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Clinical Significance of Novel, Rare and Recurrent Chromosomal Abnormalities in Acute Myeloid Leukemia Patients

P. Darshita¹, T. Pina¹, P. Dharmesh¹, V. Priya¹, P. Harsha², P.Prabhudas^{1*}

¹Cytogenetics Lab, The Gujarat Cancer and Research Institute, Asarwa, Ahmedabad-380016, India

²Professor of Medical Oncology, Head of Medical unit I, The Gujarat Cancer and Research Institute, Asarwa, Ahmedabad-380016, India

ABSTRACT

Cytogenetics as well as molecular profiling together provide framework for risk-stratification schemes in Acute Myeloid Leukemia (AML); however, the prognostic significance of many rare and novel cytogenetic abnormalities remains uncertain. Present study was carried out to explore the recurrent, rare and novel chromosomal abnormalities in AML patients. Total 430 denovo AML patients diagnosed and treated between 2017-2018 at The Gujarat Cancer & Research Institute were enrolled for the study. Bone marrow (BM) and peripheral blood lymphocytes (PBL) of all AML patients were collected for standard Conventional Cytogenetics and Fluorescence in situ hybridization (FISH) study. Short term culture and GTG banding were performed for Karyotyping. FISH was carried out using different FISH probes. Out of 430 AML patients, cytogenetic abnormalities were detected in 122 patients (28%) and 308 (72%) showed normal karyotype. Recurrent chromosomal translocations such as t(8;21)(q22;q22) (24%), t(15;17)(q22;q21) (43%), inv(16)(p13.1q22) (9%), and trisomy 8 (7%) were frequently observed. Rare chromosomal abnormalities such as del16(p10), inv(11)(q13) with t(15;17)(q22;q21), t(8;12;21)(q22;q24;q22), t(8;18;21)(q22;p11.2;q22) with loss of sex chromosome, t(3;15;17)(q26;q22;q21), t(2;14)(q22;q32) and t(3;6)(q26;p21) a novel translocation in AML-M2 subtype were observed. The combined analysis of classical cytogenetics and FISH allow better risk stratification for AML patients. Moreover, identifying recurrent, rare, and novel cytogenetic abnormalities lead us to a better understanding of the biological and clinical significance of these chromosome abnormalities and increase the utility of cytogenetics in the treatment decision.

KEYWORDS: Acute Myeloid Leukemia, Conventional Cytogenetics, Fluorescence in Situ Hybridization, Chromosomal abnormality, Leukemogenesis

***Corresponding author**

Dr. Prabhudas S. Patel

Professor & Head Cancer Biology Department, The Gujarat Cancer & Research Institute, Asarwa, Ahmedabad-380016, Gujarat, India.

E. Mail: prabhudas.patel@gcriindia.org, prabhudas_p@hotmail.com

Tel: +91 79 22688375. Fax: +91 79 22685490

INTRODUCTION

Acute myeloid leukemia (AML) is a group of hematological disorders characterized using a spectrum of clinical, morphological, immunophenotypic, cytogenetics and molecular evaluation would be more appropriate.¹ Several clinical and hematopathological parameters along with genetic characterization predict the outcome, among which cytogenetic analysis is the most important factor in classification and prognostification.²

In AML, various recurrent chromosomal aberrations have been recognized and several of which, such as translocation of (8;21)(t(8;21)(q22;q22), t(15;17)(q22;q11-12), inversion of chromosome 16 (inv(16)(p13q22)) are specific for distinct subgroups and predict a relatively favorable outcome. In contrast, in patients lacking these favorable changes, the presence of complex karyotype, monosomy of chromosome 5 (-5), deletion of long arm of chromosome 5 (del 5q), monosomy of chromosome 7 (-7) and abnormalities of 3q are defined group with relatively poor prognosis. Moreover, the remaining group of patients including those with 11q23 abnormalities, trisomy 8 (+8), +21, +22, deletion of long arm of chromosome 7 (del 7q) or other miscellaneous structural or numerical chromosomal abnormalities are not encompassed by the favorable or adverse risk groups and were found to have an intermediate prognosis.³ However, AML with normal karyotype is usually grouped in the “intermediate risk” category. The treatment outcome of AML with normal karyotype is extremely heterogeneous. Recently there has been studies done to identify molecular targets to risk stratify these patients with normal karyotype.^{4,5}

Further, recurrent aberrations and the resulting gene rearrangements are used to help define distinct disease entities within AML as defined in the new World Health Organization classification of hematological malignancies in addition to morphologic, immunophenotypic, and clinical features.² Besides establishing the type of AML, specific cytogenetic abnormalities have also diagnostic, prognostic and therapeutic importance.⁶ Therefore, identifying newer chromosomal abnormalities are crucial as they may contribute to diagnosis, prognosis and increase the utility of cytogenetics in the development of targeted therapeutic drugs.^{7,8} In the present study, we aimed at identification of different categories of cytogenetic abnormalities and their clinical relevance.

MATERIALS AND METHODS

Total 430 de novo AML patients diagnosed and treated between 2017 and 2018 at The Gujarat Cancer & Research Institute (GCRI) with age range from 2-73 years were enrolled for the study. The diagnosis and classification of acute leukemia was established according to FAB subtypes.

Bone marrow (BM) aspirate and peripheral blood samples were collected for standard Conventional Cytogenetics and FISH techniques. This study was approved by the Institutional Scientific Review Board and Ethics Committee. Prior general consent obtained from patients.

Conventional cytogenetic study

Conventional cytogenetic analysis was performed using unstimulated culture of BM or PBLC according to standard techniques.⁹ The cells were cultured and incubated at 37°C in the presence of colcemid (0.1mg/ml). The cultures were exposed to hypotonic solution (0.075 mol/L KCL) and fixed with methanol: acetic acid (3:1). The slides were prepared by air dry method. G banding was carried out using Trypsin and Giemsa stain and 20 metaphases were karyotyped and described according to the International System for Human Cytogenetic Nomenclature (ISCN) 2016.¹⁰

Fluorescence in Situ Hybridization (FISH) assay

FISH on interphase and metaphase nuclei was carried out using commercially available probes from Vysis (Abbott Molecular, Inc.). FISH assays were performed using Locus Specific Identifier probe (LSI), different Whole Chromosome Paint probes (WCP), and Centromere Enumeration Probes (CEP) according to the standard protocol of the manufacturer (Abbott/Vysis Inc., USA). The signals were captured with BX-61 Olympus fluorescence microscope (Olympus, Japan) equipped with CCD camera.

