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CASE REPORT

A new complex translocation (8;22;21)(q22;q12;q22) in *RUNX1/RUNX1T1* acute myeloid leukaemia

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Abstract

Introduction: Acute myeloid leukaemia (AML) with t(8;21)(q22;q22) producing *RUNX1-RUNX1T1* rearrangement is a distinct sub-type which is usually associated with a favourable clinical outcome. Variant forms of t(8;21) are rare. Herein we describe a novel variant of t(8;21) AML in a 25-year-old pregnant woman who presented with intermittent fever. *Case Report:* Her peripheral smear and bone marrow aspirate showed many myeloblasts. Chromosomal study revealed t(8;22;21)(q22;q12;q22) and loss of X chromosome. Fluorescence in situ hybridization (FISH) using whole chromosome painting probes confirmed the three-way translocation involving chromosomes 8, 21 and 22. *RUNX1-RUNX1T1* rearrangement was identified in FISH and reverse transcriptase polymerase chain reaction confirming the diagnosis of AML with variant t(8;21). The patient was treated with standard chemotherapy. She achieved morphological remission one month after induction chemotherapy. *Discussion:* Although the clinical significance of variant t(8;21) is not well delineated, the evaluation of 31 such cases suggests patients with variant t(8;21) have similar prognosis to those with classical t(8;21).

Keywords: RUNX1/RUNX1T, variant t(8;21), AML M2, three-way translocation

INTRODUCTION

Acute myeloid leukaemia (AML) is a clonal disorder characterised by the proliferation of immature myeloid cells in bone marrow and peripheral blood and arises from a series of recurrent haematopoietic stem cell genetic alterations accumulated with age.[1] Multiple recurrent chromosomal abnormalities and genetic mutations, identified in AML, have been used as important diagnostic and prognostic tools.[1] t(8;21)(q22;q22) is one of the most frequent chromosomal abnormalities associated with AML and is found in approximately 5-10% of all AML cases.[2,3] This translocation is closely related to AML with maturation corresponding to the M2-FAB subtype.[2] In t(8;21)(q22;q22), there is a fusion of the *RUNX1* gene on chromosome 21q22 with *RUNX1T1* on chromosome 8q22, resulting in *RUNX1-RUNX1T1* fusion gene on derivative chromosome 8. This fusion gene contributes to leukaemogenesis by transcriptional repression of normal *RUNX1* target genes.[4] Variant forms of t(8;21) are uncommon and occur in about 3% to 4% of cases of AML with t(8;21).[5] Although t(8;21) is associated with a favourable clinical outcome[6], the clinicopathological features of variant t(8;21) are not well delineated.[7] Herein we present a novel variant of t(8;21) with complex three-way translocation, t(8;22;21)(q22;q12;q22) and loss of a sex chromosome as an additional chromosomal abnormality.

CASE REPORT

A 25-year-old G2P1 woman at 9 weeks gestation presented with intermittent fever for 2 weeks. No significant abnormal findings were detected on physical examination. Her full blood count and peripheral blood analysis showed haemoglobin of 97g/L, white cell count of 6.9 x 10^9/L, platelet count of 60 x 10^9/L with 20% circulating blasts. Bone marrow aspirate was normocellular with the presence of 45% myeloblasts with evidence of granulocytic maturation. Myeloblasts exhibited lightly granular and basophilic cytoplasm with Auer rods (Fig. 1) and were positive for peroxidase cytochemical staining. Morphological features were characteristic of AML (most likely M2-FAB subtype). Immunophenotypically, the

