Chromosomal Aberration for Diagnosis and Prognosis of Acute Myeloid Leukemia Iraqi Patients

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Abstract

Background: Acute myeloid leukemia (AML) is a hematopoietic disorder in which there are too many immature blood-forming cells accumulating in the bone marrow and interfering with the production of normal blood cells. It has long been recognized that AML is a clinically heterogeneous disease characterized by a multitude of chromosomal abnormalities and gene mutations, which translate to marked differences in responses and survival following chemotherapy. This study aimed to clarify the chromosomal aberrations in early diagnosed and relapsed cases of AML.

Materials and methods: Chromosomal changes were studied in thirty Iraqi patient samples diagnosed with acute myeloid leukemia were divided into 9 newly diagnosed and 13 received chemotherapy who were incomplete remission and 8 relapsed subjects.

Results: Analysis of all chromosomal aberrations showed complex karyotype for most cells of relapsed AML patients compared with newly diagnosed patients.

Conclusions: Chromosomal abnormalities are linked to AML development and high complexity of karyotyping for relapsed group.

Key Words: AML, Relapse, Chromosomes.

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Introduction

Leukemia is the abnormal proliferation of leukocytes in the bone marrow and blood-forming organs resulting in an accumulation of undifferentiated blasts cells; it is approximately about 3.5% of all cancer incidences (Hao *et al.*, 2019). It may be classified based on the pace of progression into lymphoid and myeloid, then classified into four main subgroups depending on the type of affected blood cell (Wang *et al.*, 2019). The AML is characterized by an increase in the number of myeloid cells in the marrow and an arrest in their maturation. It occurs more commonly in adults than in children, and more commonly in men than women. AML is treated with chemotherapy. Acute myeloid leukemia (AML) is the most commonly diagnosed leukemia in adults (25%) comprises 15– 20% in children, and the incidence rate is about 1.3 per 100,000 for those under 65 and about 12.2 cases per 100,000 for those over 65 years. It is characterized by malignant clonal proliferation and differentiation of immature myeloid progenitor cells that differentiate into malignant myeloblasts that cannot function like normal blood cells.

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Consequently, bone marrow and peripheral blood get collected with leukemic blasts resulting in reducing the production of healthy and functional white blood cells (Lazarevic *et al.*, 2018).

Materials and Methods

Subjects

A case-control retrospective study was done during the period from June 2019 to Feb 2020. The total number of participants in the study was sixty subjects**.** Thirty Iraqi patient samples diagnosed with acute myeloid leukemia collected from Baghdad teaching hospital, Al-Imammin teaching hospital, and National center for research in Baghdad/Iraq were enrolled in this study. Their ages ranged (14-90) years divided into three subgroups, 16 cases newly diagnosed, 26 cases on treatment (incomplete remission) and 16 relapsed cases. For comparison thirty samples of apparently healthy individuals were enrolled in this study, their ages between (14-72) years. Chemotherapy protocol used is (3+7) and HIDC for induction and consolidation respectively. The study protocol was approved by the Ethics Committee and written informed consent was obtained from all participants before entering the study. The Ethics Committee of the Iraqi Ministry of Health approved the study.

Karyotyping Analysis

For cytogenetic analysis, 5 ml peripheral blood sample was collected from all the patients and stored into heparinized test tubes. Chromosomal analysis was performed by cultured lymphocytes in RPMI media with PHA in an incubator at 37°C for 72 hours. Metaphase harvesting was done by adding colcemid for 5 min following hypotonic KCl treatment for 1 hour and later fixation by using 3:1 methanol-acetic acid mix. Cells treated with trypsin enzyme before staining with Giemsa stain chromosomal aberrations was examined and analyzed by cytovision, (Leica) according to ISCN, 2016.

Statistical Analysis

The Statistical Analysis System- SAS (2012) program was used to detect the effect of difference factors in study parameters. Chi-square test was used to significant compare between percentage 0.05 and 0.01 probability in this study.

Results

Results showed very complex chromosomal analysis in most cells of patients especially relapsed, and many chromosomal aberrations were noticed in these cells, and showed numerical chromosomal aberrations in monosomy and trisomy in some chromosomes and showed a significantly higher frequency of monosomic chromosomes (30)% was observed in AML groups and distributed as follows (five relapsed cases, four cases which were on treatment, and only one case was newly diagnosed) in different chromosomes. However, chromosome 7 was mostly frequented especially in relapsed cases.