Treatment regimen

Induction therapy with Cytarabine (Ara-C) and an anthracycline remains a standard of care in AML. The standard combination is the 7 + 3 protocol, with a 7 days continuous infusion of Cytarabine at the dosage of 100 mg/m²/day on days 1–7 and Daunorubicin at 20 mg/m²/day on days 1–3. Induction was considered successful if CR was achieved (CR; BM blasts <5%). Further post remission therapy was given in the form of 3–4 cycles of HiDAC (High-dose Ara-C) followed by allogeneic hematopoietic stem cell transplantation. In AML M3 FAB subtype, induction was given with Daunorubicin for 3 days along with All-Trans Retinoic Acid (ATRA)/ Arsenic Trioxide. For consolidation, two blocks each of 21 days including Daunorubicin for 3 days with ATRA for two weeks or with Arsenic Trioxide, followed

by maintenance with 6-Mercaptopurine (6MP) continuous daily, Methotrexate (MTX) weekly along with ATRA for 15 days every 3 months or with Arsenic Trioxide. Oral chemotherapy included drugs 6-Thioguanine (6TG), 6MP and Hydroxyurea.

RESULTS

The study comprised of 430 AML patients. There were 243 (57%) men and 187 (43%) women with age range of 2-73 years. Out of 430 patients, chromosomal abnormalities were observed in 122 (28%) patients and 308 (72%) showed normal karyotype. Most of the patients were categorized as AML undifferentiated subgroup (which showing >20% blasts and lack specific markers) followed by AML-M2 subtype. Clinical characteristics of all patients are summarized in Table 1.

Table 1. Characteristics of total 430 AML Patients and frequency according to FAB classification

Characteristics	N (%)
Total patients	430 (100)
Gender	
Men	243 (57)
Women	187 (43)
Age (Median: 36 years)	
<36 years	206 (48)
≥36 years	224 (52)
Chromosomal Abnormalities	122 (28)
Normal Karyotype	308 (72)
FAB Subtype	
AML-M1	56 (13)
AML-M2	80 (19)
AML-M3	73 (17)
AML-M4	63 (14)
AML-M5	51 (12)
AML- Undifferentiated	107 (25)
Hematological characteristics	Median value
Hemoglobin Level (Hb) (gm/dl)	7.4
White Blood Cell (WBC) Count X 10 ³ /cmm	16.25
Platelet Count X10 ³ /cmm	31.5
Blast Count (%)	66

Recurrent, Rare, and Novel chromosomal abnormalities in AML

The present study documented several recurrent (n=115), rare (n=6) and novel (n=1) chromosomal translocations along with clinical details using the global cytogenetic databases of cancer chromosomes [<http://cgap.nci.nih.gov/Chromosomes/Mitel Search>].¹¹

Till date, there are total 70,469 cases & 32,551 gene fusions because of chromosomal abnormalities reported in Mitelman database [Updated on July 15, 2020]. As per the norms of Atlas of Genetics and Cytogenetics in Oncology and Hematology [<http://atlasgeneticsoncology.org>]¹², for a case that is reported for the first time (not reported in literature) is considered as a novel case, if a case is reported less than 20 times, it is considered a rare translocation (rare case) and if a case is reported more than 20 times it is considered as a recurrent abnormality. In the current study, we reported recurrent, rare and as well as novel chromosomal findings that are listed in the Table 2.

Table 2. AML cases with Recurrent, Rare and Novel chromosomal translocations

Recurrent	t(8;21)(q22;q22); t(15;17)(q22;q21); inv(16)(p13.1q22); 11q23 rearrangement; Trisomy 8; Trisomy 21
Rare	t(8;12;21)(q22;q24;q22); -Y,t(8;18;21)(q22;p11.2;q22); t(2;14)(q22;q32); inv(11)(q13),(15;17)(q22;q21); t(3;15;17)(q26;q22;q21); del16(p10)
Novel	t(3;6)(q26;p21)

Recurrent structural chromosomal abnormalities:

1. t(8;21)(q22;q22); *RUNX1-RUNX1T1*

The t(8;21)(q22;q22) was observed in 29 patients (24%), predominantly in young individuals with a median age of 37 years as compared to pediatric patients (Figure 1 A & B). In cases with t(8;21) the median values of Hemoglobin (Hb), White Blood Cell (WBC), platelets and blast cells were 7.4 gm/dl, 13000/cmm, 20000/cmm and 55% respectively. The standard combination of 7+3 induction followed by consolidation therapy was given for the patients with t(8;21)(q22;q22). In addition to t(8;21), loss of X or Y chromosome were observed in 10 patients and one case showed presence of trisomy 4 (Table 3) (Figure 1 C, D & E). Hematological response was seen in most of the cases with t(8;21) that were treated with induction and consolidation therapy, however patient with t(8;21) with trisomy 4 was treated with MTX and 6MP did not show hematological response and expired within 6 month of diagnosis. The Complete Blood Count (CBC) report for this case showed 7.2 gm/dl of Hb, 11700/cmm of WBC count, 9000/cmm of platelets and presence of 28% blasts.