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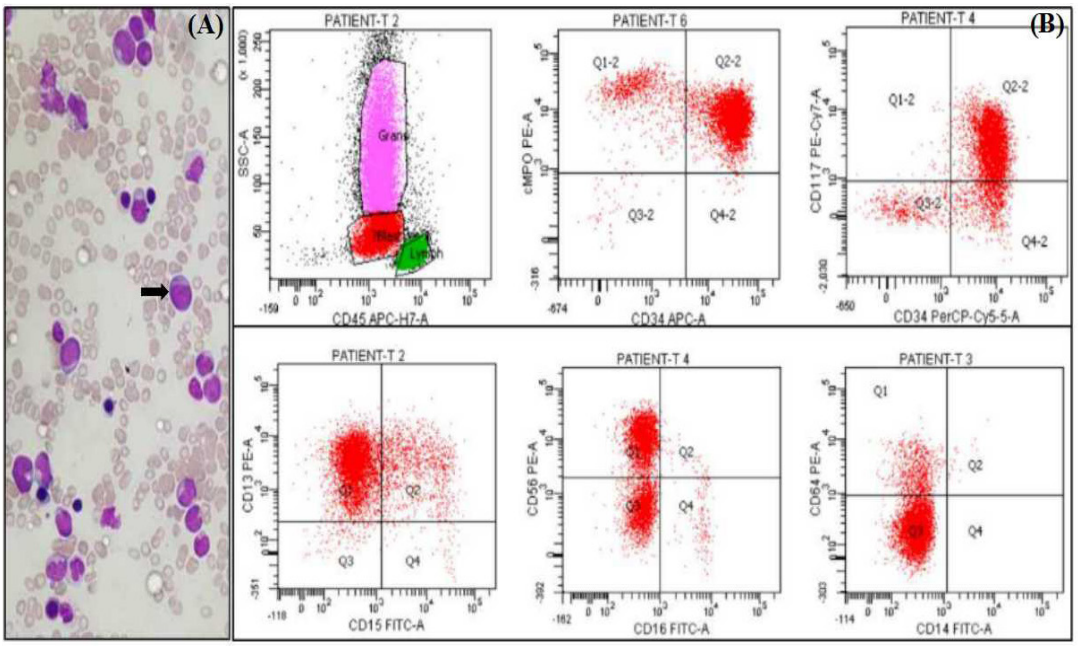

FIG. 1: (A) Bone marrow aspirate exhibiting many myeloblasts with mildly basophilic cytoplasm including one with Auer rod (arrow) [May-Grunwald Giemsa stain, x40]. (B) Flow cytometric immunophenotyping of bone marrow aspirate showing blast cells are CD34(+), cytoplasmic MPO(+), CD117(+), CD13(+), CD56(partial +), CD15(-), CD16(-), CD64(-) and CD14(-).

blasts were positive for CD34, CD117, HLA-DR, cMPO, CD13, CD33 and CD56 (Fig. 1).

The patient's cytogenetic analysis showed presence of an abnormal clone with a three-way translocation involving chromosomes 8, 21 and 22 as a stemline abnormality noted in 22 metaphases and loss of X chromosome as an additional chromosomal anomaly observed in 13 out of 22 abnormal metaphases. Her karyotype was reported as 46,XX,t(8;22;21)(q22;q12;q22)[9]/45,X,sl,-X[13]/46,XX[1], according to the criteria of ISCN 2016 (Fig. 2). Bone marrow