Furthermore, six (20)% out of (30) AML patients recorded trisomy distributed as follows (three were on treatment, two cases were relapsed and only one case was newly diagnosed) in different chromosomes, however chromosome 21 and 22 were most frequented especially in relapsed cases Table (1) Figure(1).

Table 1. Frequency of numerical aberrations in AML groups

Numerical Aberrations	AML Groups				
	Newly diagnosed	**On treatment**	**Relapse**	**Frequency**	**%**
Monosomy	**1**	**4**	**5**	**10**	**33**
Trisomy	**1**	**3**	**2**	**6**	**20**
Chi-Square (χ^2)	**-**	**-**	**-**	**-**	**4.924 ***
* (P≤0.05).					

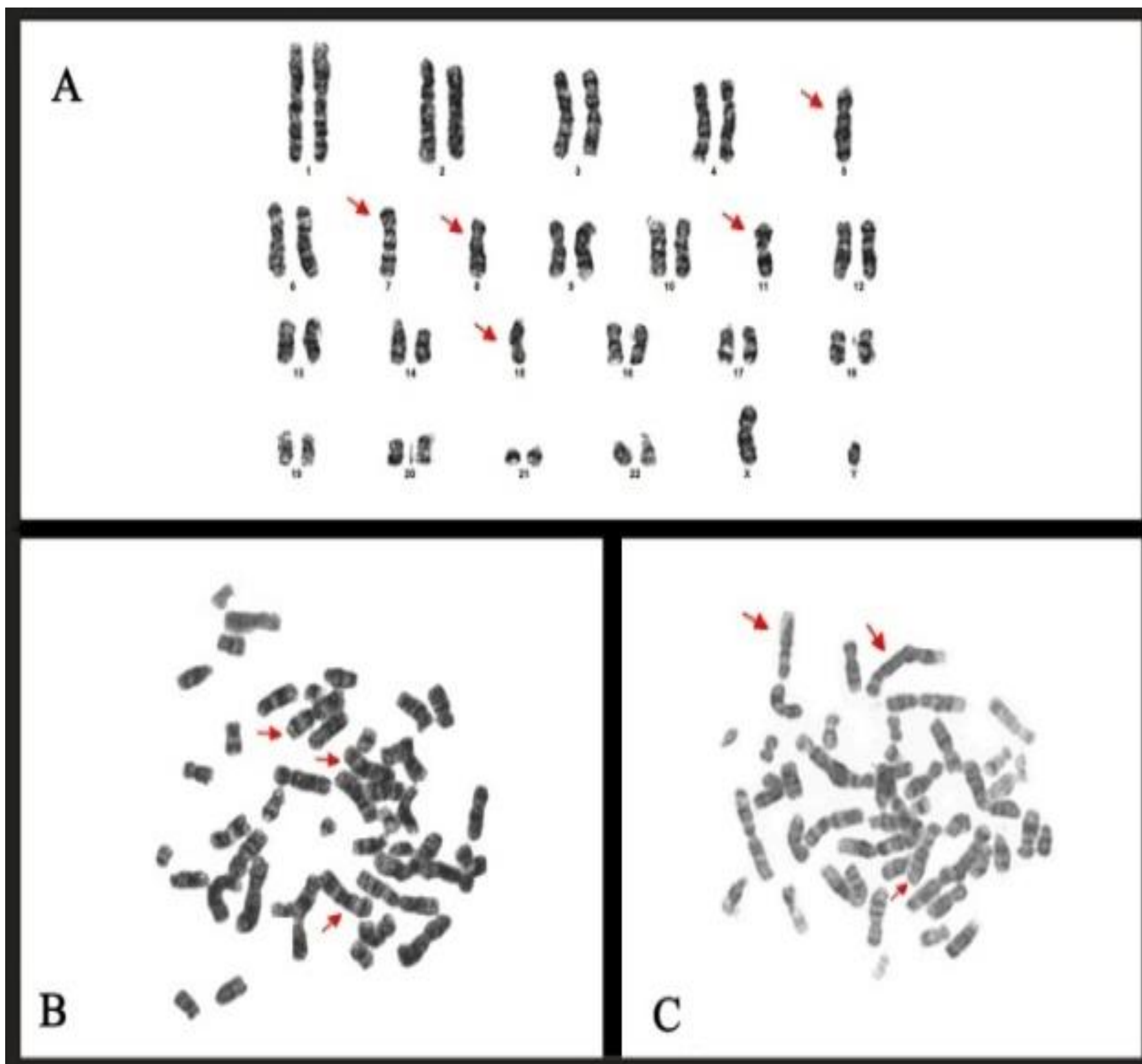
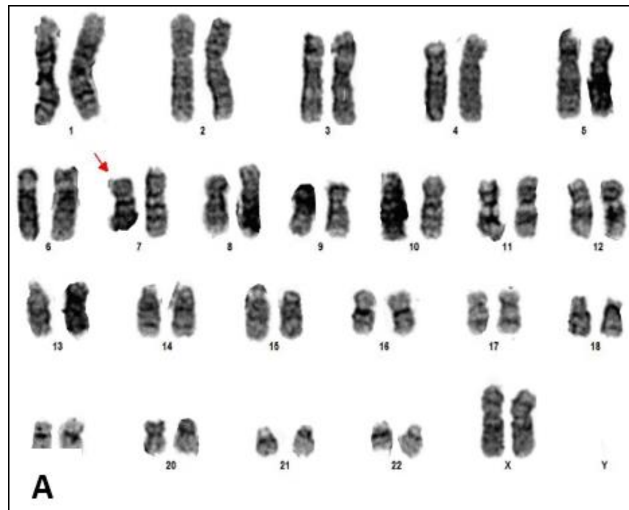


