

A Rare Cytogenetic Presentation of Acute Myeloid Leukemia (AML-M2)

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Abstract- Acute myeloid leukemia (AML) with t(8;21)(q22;q22) generating the AML1/ETO fusion gene on 8q22 is a distinct type of AML t(8;21) category (WHO)/AML-M2 (FAB), generally associated with a favourable prognosis. Variant additional chromosomal abnormalities are frequently reported. We report three adult cases of this category with unusual karyotype. Bone marrow cytogenetics of case no. 1: 45,X,-Y, t(8;21)(q13;q22) with a novel breakpoint of chromosome 8 at (q13). Case no. 2: 46,X,t(X;2)(q22;q37),t(3;7)(q21;q36),t(5;14)(p15;q11),del(8)(q22) a complex rearrangement without the involvement of chromosome 21. Case no. 3: 49,XX,+5, t(8;21)(q22;q22), +16, +der(21)t(8;21)(q22;q22) with additional der(21). Endometrial in this case which was positive for myeloperoxidase (MPO) and CD117 conforming the AML infiltration. All are morphologically AML with t(8;21). Relevant literature in cytogenetic of AML-M2 is reviewed. The molecular mechanism involved in unusual rearrangements and clinical significance of them are subjected for further studies.

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Keyword: Acute myeloid leukemia; Breakpoint; Complex karyotype; Variant

Introduction

Acute myeloid leukemia (AML) is a heterogeneous disease. Diagnostic karyotype is one of the most independent prognostic indicators which serves to identify biologically distinct subsets of disease and has been widely adopted to provide the frame work for risk-adopted treatment approaches (1). t(8; 21)(q22;q22) is a frequent non-random cytogenetic abnormality in AML and is strongly associated with French-American-British (FAB) subtype M2 (WHO) and has distinctive clinical and morphologic features associated with a favourable prognosis with high remission rate and a long median survival. The t(8;21)(q22;q22) is a balanced reciprocal translocation associated with about 6% of AML-M1 and up to 92% with AML-M2 cases (2-4). This translocation also reported in a small portion of M0, M1 and M4 subtypes. This results in the fusion of AML1 gene from chromosome 21q22 with the ETO gene on chromosome

8q22. The t(8;21)(q22;q22) is frequently associated with additional chromosomal abnormalities and loss of sex chromosome being the most recurrent one. Approximately, 3-4 % of AML associated with variant t(8;21)(q22;q22) (2,3,5,8). Clinically AMLs with t(8;21) associated with good response to chemotherapy. Here, we report three cytogenetically interesting cases of AML-M2 presented to our institution in the year 2011.

Materials and Methods

The bone marrow cells were cultured for 24 and 48 hours without mitogens in RPMI-1640 (Gibco) medium supplemented with 15% fetal bovine serum (Gibco) at 37°C. After incubation, the cells were exposed to colcemid (Gibco, 0.10µg/ml) for 30 minutes, then hypotonised by 0.075 M KCl for 20 minutes, and fixed with Carnoy's fixative (methanol: glacial acetic acid 3:1) and kept in refrigerator overnight. Next day the air

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dry slides were made. The chromosomes were G-banded with trypsin-digestion method and stained with Giemsa (6). Metaphases were captured and analyzed by Image Analysis System. The karyotypes were interpreted according to ISCN 2009 (7). Other laboratory details were obtained by going through the case records. Each case was classified according to WHO and FAB classification system.

Results

We identified three cases of variant $t(8;21)(q22;q22)$. The presenting symptoms were variable and included recurrent infection, weight loss, fatigue, easy bruising and the third case with unusual vaginal bleeding. The clinical, hematology, morphology, cytochemistry and

cytogenetic data are summarized in Table 1. All three patients showed abnormal karyotype with additional abnormalities.

In case one, the metaphases were hypodiploid, with the loss of Y chromosomes. The chromosome 8 and 21 were involved in translocation, but breakpoint of 8 was at (q) (13) appears unusually short and chromosome 21(q) was longer than usual (Figure 1).

In case two diploid complex karyotype with three independent reciprocal translocations and $del(8)(q22)$ with normal chromosome 21 was observed (Figure 2).

The third case demonstrated hyperdiploidy, with trisomy 8, 15 as numerical abnormalities, additional derivate (21) as numerical and structural abnormality along with $t(8;21)(q22;q22)$ (Figure 3).