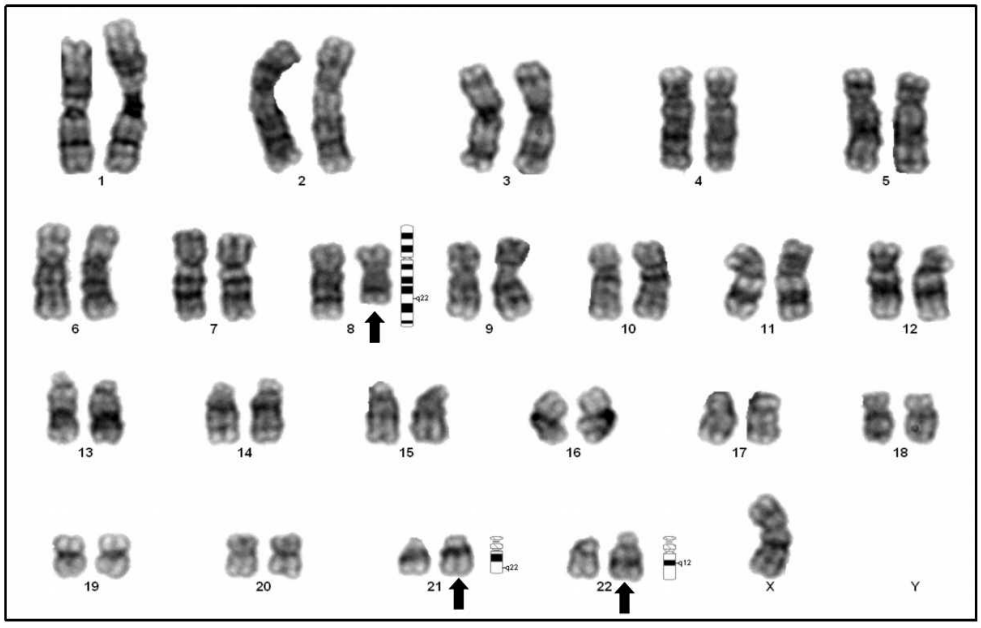

FIG. 2: G-banded karyotypes of bone marrow cells: 45,X,-X,t(8;22;21)(q22;q12;q22) [Arrows indicate derivative chromosomes 8, 21 and 22].

sample for conventional cytogenetic analysis (CCA) and FISH collected in a heparinised container was cultured overnight in Chang medium BMC (Irvine Scientific) which was supplemented with fetal bovine serum and 10% giant cell tumour-conditioned medium without addition of any stimulating agents. The chromosomes in cell culture were then harvested on the following day and stained with Giemsa banding technique. G-banded chromosomes from each culture were analysed and karyotyped by using automatic Cytovision karyotyping software. In general, 20 metaphases from each specimen were analysed as per the International System for Human Cytogenomic Nomenclature (ISCN).[8] Typical CCA for bone marrow sample was based on a resolution of 300-400 bands.

FISH study was carried out on cell suspension prepared from harvested chromosomes according to the manufacturer's recommendations. FISH analysis on 200 interphase nuclei and 5 metaphase spread using *RUNX1/RUNX1T1* dual colour dual fusion probes (MetaSystems, Altlussheim, Germany) confirmed the presence of *RUNX1-RUNX1T1* fusion signals on the derivative chromosome 8 in 92% of (184/200) interphase nuclei examined (Fig. 3). FISH