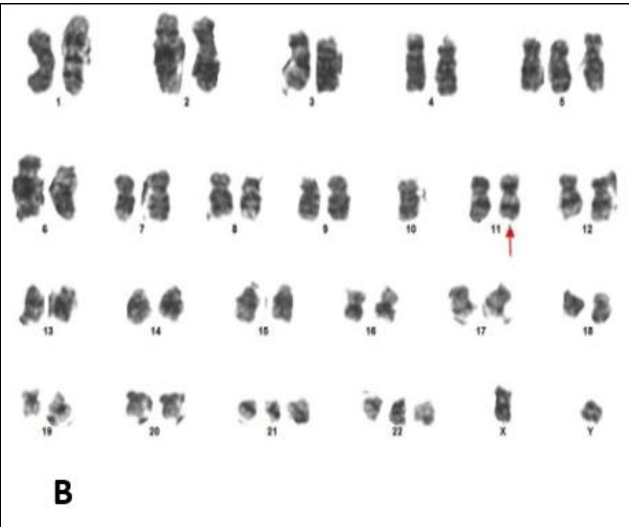
Figure 1., A: Monosomy chromosomes 5,7,8,11 and 15 B: Trisomy chromosome 3 and C: Trisomy chromosome 6 (red arrows)

Results showed highest frequency of deletion was observed in 7p in on treatment AML cases (30)% followed by relapsed cases (16.6)% with significant differences. Also, frequency of 11q deletion was distributed as follows (3. 3% in newly and 16.6% in treated and 6.6% in relapsed cases) respectively. On other hand, frequency of 17q deletion was 20 % in relapsed cases while it was 13.3% in on treatment and 6.6% in newly diagnosed and statistical analysis revealed signification differences at $p<0.05$ Table (2) Figure (2).

Table 2. Frequency of deletion in different arms of chromosomes

AML groups				
Deletion	**Newly diagnosed No. (%)**	**On treatment No. (%)**	**Relapsed No. (%)**	**P-value**
7p	-	9(30)	5(16.6)	0.0026 **
11q	1(3.3)	5(16.6)	2(6.6)	0.0362 *
17q	2 (6.6)	4(13.3)	6(20)	0.0272 *