Table 1. Clinical, haematological and cytogenetic findings of patients of AML-M2.

Case	Age	Sex	HB g/dl	WBC	PLT	MPO	PAS	Morphology	Karyotype
1	23	Male	8	11.4	34	+ve	-ve	AML-M2	45, X,-Y, $t(8;21)(q13;q22)$
2	17	Female	9	12	33	+ve	-ve	AML-M2	46, X, $t(X;2)(q22;q32)$, $t(3;7)(q21;q36)$, $t(5;14)(p15;q11)$, $del(8)(q22)$
3	39	Female	7.2	11.3	34	+ve	-ve	AML-M2	49, XX,+8, $t(8;21)(q22;q22)$, +15, +der(21)t(8;21)(q22;q22)

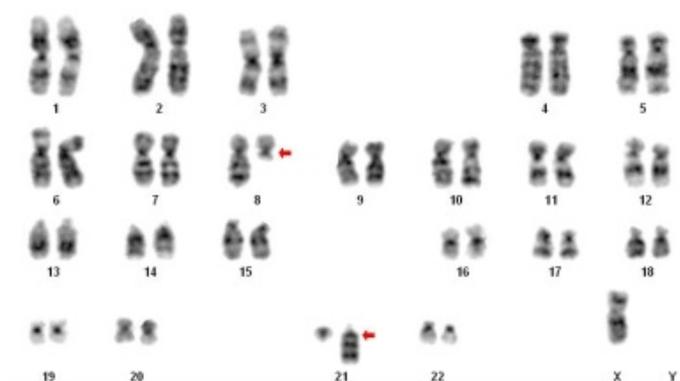


Figure 1. Karyotype of case no. 1: 45,X,-Y, $t(8;21)(q13;q22)$.

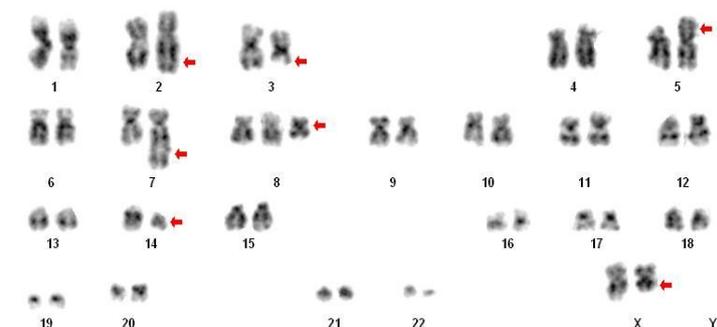


Figure 2. Karyotype of case no. 2: 46,X, $t(X;2)(q22;q32)$, $t(3;7)(q21;q36)$, $t(5;14)(p15;q11)$, $del(8)(q22)$

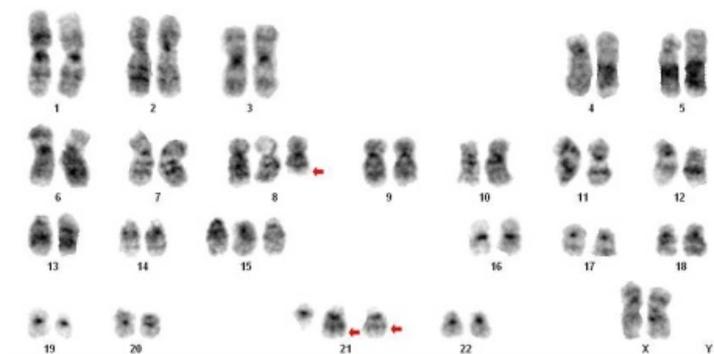


Figure 3. Karyotype of case no. 3; 49,XX,+8,t(8;21)(q22;q22), +15,+der(21),t(8;21)(q22;q22)