Table 3. No. of patients with t(8;21)(q22;q22)

Abnormality	No of patients (%)
Total patients with t(8;21)(q22;q22)	29 (24)
Sole t(8;21)(q22;q22)	18 (15)
t(8;21) with additional chromosome abnormalities	11 (09)

2. t(15;17)(q22;q12); PML-RARA

In present study, t(15;17)(q22;q12) was seen in 53 patients (43%) (Table 4). Translocation t(15;17)(q22;q21) as a sole abnormality was observed in 50 patients out of the total 53 cases. (Figure 1 F & G). Median values for patients with t(15;17) were Hb 80.05 gm/dl, WBC count 28450/cmm, platelet 17000/cmm and 72% of blast cells. Two cases showed +8 as an additional chromosomal abnormality who had lower haemoglobin levels and high WBC counts. Among these two patients one patient treated with Arsenic Trioxide 10 mg along with 7+3 protocol (ATRA + Daunorubicin) with 6MP 50 mg and MTX 2.5 mg for 9 month showed hematological response whereas other patient treated with 7+3 protocol was lost to follow-up (LFU). However, one patient with + 21 as an additional chromosomal abnormality treated with 7+3 protocol did not show hematological response and expired during 1st cycle of treatment.

Table 4. No. of patients with t(15;17)(q22;q12)

Abnormality	No of patients (%)
Total patients with t(15;17)(q22;q12)	53 (43)
Sole t(15;17)(q22;q12)	50 (41)
t(15;17) with additional chromosome abnormalities	03 (02)

3. Inv(16)(p13;q22); CFBF-MYH11

Inv(16)(p13;q22) was found in 11 patients (9%) of AML cases with the AML- M4 subtype. The median values of hematological reports were Hb 7.4, WBC 45500/cmm, platelet 24000/cmm and 62% blast count of inv(16) patients. Out of 11 patients, sole inv(16)(p13;q22) was observed in 10 patients (Table 5) (Figure 1 H & I) and additional changes with inv(16)(p13;q22) was found in 1 patient (Table 5) (Figure 1 J).

Out of 11 cases, inversion 16 was identified karyotypically (Figure 1 H) and by FISH in 3 cases. Remaining 8 patients showed presence of inversion 16 by the application of FISH using LSI CFBF dual color break apart rearrangement probe. One yellow signal was present on normal chromosome 16, one

green on q arm of derivative of chromosome 16 (der(16)) and one orange signal was observed on p arm of the same der(16) indicating inv(16) (Figure 5 B).

Table 5. No. of patients with Inv(16)(p13;q22)

Abnormality	No of patients (%)
Total patients with inv(16)(p13;q22)	11 (9)
Sole inv(16)(p13;q22)	10 (8)
Inv(16) with additional abnormalities: trisomy 22 with inv (16)	01 (01)

Out of 10 patients 3 were lost to follow-up (LFU) and 7 responded to treatment (Cytarabine 100mg/m²/day followed by high dose of Cytarabine (1gm) whereas patient with inv(16) with the trisomy 22 had the treatment with cytarabine 100mg/m²/day followed by high dose of Cytarabine (1gm) and Filgrastim 300MCF-FIL for 3 months and showed hematological response.

4. 11q23 rearrangements; MLL (KMT2A-MLL Mixed Lineage Leukemia,11q23 locus)

In the 11q23 rearrangements, del 11(q23) (n=4) were detected in different subgroups of AML. In patients with deletion 11q23, FISH results showed one Green and one Yellow signal pattern using *MLL* probe (Figure 2 D) and deletion in long arm of chromosome 11 by the conventional cytogenetics (Figure 2 C). Out of 4 patients with del 11(q23), 2 patients expired during the treatment course (7+3 protocol and TAB 6 MP (50 mg) and remaining 2 patients were LFU.

5. t(5;11)(q33;p15) ;11p15.4 rearrangement, NUP98

The translocation of chromosome 5 and 11 (t(5;11)(q33;p15)) was observed in 1 patient with Hb 8 gm/dl, WBC count 298300/cmm, platelet 60000/cmm and 70% blast count. Conventional cytogenetic results showed 46,XY,t(5;11) and results were confirmed using WCP probe 5 and 11. (Figure 2 A & B). Patient with t(5;11) with AML-M2 expired within 15 days of diagnosis before starting treatment.