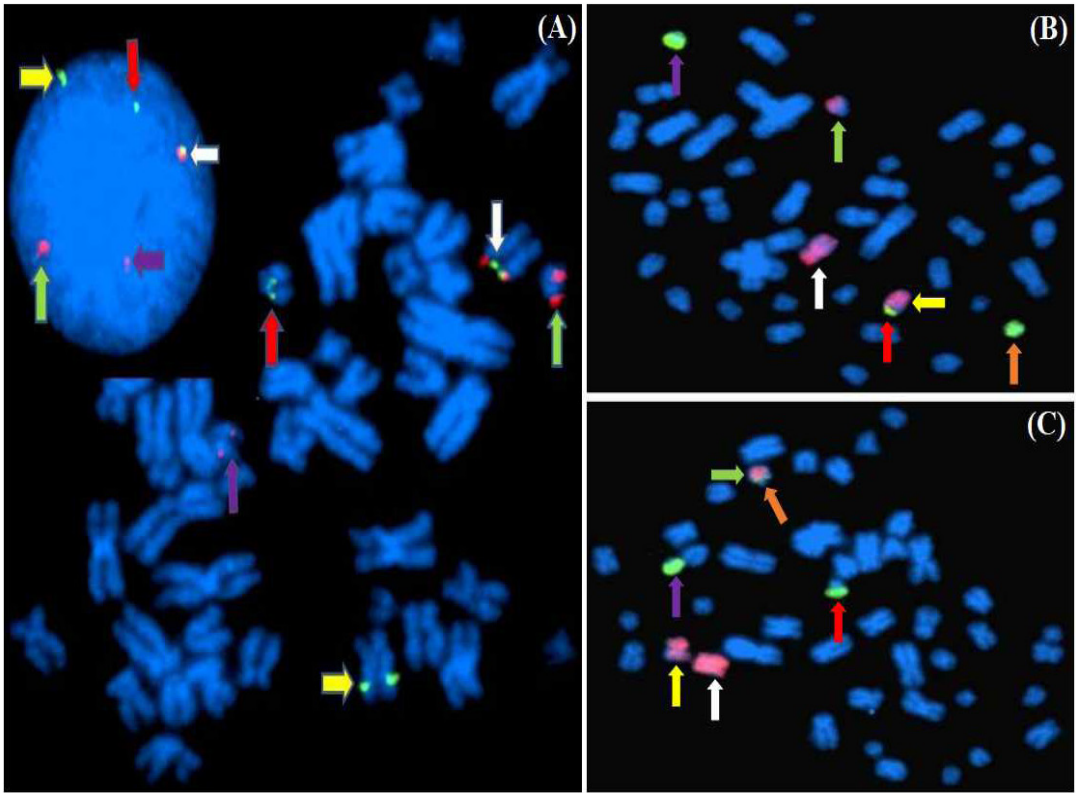

FIG. 3: (A) FISH analysis using *RUNX1-RUNX1T1* dual colour dual fusion probe on metaphase spreads and interphase nuclei: Yellow arrows indicate *RUNX1T1* signals (green) on normal chromosome 8, white arrows indicate *RUNX1-RUNX1T1* fusion signals (red/green) on der(8)t(8;21)(q22;q22), green arrows indicate *RUNX1* signals (red) on normal chromosome 21, purple arrows indicate partially deleted *RUNX1* signals (smaller red signal) on der(21)t(21;22)(q22;q12) and red arrows indicate partially deleted *RUNX1T1* signals (smaller green signal) on der(22)t(8;22)(q22;q12). (B) WCP FISH results for chromosomes 8 and 21 where WCP8 (red) paints normal chromosome 8 [white arrow], part of der(8)t(8;21)(q22;q22) [yellow arrow] and translocated part of der(8) on der(22)t(8;22)(q22;q12) [green arrow] and WCP21 (green) paints normal chromosome 21 [purple arrow], part of der(21)t(21;22)(q22;q12) [orange arrow] and translocated of chromosome 21 on der(8)t(8;21)(q22;q22) [red arrow]. (C) WCP FISH results for chromosomes 8 and 22 where WCP8 (red) paints normal chromosome 8 [white arrow], part of der(8) t(8;21)(q22;q22) [yellow arrow], and translocated part of der(8) on der(22)t(8;22)(q22;q12) [green arrow] and WCP22 (green) paints normal chromosome 22 [purple arrow], part of der(22)t(8;22)(q22;q12) with small residual green signal [orange arrow] and translocated part of chromosome 22 on der(21)t(21;22) (q22;q12) [red arrow].

analysis with whole chromosome painting probes (WCP) for chromosomes 8, 21 and 22 (Cytocell, Cambridge, United Kingdom) confirmed the presence of der(8)t(8;21)(q22;q22), der(22) t(8;22)(q22;q12) and der(21)t(21;22)(q22;q12) in all 12 metaphase cells (Fig. 3). The signal patterns of *RUNX1-RUNX1T1* FISH analysis on three derivative chromosomes and their homologous normal chromosomes were illustrated with ideograms (Fig. 4). Matched metaphase FISH using *BCR-ABL* dual colour dual fusion probes (MetaSystems, Altlussheim, Germany) exhibited normal hybridisation patterns with intact *BCR* gene on derivative chromosome 22, indicating the breakpoint is distal to 22q11.23 (Fig. 5). Regarding matched metaphase FISH study, the position of metaphase of interest was first identified by England Finder after G banding of the slide. The slide was then washed with xylene and ethanol, fixed with Carnoy's fixative, immersed in methanol and finally applied FISH probes. After FISH procedure was completed, the metaphase of interest was searched again with England_Finder. FISH image of particular metaphase was analysed in comparison with G-banded metaphase and karyotype.

Reverse transcriptase polymerase chain reaction showed the presence of *RUNX1-RUNX1T1* fusion transcripts with negative *FLT3* and *NPM1* mutations. Hence, the patient was diagnosed as *RUNX1-RUNX1T1* AML with variant t(8;21). She was treated with induction therapy consisting of cytarabine (100mg/m^2, continuous infusion, days 1-7) along with intravenous daunorubicin (60mg/m^2, days 1-3). She also underwent medical termination of pregnancy at 14 weeks of gestation using prostaglandin analogues. The patient achieved complete morphological remission 1 month after induction therapy and completed further two cycles of consolidation therapy. She was clinically stable until the latest follow-up in the clinic.