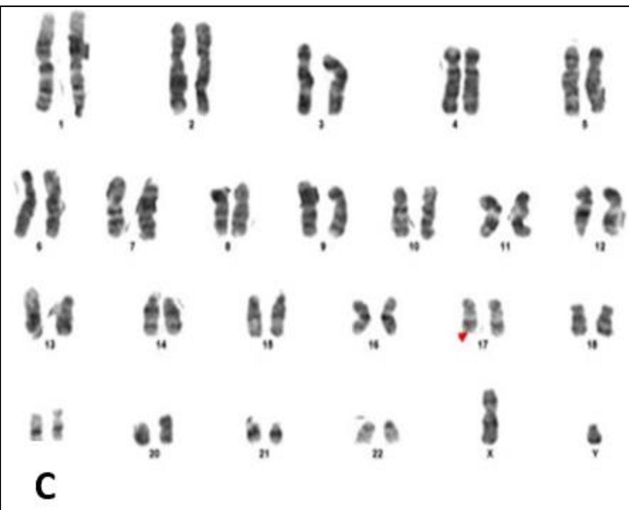


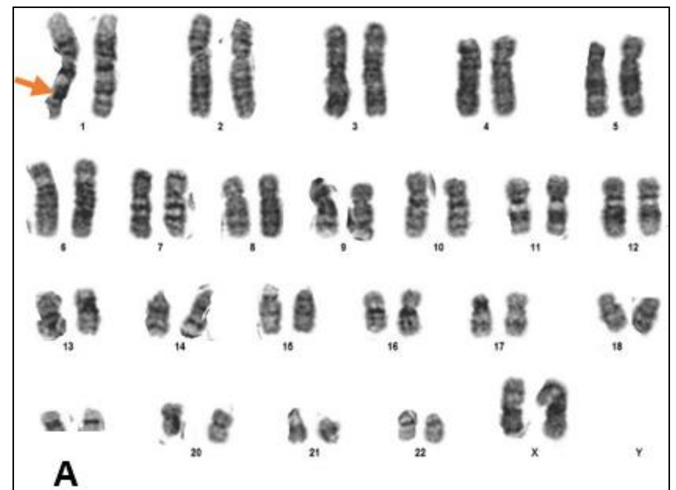
Figure 2. A: Deletion of 7p, B: Deletion of 11q, C: Deletion of 17 q (red arrows) in AML groups.

Other structural chromosomal abnormalities were revealed as duplication in different arms of chromosomes showed in Table (3)

Table 3. Frequency of duplication in different arms of chromosomes

AML groups				
Aberration	**Newly diagnosed No. (%)**	**On treatment No. (%)**	**Relapsed No. (%)**	**P-value**
Duplication 1q	**-**	**3(10)**	**1(3.3)**	**0.0417 ***
Duplication 3q	**1(3.3)**	**2(6.6)**	**1(3.3)**	**0.294 NS**
Duplication 10q	**2(6.6)**	**7(23.3)**	**2(6.6)**	**0.0092 ****

Frequency of duplication long arm of chromosome 1 (1q) was 10% in on treatment patient and 1% in relapsed cases (figure2). Other frequency of duplication in 3q recorded 6.6% in on treatment AML and 1% in newly diagnosed and relapsed cases, Figure (3).



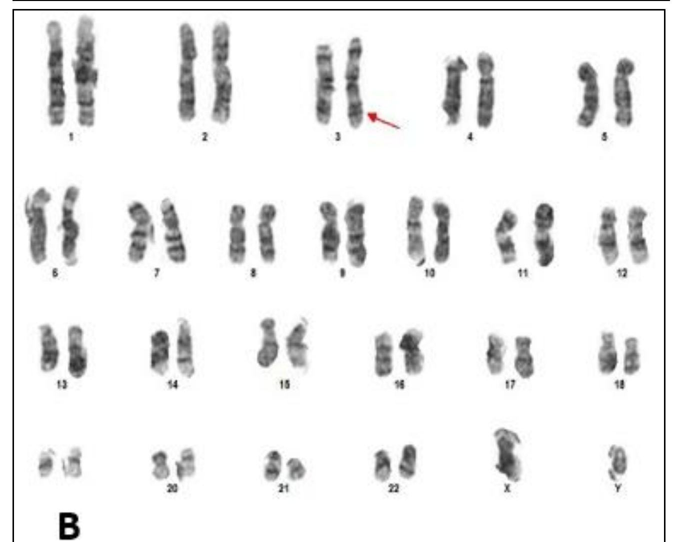


Figure 3. A: Duplication of 1q, B: Duplication of 3q in AML groups.