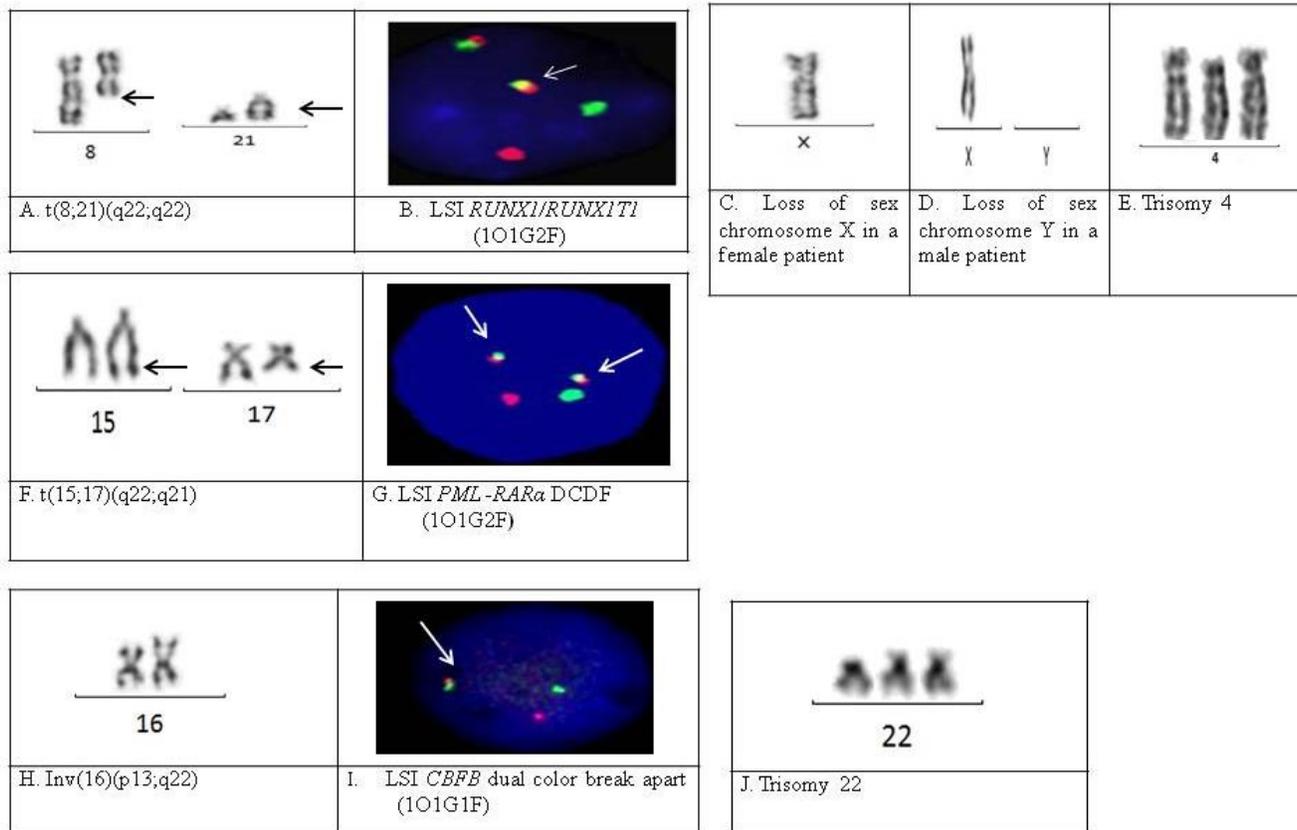


Figure 1: (A & B) Representative G banded partial karyotype of t(8;21) and FISH signal pattern using LSI *RUNX1/RUNX1T1* probe (C, D & E) Representative G banded partial karyotype of most common additional chromosomal changes with t(8;21) (F & G) Representative G-banded partial karyotype of t(15;17) and FISH signal pattern using LSI *PML-RARα DCDF* (H & I) Representative G banded Partial karyotype showing inv(16)(p13;q22) and FISH image using LSI *CBFB* dual color break apart probe (J) Representative G-banded Partial karyotype showing additional chromosomal abnormality with inv(16)

6. Deletion 5q and 7q

Del 5q (n=1) and del 7q (n=3) were detected in different subgroups of AML (Figure 2 E & F). Patient with del 5q with AML-M5 expired within 15 days of diagnosis. Out of 3 patients with del 7q, 2 patients expired within 1 month of diagnosis from that 1 patient had taken 2 doses of Hydroxyurea 500 mg and other had 2 cycles of Cytarabine and remaining 1 patient was lost to follow-up (Figure 2 G & H).

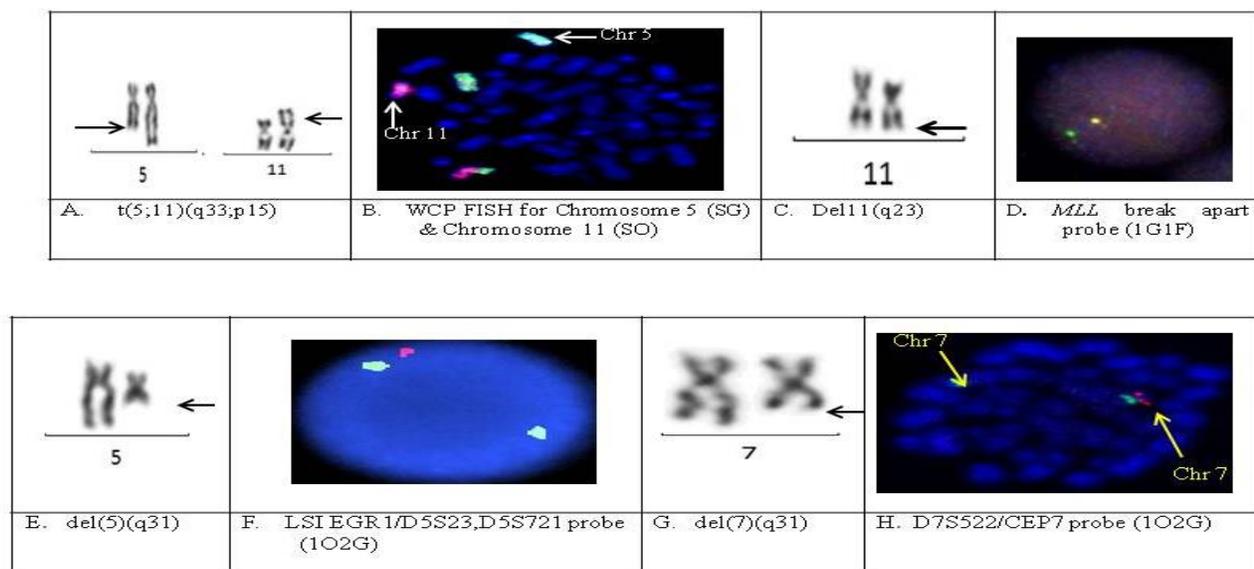


Figure 2: (A, B, C & D) Representative G banded Partial karyotype showing $t(5;11)(q33;p15)$ and $del 11(q23)$ and FISH image using WCP FISH probe and LSI *MLL* break apart probe (E, F, G & H) Representative G banded Partial karyotype showing $del 5q$ and $del 7q$ and FISH signal pattern using LSI EGR1/D5S23, D5S721 and LSI D7S522/CEP7 probe

Recurrent Numerical Chromosomal abnormalities:

Total 13 patients showed numerical chromosomal abnormalities (Figure 3 A). The most frequent numerical chromosomal abnormality observed was trisomy 8 (n=8) followed by trisomy 21 (n=2), monosomy 7 (n=2) and hyperdiploidy (n=1) (Figure 3 B, C & D). The patients with trisomy 8 and monosomy 7 showed poor prognosis. Patients with Trisomy 8, median of CBC reports were Hb 8 gm/dl, WBC 21250/cmm, platelet count 35500/cmm and 85% blast cells among which 1 patient had 7+3 (expired during treatment) and 5 patients were expired before starting of the treatment whereas 2 patients were LFU. In case of monosomy 7, patients with lower Hb and higher WBC count expired during the induction phase of treatment. Moreover, patients with trisomy 21 showed intermediate prognosis as out of 2 patients with trisomy 21, 1 patient with Hb 7.8 gm/dl, WBC 15000/cmm and 77% blast cells treated with 7+3 protocol and but did not respond to treatment and expired within 1 month and other patient with Hb 12.9 gm/dl, WBC 56900/cmm and 21% blast cells treated with Cytarabine 100 mg followed by filgrastim 300MCG-FIL and Etoposide +6MP for 9 months and expired during the treatment.