DISCUSSION

Variant t(8;21) is uncommon. Majority of reported cases (24/31, 77%) of variant t(8;21) are caused by three-way translocations, involving chromosome 8, 21 and a third chromosome.[9-14] Nearly all chromosomes except chromosomes 7, 22 and Y have been involved as the third chromosome in previously reported cases of variant t(8;21).[9-14] To the best of our knowledge, this is the first reported case of a novel variant involving chromosomes 8, 21 and 22. Band 22q12, involved in this case, is an important chromosomal region as it harbours candidate genes such as *MN1* (Meningioma 1) and *EWSR1* (Ewing Sarcoma breakpoint region 1) genes which are critical in pathogenesis of malignancies.[15,16] Furthermore, gene rearrangements involving *ESWR1* and *MN1* have both been reported to be associated with acute myeloid leukaemia.[15,16] However, in this case, the possibility of additional gene rearrangement and their significance cannot be confirmed without further investigations such as FISH or gene sequencing.

According to WHO classification, AML with t(8;21) is recognised as a distinct type of AML, commonly occurring in young patients.[17] This translocation confers a high sensitivity to standard chemotherapy used for AML.[18] Accordingly, in t(8;21) positive AML, the rate of complete remission reached up to 98% with 5-year overall survival rate of 69% and 5-year

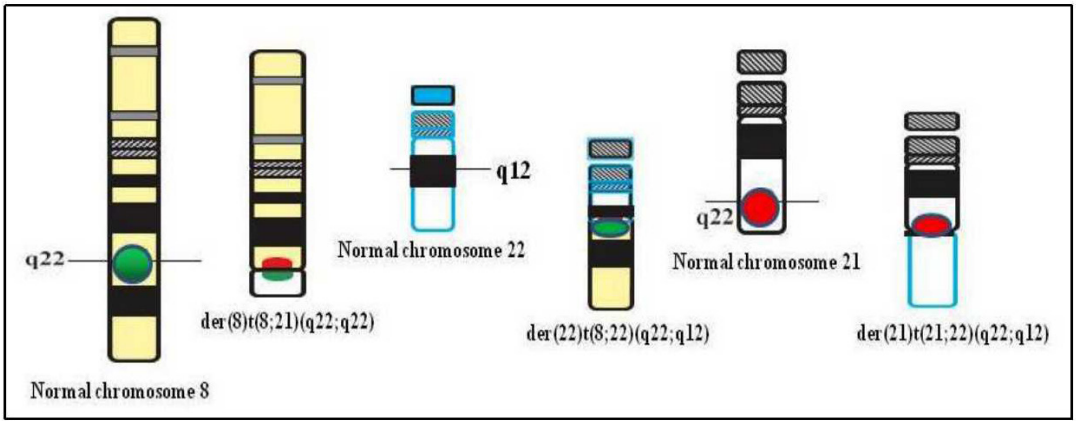

FIG. 4: Ideograms of G-banding patterns for the three-way translocation t(8;22;21)(q22;q12;q22) at 300-band levels. The three derivative chromosomes, their homologous normal chromosomes and their signal pattern on *RUNX1-RUNX1T1* FISH analysis are shown.