Finally, the enticing 10q duplication was distributed as follows (6.6 % in newly diagnosed, 23.3% in on treatment and 16.6% relapsed cases). It may refer for the importance of duplication 10q as candidate predicted biomarker for relapsed AML cases, Figure (4).

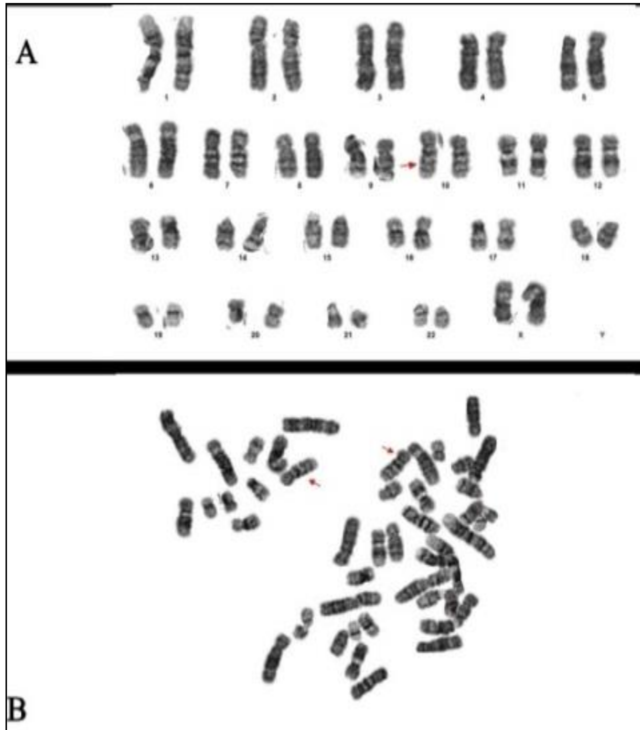


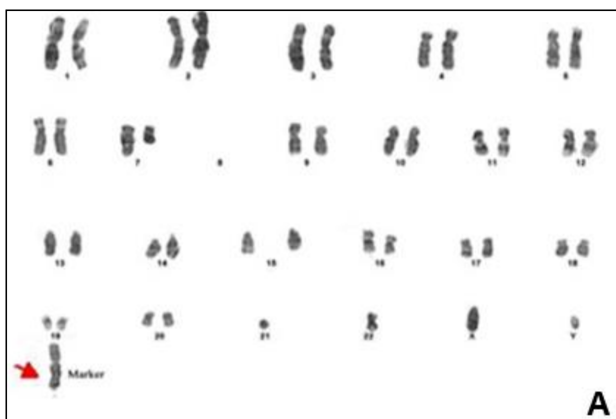
Figure 4. A: Duplication 10q in karyotype B: Duplication 10q in metaphase (red arrows) in AML groups.

Other Structural Chromosomal Aberrations

Other structural chromosomal aberrations (SCA) were appeared first as marker chromosomes, which were observed in five cases frequency 16.6%, most of these cases were relapsed showed in Figure (5A). The second SCA was the Double Minute which were observed in 3 relapsed cases only with frequency10%, Figure (5B), Table- 4.

Table 4. Different structural chromosomal aberration in AML groups

AML groups					
Structural aberrations	**Newly diagnosis**	**On treatment**	**Relapsed**	**Frequency**	**%**
Marker	1	1	3	5	16.6
Double minute	-	-	3	3	10



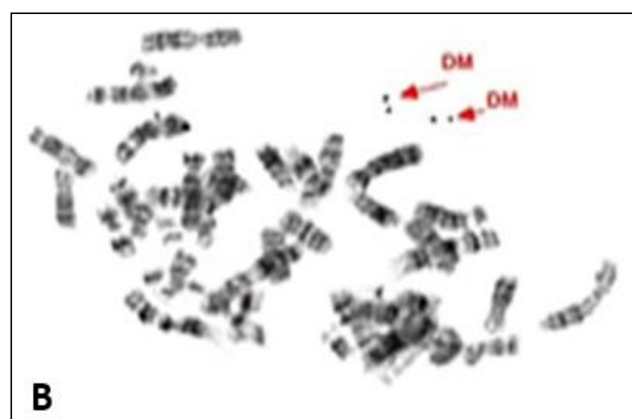


Figure 5. A: Marker chromosome, B: Double minutes (red arrows).