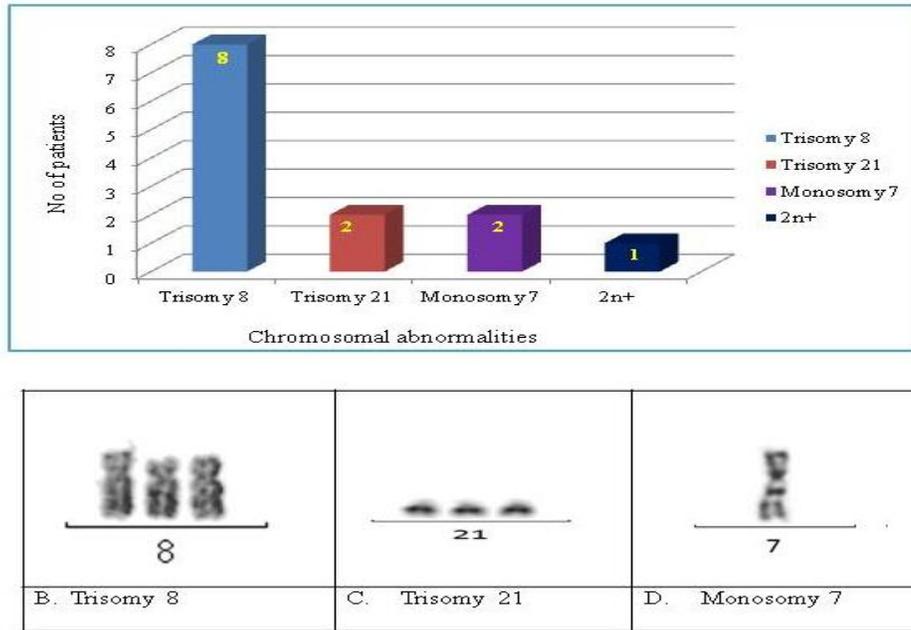


Figure 3: (A) Frequency of numerical chromosomal abnormalities (B, C & D) Representative G banded Partial karyotype of trisomy 8, trisomy 21 and monosomy 7

Noteworthy cases (Novel and Rare) observed in the present study:

Case reports of the patients with rare translocation

Table 6. Karyotype results of patients with rare translocations

Case No.	Age (year)/ Gender	Chromosomal abnormalities	AML subtype	Event
1	16/Male	46,XY,t(8;12;21)(q22;q24.2;q22) [7]	AML-M2	Expired within 2 months of diagnosis
2	6/Male	45,X,-Y,t(8;18;21)(q22;p11.2;q22) [11]	AML-M2	LFU
3	65/Male	46,XY,t(2;14)(q22;q32) [10]	AML-M2	HR
4	34/Female	46,XX,del(16)(p10) [15]	AML-M1	Expired within a 11 months of diagnosis
5	50/Female	46,XX,inv(11)(p15q13),t(15;17)(q22;q21) [8]	AML-M3	Expired within a week of diagnosis
6	31/Male	46,XY,t(3;15;17)(q26;q22;q21) [12]	AML	Expired within 4 months of diagnosis
HR: Hematological Response LFU: Lost to Follow-Up				

Case no 1: A 16 years/male patient presented with hematological reports; Hb 7.7 gm/dl, WBC 6300/cmm, blast cells 50%, and platelet count 16000/cmm. The BM report was AML-M2. Karyotype study revealed 46, XY,t(8;12)(q22;q24.2) by G- banding (Figure 4A). In order to rule out a variant t(8;21) translocation FISH study was carried out using *RUNX1/RUNX1T1* FISH probe. FISH analysis disclosed a 3-way translocation involving chromosomes 8, 12, and 21 and showed presence of a masked variant signal pattern (two orange, two green and one yellow signals) for t(8;21)(q22;q22) (Figure 4 B). Hence the final karyotype result based on FISH findings was revised as t(8;12;21)(q22;q24.2,q22). The patient expired after 2 months of diagnosis.

Case no 2: A 6 years/male patient presented with hematological reports; Hb 8.5 gm/dl, WBC 5200/cmm, blast cells 75%, and platelet count 22000/cmm. The BM report revealed AML-M2. The karyotype study revealed 45,X,-Y,t(8;18;21)(q22;p11.2;q22) by G- banding (Figure 4 C) and FISH analysis confirmed variant signal pattern two orange, two green and one yellow signals using *RUNX1/RUNX1T1* probe (Figure 4 D). The patient is LFU at present.

Case no 3: At our institute, a 65 years/male patient presented with fever, cough, and bleeding. His BM and Immunophenotyping (IPT) reports revealed AML-M2 subtype. The CBC report of patient was Hb 11.6 gm/dl, WBC 20100/cmm, blast cells 50% and platelet 283000/cmm. FISH studies for *RUNX1/RUNX1T1* (Figure 4 F) and inversion 16 (*CBFBeta* gene) (Figure 4 G) were negative. Karyotype analysis revealed a rare translocation t(2;14)(q22;q32) (Figure 4 E). Patient had taken 2 doses of Hydroxyurea and showed hematological response.

Case no 4: A 34 years/female patient with complain of fever visited at our institute. BM and IPT reports showed AML-M1 FAB subtype, Hb with 9.8 gm/dl, WBC count 40500/cmm, platelet count 21000/cmm and 90% blast cells. Conventional cytogenetic study revealed deletion in chromosome 16 in p arm at break point; del(16)(p10) (Figure 5 A). The patient was on 7+3 and Filgrastim 300MCG-FIL treatment; however, he did not show the hematological response and expired after 11 months of diagnosis.