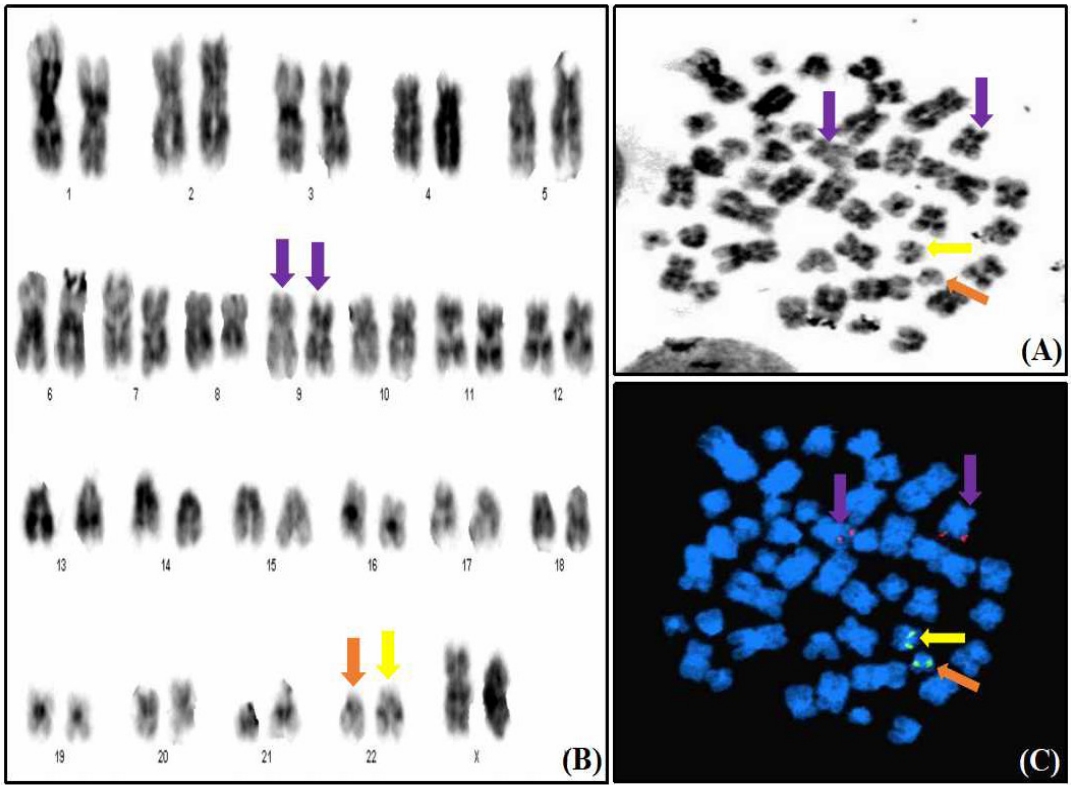

FIG. 5: Matched metaphase FISH study: (A) G-banded metaphase spread and (B) G-banded karyotypes showing normal chromosomes 9 (purple arrows) and normal chromosomes 22 (orange arrows) and der(22) (yellow arrows). (C) FISH analysis using *BCR-ABL* dual colour dual fusion probe on same metaphase spread exhibiting two normal *ABL* signals (red) on chromosomes 9 [purple arrows] and two normal *BCR* signals (green) on normal chromosome 22 and der(22) [orange and yellow arrows respectively].

relapse rate of 29%.[6] Additional chromosomal abnormalities, such as loss of sex chromosome or deletion chromosome 9q, are frequently present in cases of t(8;21) positive AML and these abnormalities are not known to have any prognostic significance.[6] Regarding AML cases with variant t(8;21), their clinical significance is less well delineated and prognosis appears to be controversial.[7,19] There was an earlier literature review with 24 cases of variant t(8;21) published in 2011.[8] Seven more cases including the present one have been reported since that review.[10-14] Among these 31 reported cases, information on the achievement of remission was available on 24 patients and all 24 of them attained complete remission. Of 21 cases with details on disease relapse and survival status, 5 patients (24%) relapsed and 3 patients (14%) expired within the follow-up period.[9-14] Although the follow-up period in these 31 reported cases is variable and relatively short (with a mean follow-up of 19.9 months, (range of 1 to 75 months), AML patients with variant t(8;21) do not appear to be associated with poorer clinical outcomes compared to AML cases with classic t(8;21).[9-14]

In summary, we describe a new variant of t(8;21), t(8;22;21)(q22;q12;q22), in a 25-year old pregnant woman with AML. The morphological features were in favour of the M2-FAB subtype. The presence of *RUNX1-RUNX1T1* gene rearrangement was confirmed using FISH and RT-PCR. The evaluation of 31 AML cases with variant t(8;21) showed that their prognostic outcome might not be different from AML cases with classical t(8;21).[9-14] However, due to the rarity of this chromosomal abnormality, the registry of such variant t(8;21) cases together with a proper follow-up and further genetic exploration are required to elucidate the clinicopathological features and prognostic significance of this rare AML subgroup.

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