Discussion

AML has been characterized by genetic disorders events leading to the transformation of the normal hematopoietic cell, clonal chromosomal abnormalities are found in approximately 80% of children with AML. These shown correlations between specific recurrent chromosomal abnormalities and clinical-biological characteristics and outcomes (Kronke *et al*., 2013).

Numerical chromosomal aberrations were found in groups of AML patients in monosomy and trisomy chromosome in different chromosomes, however monosomy chromosome 7 was mostly frequented in relapsed cases, this result like Mehta *et al.* (2010) who found unbalanced chromosomal aberrations in monosomy 7 and 7q deletions associated with increased risk of Fanconi anemia treated patient which progression into MDS-AML. This loss of one copy of chromosome lead to loss of specific genes, or alter the dosage of some genes resulting in inactivation of tumor suppressor genes or activation of oncogene and defective DNA repair (Kawankar and Vundinti, 2011). Also, AML with trisomy 8 seems to be associated with mutations in DNA methylation genes; these alterations probably have stronger implication for leukemic pathogenesis. Further, band 21q22 that contains the ERG oncogene, are also found in AML, thus overexpression of the chromosome 21 gene CHAF1B has been shown in stem cell signature in AML (Hemsing *et al*., 2019).

Structural chromosomal aberrations which found in groups of AML patients, was deletion and duplication in different arms of chromosomes, such as short arm of chromosome 7 was shown deleted most frequency in relapsed cases as well as in patient whom on treatment,7p harbor different developmental regulator genes, including HOXA gene cluster on 7p15 as a regulator gene and epidermal growth factor (EGF) receptor gene which was reported of biological significance in pancreatic tumors (Fryssira *et al*., 2011).

Chromosome 11 abnormalities are found in several hematological malignancies, including AML. The proto-oncogene MLL in 11q23.3 is frequently altered and may play a role in initiation or progression of myeloid malignancies (Sarova *et al*., 2013). In addition, ATM gene at 11q23, which responsible of DNA damage and contributed to the increased need for transfusion in patients with MDS (Lovatel *et al*., 2018).

Finally, the 17q11 region was deleted in mostly relapsed cases, was contains NF1gene, as a tumor-suppressor, which have been reported in many adult myeloid malignancies and deletion in this gene contributes to leukemic transformation or progression to juvenile myelomonocytic leukemia with secondary event in AML (Boudry-Labis *et al*., 2013).

Duplication in 1q arm was like result Marchesin, (2017) who found that amplification of 1q21 in multiple myeloma (MM) and is correlated with disease progression and drug resistance. This region contains a number of putative oncogenes and genes that may show the simultaneous amplification including MCL1, IL6R, BCL9, CKS1B, ANP32E, ILF2, and ADAR122–24 (Sawyer *et al.,* 2019). Lee (2019) reported that patients had MDS with pathogenic mutations of the many genes and developed 1q JTs at the time of progression from MDS to AML .In addition to 1q21-1q32 might harbor oncogenes or tumor suppressor genes that are pathogenetically relevant to both chronic and advanced phases of myeloproliferative neoplasms MPN (Caramazza *et al*., 2010). While Chromosome 3 which contain Fragile site on 3q21-29 represented chromosomal breakpoints that result in chromosomal rearrangement and is associated with leukemia (Scully *et al.,* 2003).

10q duplication which novel occurrence in leukemia showed here in all groups, this contains many tumor suppressor genes such as ERCC6, PTEN, and DMBT1 which putatively implicated in the most common type of malignant glioma (Chen *et al*., 2015). PTEN have essential role in development of myeloid malignancies especially AML (Morotti *et al*., 2015).

Marker chromosomes are found only in relapsed patients, these abnormal structural chromosomes that are rearranged to a level that difficult to characterize and prohibit its distribution to one of the known chromosomes and indicating gross structural chromosomal damage. This aberration is more frequent in AML among elderly patients (Bochtler *et al*., 2017). Also, double minutes (d min) which was observed in only relapsed patients. D min results from gene amplification and overexpression of some cellular oncogenes, which may lead to formation of extrachromosomal double minutes (Macchia *et al*., 2010).

From these results, deletion 7p, deletion 17q, and duplication 10q may be considered as candidate predicted biomarker for relapsed AML cases.

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