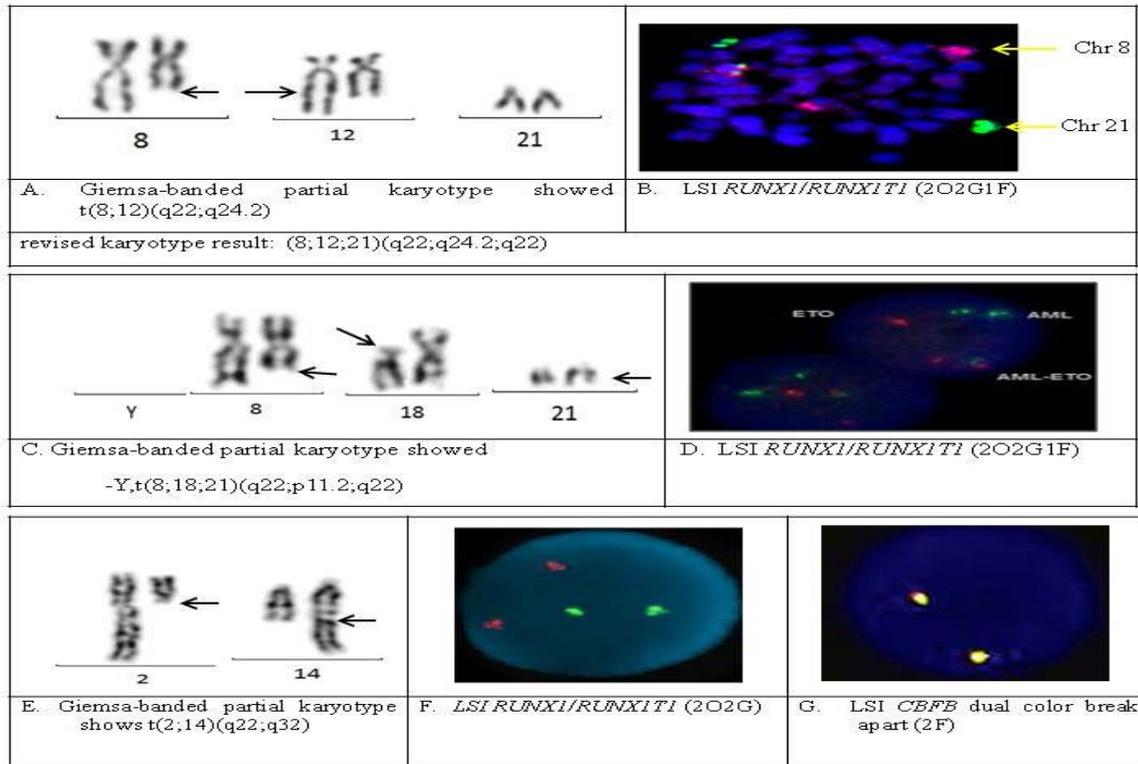


Figure 4: (A & B) Partial G-banded karyotype of t(8;12)(q22;q24.2) and FISH signal pattern using LSI *RUNXI/RUNX1T1* probe (C & D) Partial G-banded Karyotype of -Y,t(8;18;21)(q22;p11.2;q22) and FISH signal pattern using LSI *RUNXI/RUNX1T1* probe (E, F & G) Partial G-banded karyotype of t(2;14)(q22;q32) and FISH signal pattern using LSI *RUNXI/RUNX1T1* probe and LSI *CBFB* dual color break apart probe

Case no 5: A 50 years/female with APML (Acute Promyelocytic Leukemia) who was registered at our institute due to high-grade fever. Peripheral blood examination showed Hb levels of 6.7 gm/dl, platelet count was 7000/cmm, and a WBC count of 39,800/cmm with 91% blasts.

The karyotype study revealed 46,XX,der(11),t(15;17)(q22;q21)[20] (Figure 5 B). FISH results using LSI *PMLRARA* probe showed one orange, one green, and two fusion signals confirmed translocation between chromosome 15 and 17. The metaphase FISH results WCP 11 with spectrum orange (SO) and Centromere Enumeration Probe for chromosome 11 (CEP 11) with spectrum green (SG) revealed inversion of chromosome 11 and confirmed the diagnosis (Figure 5 C & D). Based on the FISH studies the revised karyotype result was 46, XY,inv(11)(p15q13),t(15;17)(q22;q21)[20] (Figure 5). The patient received standard chemotherapy of Daunorubicin combined with ATRA. The patient expired after 7 days of diagnosis during the induction period.

Case no 6: A 31 years/male patient presented with hematological reports; Hb 9.1 gm/dl, WBC 10100/cmm, blast cells 71%, and platelet count 20000/cmm. The BM report revealed AML undifferentiated type. The karyotype study revealed $t(3;15;17)(q26;q22;q21)$ by G-banding (Figure 5 E) and FISH analysis showed variant $t(15;17)(q22;q21)$ with two orange, two green and one yellow signal pattern using *PML-RARA α* probe (Figure 5 F). However, the patient was subjected to 7+3 protocol and he did not respond to treatment and expired within 4 months.