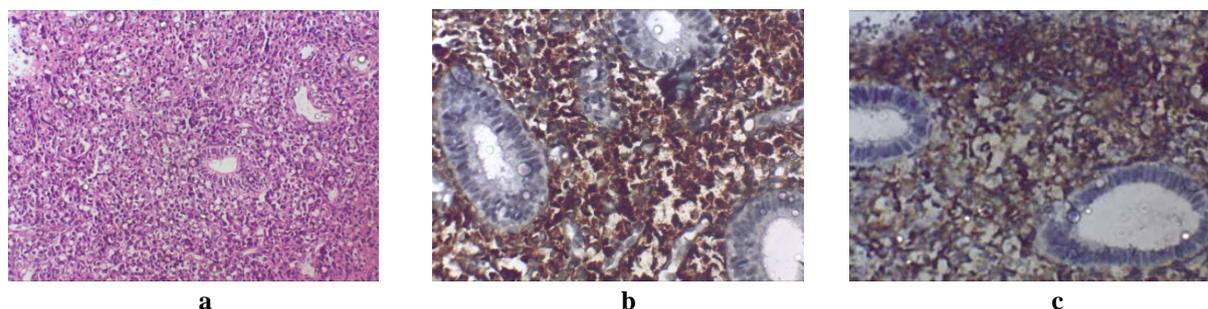


Figure 4. Case no. 3.

- H&E sections of endometrium showing the infiltration of blasts.
- The blasts were positive with MPO.
- The blasts showed strong positivity with CD117.

Histopathologically, endometrium of this case showed large foci of infiltration by blasts. The cells were polygonal with large round nuclei with 1-2 nucleoli and scanty clear cytoplasm. On immunohistochemistry blasts were positive for myeloperoxidase (MPO) and CD117 confirming the AML infiltration to endometrial tissue (Figures 4a, 4b and 4c).

Discussion

The t(8;21)(q22;q22) is seen in 92% of all AM-M2, some cases of AML-M4 and less commonly in M0 and M1(6%) (9). The AML1 rearrangement or AML1/ETO fusion translocation in complex variants of t(8;21)(q22;q22) are occasionally observed in a small percentage of AMLs. Most of them are three way translocations like t(8;13;21) (q22;q14;q2), t(8;21;14) (q22;q24;q24) and t(2;21;8) (q36;q22;q22) (5). Very rarely, a four way variant complex translocation is reported (4). Variants showing good response to therapy as in typical t(8;21)(q22;q22) cases (3) but others have reported that variants have a worse prognosis (9). The

additional numerical and structural changes reported to be two- third and one- third cases respectively (10). In all reported cases the breakpoint on chromosome 8 was q(22), the locus of ETO gene. The relevance of the der(8) chromosome, which contains the AML1/ETO fusion gene in the pathogenesis AML-M2 is reported by several studies (4). Huang *et al.* report a case with der 8 appears longer than normal chromosome 8 due to duplication and insertion (3). The first case of present study even though the translocation between 8 and 21 the breakpoint of chromosome 8 is on q(13) the locus for the unknown gene. The clinical relevance of the novel breakpoint is not known.

Vunditi *et al.* (2) reported a case with three way translocation t(X;21;8)(p22;q22;q22), where ETO gene is translocated to X(p22) region. It was presumed to be Xp(22.1) region translocated to chromosome 21 and AML1 translocated to 8q22. Derivative 8(q22) or del 8(q22) with normal chromosome 21 have been reported in several cases. Very rarely, der(21)(q22) with normal 8 is reported (8). In second case of our study, del 8(q22) might possibly be the derivative of t(8;21) and any one among three translocations of complex karyotype were

involved in rearrangement. The molecular analysis like FISH or SKY may help in the identification of chromosomes which are involved in the masked type of variant t(8;21)(q22;q22).

Extramedullary deposits of AML can occur at any site in the body like, lymph node, bone, soft tissues and testis (11). AML infiltration of the female genital tract is very rare and even rare in the symptomatic presentation, with very few such reports in the literature, Myagi *et al.* reports a case with extramedullary myeloblastoma of the uterus with variant t(8;17)(q22;p13) by G-banding and three way translocation involving chromosomes 8,17 and 21 by SKI and identified a masked type of variant (8). Third case of present study with an extra copy of der(21)(q22) as an additional abnormality along with t(8;21) is a rare karyotype feature. Though molecular evaluation of der(21) was not possible, to the best of our knowledge this is the first case of der(21) as an additional abnormality in AML-M2 with endometrium involvement from our centre. Unfortunately all three cases were lost for follow up. Molecular mechanisms involved in unusual rearrangements are a subject for further studies and the clinical significance of them may be determined with larger studies with longer follow up.

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