

Conclusion: Detection of PML/ RARA translocation helps in rapid diagnosis as well as treatment of patient with APML. Further it will also help in prognosis and follow up to detect residual disease in these patients.

Key-words: *Acute promyelocytic leukemia, PML/RARA gene, FISH, Cytogenetics, ATRA*

INTRODUCTION

Acute Promyelocytic leukemia (APML) is a type of acute myeloid leukemia belongs to M3 type of FAB classification. APML is a medical emergency since there is high chance of patient going to disseminated intravascular coagulation. [1-2] Hence diagnosis and timely management becomes mandatory. APML is caused by arrest of dividing granulocutes at promyelocyte stage during granulopoesis. This arrest is caused by translocation between chromosomes 15 and 17 which has PML (Promyelocytic Leukemia) gene and RARA (Retinoic receptor Alpha) gene respectively. This results in PML/RARA fusion gene which produces abnormal retinoic acid receptors leading to continuous repression of RARA target genes leading to block at promyelocytic stage. APML is characterised by presence of circulating abnormal promyelocytes in peripheral blood and bone marrow. Diagnosis of PML/RARA Translocation is necessary since this forms key tool in treatment. With the advent of Al Trans Retinoic acid (ATRA) and arsenic trioxide(ATO) there is dramatic change in patient response and survival. [3] ATRA works by terminal differentiation of promyelocytes into

myelocytes and ATO will destruct abnormal promyelocytes.PML/RARA translocation can be diagnosed by karyotyping, Fluorescence In Situ Hybridization (FISH) and real time PCR. [4] FISH is rapid diagnostic test can be done on interphase cells and can be reported within 24 hours which will help in timely treatment. Aim of present study was to estimate presence of PML/RARA translocation in patients diagnosed with APML using FISH.

MATERIALS AND METHODS

Ethical clearance was obtained from Institutional Ethical Committee. Informed consent was taken from patient or his /her relatives before test. Patient age group ranged 18-50 yrs (Both males &females). FISH was carried on 20 Bone marrow samples referred to Division of Human Genetics, Department of Anatomy, SJMC from March to August 2018 for a period of six months. Percentage of patients showing positive results was calculated.

Bone marrow samples were subjected to standard unstimulated lymphocyte culture. Incubation was done for overnight and 24 hours followed by harvesting and fixation on slide. This was followed by standard Protocol for FISH with 1 hour hybridisation of commercially available PML/RARα probe from cytocell aquarius and observed under fluorescent microscope. Analysis was done by automated karyotyping system. Around 200 cells were analysed .If fusion is present in more than 10% cells result was considered positive.

RESULTS

Results were described as positive or negative. Negative result is indicated by 2 red (Chromosome 15-PML gene), and 2 green signals(Chromosome 17-RARα gene) .Positive result is indicated by 1 red ,1 green and two fusions sowing translocation between PML/RARA gene..