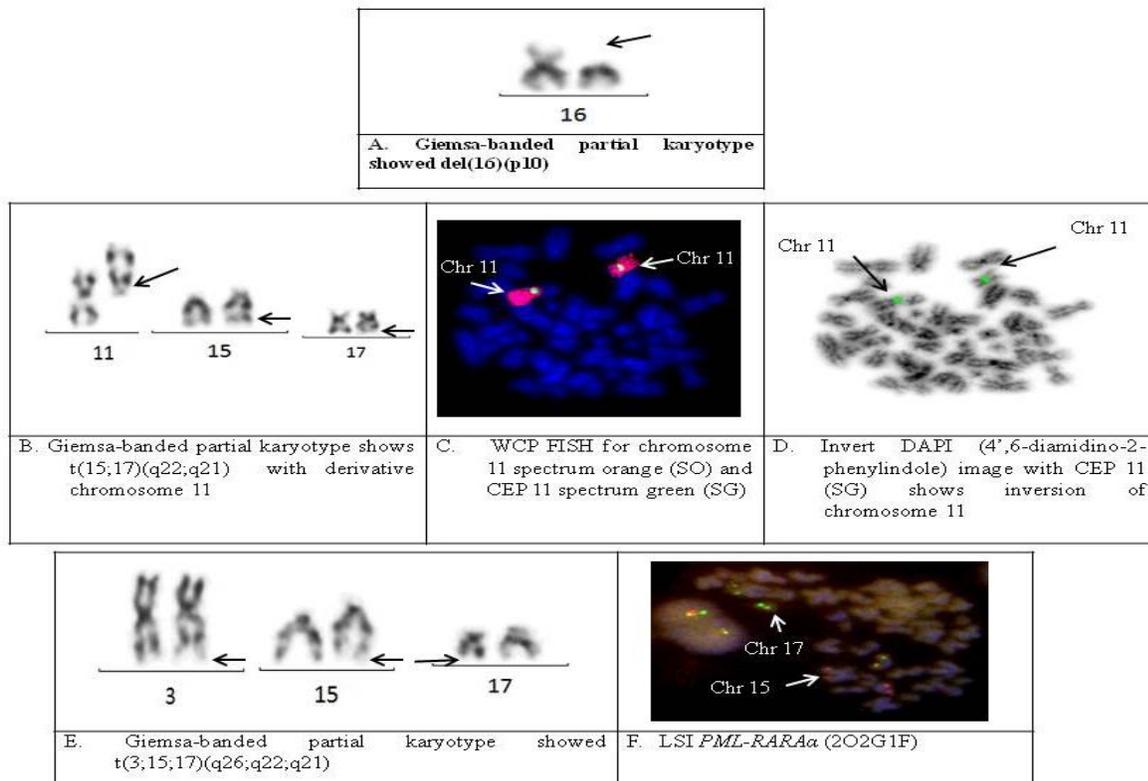


Figure 5: (A) Partial G-banded karyotype of $del(16)(p10)$ (B, C & D) Partial G-banded karyotype of $der(11),t(15;17)(q22;q21)$ and FISH signal pattern using WCP FISH probes (E & F) Partial G-banded karyotype of $t(3;15;17)(q26;q22;q21)$ and FISH signal pattern using LSI *PML-RARA α* FISH probes

Case Report of patient with novel translocation

In our study we reported one novel case with $der(6)t(3;6)(q26;p21)$ of AML-M2 subtype. A 3-years/male child suffering from low grade fever, weakness and blood loss was referred to our institute. The laboratory investigations revealed Hb 8.4 gm/dl, WBC 9000/cmm and platelets count 8000/cmm.

Conventional cytogenetic was carried out from blood and BM samples and showed 46,XY,der(6)t(3;6)(q26;p21) [20] (Figure 6 A). FISH result using *RUNX1/RUNX1T1* probe revealed 2O2G signals which indicated absence of translocation between chromosome 8 and 21 (Figure 6 B). For confirmation of t(3;6)(q26;p21), WCP FISH probes WCP 3 (SG) and WCP 6 (SO) were applied. Results showed that the q arm of chromosome 3 (SG) had translocated to p arm of chromosome 6 (SO) and one orange color chromosome showed normal chromosome 6 and one green color chromosome showed normal chromosome 3 (Figure 6 C).

The patient was treated with 6-thioguanine-40mg-Thio at 19 days of interval (2 cycles) and expired within one month of diagnosis. Our report of translocation t(3;6)(q26;p21) in AML- M2 subtype is a novel findings in literature study and can be represented as a first report of the Mitelman Cancer chromosomal abnormality database.

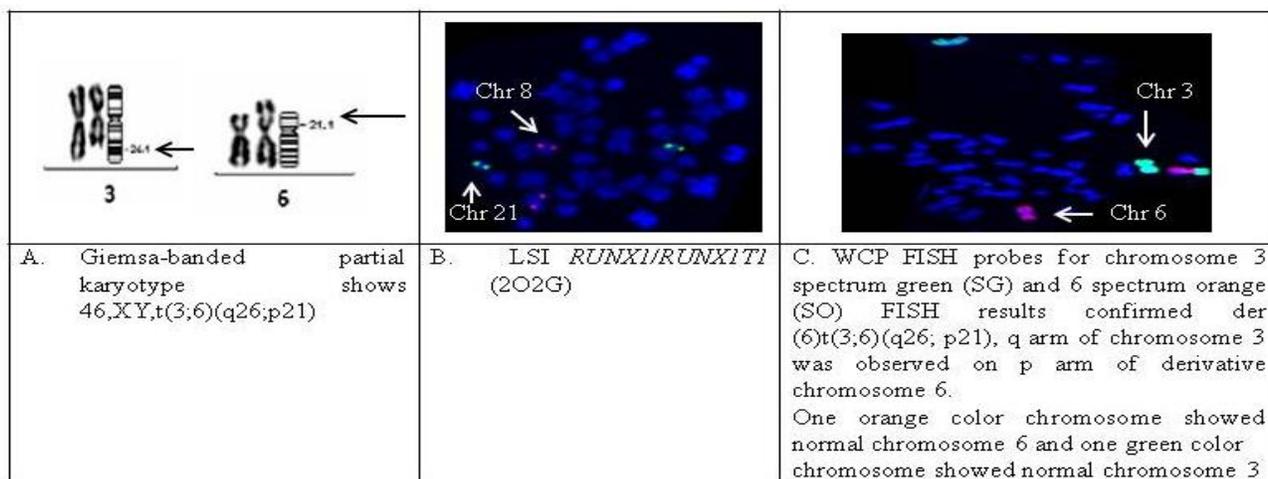


Figure 6: (A, B & C) Partial G-banded karyotype of t(3;6)(q26;p21) and FISH signal pattern using LSI *RUNX1/RUNX1T1* and WCP FISH probes

DISCUSSION

AML is a heterogeneous disease regarding its biology and its clinical course. In our study chromosome abnormalities were detected in 28% of AML patients which is similar to the Meng et al, 2013 study (30%) whereas Martens et al, 2010 and Klaus et al, 2004^{13,14} have reported higher chromosome abnormalities in AML (more than 50%) and this can be attributed to the difference in geographical regions.^{3,13-14}