Table 1: Prevalence of PML/RARA translocation in our patients

	Positive	Negative	Percentage
Sample size(n=20)	18	2	90%

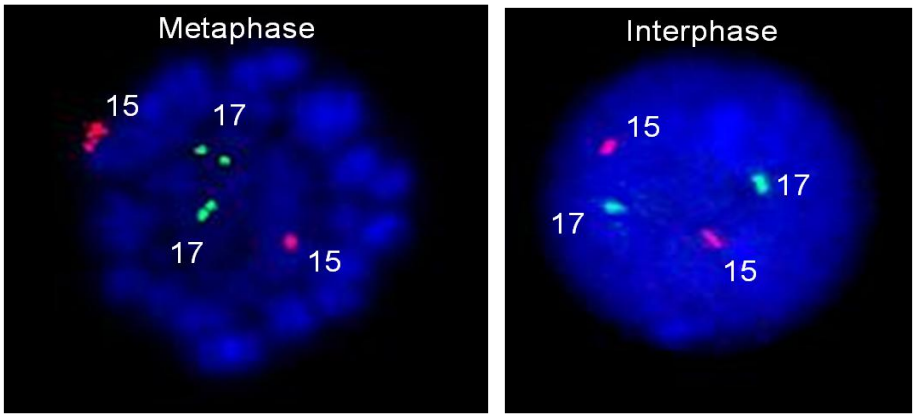

Fig 1:Metaphase and interphase showing normal pattern of PML and RARA gene

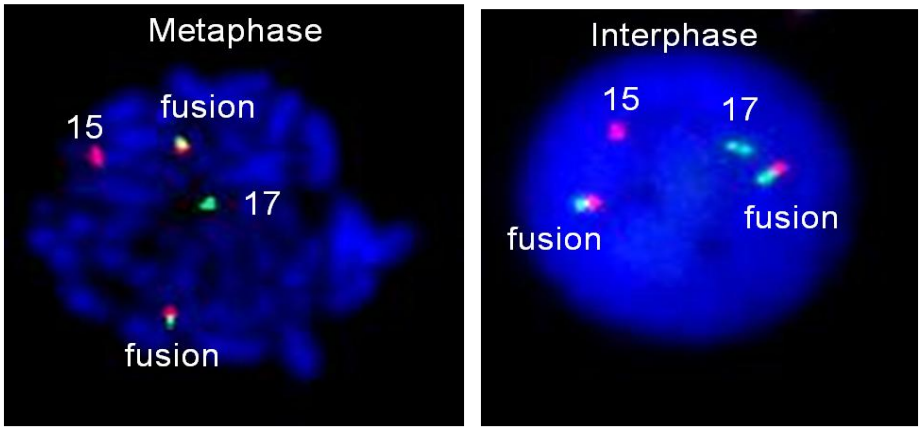

Fig 2: Metaphase and interphase showing PML and RARA gene fusion

DISCUSSION

Presence of PML/RARA Translocation is fundamental for treatment of patients diagnosed with APML. In present study, 90% of cases showed positive for PML/RARA fusion by FISH method. This showed that there is high detection rate with FISH. Positive results warrant immediate therapy with ATRA and ATO.

Negative results can be due to Submicroscopic insertion of RARα to PML or due to cryptic translocations involving t(5;17), t(11;17) involving genes NPM/ RARα(11, PLZF/RARα or NuMA/RARα. These cryptic translocations can be detected by RT-PCR. According to Julia Adams et al who did review of variant translocations, most patients having APL with variant translocations can also be successfully treated with ATRA and ATO; however, the prognosis may not be as favorable as in patients with PML/RARA fusion. Hence positive results with FISH shows good prognosis.

Chauffaille et al did comparison with cytogenetics, FISH and RT-PCR. When the three tests were compared at diagnosis, karyotyping presented the translocation in 80% of the tested samples while FISH and RT-PCR showed the PML/ RARA rearrangement in 100% of them. They concluded that FISH was technically more useful at diagnosis due to its rapid execution, sensitivity and accuracy. After treatment, RT-PCR is the method of choice. [5]

Iqbal S did FISH and RT PCR in 37 patients with APML. Out of FISH using the PML and RAR a probes was successful in 34/37 patients (92%). In 22/34 patients in whom a result was obtained, BM FISH confirmed the presence of the PML/RAR a fusion. [6] Pollampalli et al did FISH on 52 patients in which 48 patients showed positive results. They confirmed that FISH offers some unique advantages over RT-PCR, where a single probe covers multiple dispersed breakpoints in chromosomal regions and can detect additional aberrations. [4]

In an Indian study done by Sivakumar et al, 25 out of 23 patients showed positive results for PML/RARA fusion. FISH was useful in detecting PML-RAR alpha fusion in cytogenetically normal patients and those in when karyotyping was a failure and can be used in routine analysis for rapid confirmation of t(15;17) in patients with acute myeloid leukemia. [7] Nikolay D et al did FISH analysis of 172 patients diagnosed with APML out of which 169 showed positive results for PML/RARA fusion which had sensitivity upto 97%. [8] In all previous studies sensitivity of FISH in detecting translocation was above 80%.Hence detection of PML/RARA translocation by FISH forms basis for management of patients diagnosed with APML.

CONCLUSION

FISH for PML/RARα plays important role in diagnosis, treatment and prognosis in patients with APML. Negative results should be subjected to RT- PCR to rule out cryptic translocations.

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