Yang et al, 2017 found that t(8;21)(q22; q22) is a common recurrent chromosomal translocation seen in nearly 10-15% of AML, while in our study it was observed in 24% of cases.¹⁵ The t(8;21)(q22;q22) involves the *RUNX1* gene located on 21q22 and the *RUNX1T1* gene on 8q22, generating the *RUNX1- RUNX1T1* fusion transcript.¹⁶ It acts through inhibiting apoptosis by up-regulating the expression of anti-apoptotic *BCL2*.^{17,18} Trisomy 4 is a rare numerical abnormality in AML patients with t(8;21), which is associated with *c-KIT* gene involvement. In adults, *c-KIT* mutation carries an unfavorable clinical outcome.¹⁹ Similarly in the current study 63 years old female patient with trisomy 4 and t(8;21) had poor clinical outcome.

In present study the sole t(15;17)(q22;q21) was observed in 41 % patients, while t(15;17)(q22;q21) along with +8 and +21 were detected in 1% each. Contradictory to our results, the most common secondary change is +8 which is seen in 36% according to Cerevra et al, 2010.²⁰ Our study highlights the poor prognosis of additional chromosomal abnormalities in t(15;17) positive case. Consistent with the results from previous reports (ranging from 5% to 13%), inv (16) was found in 9% of patients in the present study.^{15, 21-22} The German-Austrian AML study Group (AMLSSG) have also stated that trisomy 22 was associated with more favorable outcome.²³ Our study also reported trisomy 22 as a favorable marker when associated with inv (16). Patients harboring an inv(16)/t(16;16) have a favorable prognosis, showing long periods of complete remission and high overall survival rates up to 70%.²⁴

Winters et al, 2017 reported that ~10% of all leukemias harbor *MLL1* translocations. Of these, the majority of patients involved are younger than 1 year of age at diagnosis (primarily ALLs) and young-to-middle-aged adults (primarily AMLs).²⁵ Similarly 3% of the patients in the present study showed *MLL* rearrangements with the age range 15-50 years. *MLL* fuses with different partner genes and the identification of fusion partner genes influences the prognosis of AL.²⁶ Further, there could be a possibility of presence of tumor suppressor gene on the long arm of chromosome 5 (*CTNNA1* and *HSPA9*) that are associated with MDS/AML leading to disease progression and poor outcome.²⁷ Additionally, in one of our previous studies reported by Trivedi et al, 2016, none of the patient with del 5q achieved remission and expired within one month supporting our findings.²⁸ Moreover, the presence of a putative myeloid leukemia suppressor gene in the commonly deleted genomic segment 7q22 and even multiple genes in 7q22 -31 have a role in leukemogenesis.

In the present study, patients with trisomy 8 showed poor prognosis. Genes with possible significance in leukemogenesis located on chromosome 8 include *C-MYC* on 8q24, *C-MOS* on 8q22,

MOZ on 8p11, and *ETO* on 8q22. Trisomy 8 could represent an alternative mechanism for increasing *C-MYC* gene dosage to achieve amplification of *C-MYC* oncogene leading to poor prognosis.²⁹ Trisomy 21 represent intermediate prognosis in the current study. The candidate genes located on chromosome 21 having a role in leukemogenesis are *RUNX1* gene present on 21q22 and two members of the *ETS* transcription factor family, *ETS2* and *ERG*, located at 21q22.³⁰

In literature studies AML variants of translocation t(8;21) are observed in 3-4% of cases involving chromosome 1,2,4,5,6,7,8,10,12,13,15,17,18,19 or 20. But the prognosis of variants of t(8;21) is controversial.³¹ The determination of the significance of variants is important because it helps to assess the prognosis in terms of therapy outcome. Abreu et al, 2017³² stated that the different clinical outcomes observed between variants t(8;21) are directly linked to the involvement of genes located on the third and/or fourth chromosome. In our study, both the patients showed varied prognosis, as in Case 1 patient expired within 2 months of diagnosis whereas other patient showed hematological response during the treatment (Case 2). Torkildsen et al, 2015 reported abnormal expression of *BCL11B* coding regions subjected to control by the *ZEB2* promoter seems to be the leukemogenic mechanism behind the translocation t(2;14)(q22;q32) in AML (Case 3). The *ZEB2* gene (2q22 region) codes for a protein which is a member of the *Zfh1* family of 2-handed zinc finger/homeodomain proteins and *BCL11B* gene and its paralogue *BCL11A* code for krueppel-like C2H2-type zinc finger proteins of the *BCL11* family of transcription factors.³³

Furthermore, we observed APLM patient with co-occurrence of inv(11)(p15q13) and t(15;17). The gene present on chromosome band 11q13 is *PRAD1* and encodes cyclin D1. Cyclin D1 plays an important role in control of the cell cycle and overexpression of cyclin D1 may be involved in disease progression in this case.³⁴ In the present case, patient expired within 7 days of diagnosis, so it is assumed that APLM with inv(11)(p15q13) might have poor prognosis (case 5).³⁵ Also, patient with novel translocation der(6)t(3;6)(q26;p21) showed poor prognosis.³⁶ In myeloid malignancies involvement of 3q26 in balanced rearrangements is highly suggestive of *EVII* and/ or *MDS1/EVII* rearrangements that are associated with poor prognosis.^{37,38}

CONCLUSION

In the present study, some recurrent cytogenetic abnormalities showed the favourable prognosis that responded well to the treatment such as t(8;21), t(15;17) and inv(16) whereas the treatment outcome for trisomy 8, *MLL* rearrangements and del7q/del5q were poor. Moreover, in cases showing presence of

rare and novel cytogenetic abnormalities, chromosome and the gene involved on the chromosome will enable determination of prognostic significance and further help to provide insights into the mechanisms of disease pathogenesis in AML. So, the evaluation of AML patients with combination of classical cytogenetics and FISH will allow better risk stratification by identifying recurrent, rare, and novel chromosomal abnormalities.

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