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Chromosomal Abnormalities in Chromosome 5 and Chromosome 8 in Iraqi Patients with Acute Myeloid Leukemia

Amal Mohammed Ali[1], Asmaa Amer Almukhtar[1], Noor Hashim Ismail[2], Ayeda M.Majeed[2], Noor Thaer[1], Fatima Abd Mutlak[1], and Shaimaa Abdul Alrahman[1]

1 Department of Medical Genetics, Iraqi Center for Cancer and Medical Genetics Research, Mustansiriyah University, Baghdad, Iraq

2 Department of Cancer Research, Iraqi Center for Cancer and Medical Genetics Research, Mustansiriyah University, Baghdad, Iraq

Abstract

Acute myeloid leukemia (AML) is the mostly diagnosed leukemia in adults, resulting from accumulate immature blasts in the bone marrow that are replaced instead of normal blasts that cannot function like normal blood cells. AML resulting from genetic abnormalities, including multiple gene mutations and chromosomal aberrations, are found in approximately 80% of children with AML. These shown correlations between specific recurrent chromosomal abnormalities and clinical-biological characteristics and outcomes, also, novel therapies are being developed that target some of the identified genetic defects. The aim of this study was to investigate and record the role of chromosome 5 and 8 aberrations in the development of newly diagnosed and relapsed cases of AML in Iraqi patients. Chromosomal changes were studied in peripheral blood samples from 30 Iraqi patient samples diagnosed with acute myeloid leukemia and divided into 9 newly diagnosed and 13 chemotherapy-treated subjects who were incomplete remission and 8 relapsed subjects. The results of the chromosomal analysis of this study revealed numerical and structural abnormalities of both chromosomes: 5 and 8 in 16 (53.33%) and 12 (40%), respectively, within a complex karyotype and high frequencies in numerical abnormalities of chromosomes for chromosome 5 and structural abnormalities for chromosome 8. Chromosomal aberrations involving chromosomes 5 and 8 are linked to AML development and prognosis, and their presence in a complex karyotype can lead to disease progression and the worst prognosis, especially for abnormalities on chromosome 5, which were detected the most frequently. Future molecular genetic tests and complete karyotype analysis should enhance AML diagnosis and treatment.

Keywords: *AML, chromosome abnormalities, deletion 5q, trisomy chromosome 8*

Introduction

Acute myeloid leukemia (AML) is an aggressive myeloid leukemia recognized by the accumulation of abnormal blast cells in the peripheral blood and bone marrow (1). This disease has thus a series of genetic aberrations and is associated with large chromosomal aberrations, as well as mutations in genes and aberrant gene expressions (2,3). These genetic alterations involve oncogenes or lead to loss of suppressor genes (4,5). Such cytogenetic changes are important prognostic and predictive markers of AML, which have the potential to serve as novel therapeutic targets for personalized medicine (6). Thus, chromosomal abnormalities are recognized as an important factor in diagnosis and as an independent prognostic indicator. Accordingly, treatment in most therapeutic protocols for AML is based on chromosomal and molecular abnormalities of leukemic cells (2, 7-9).

Chromosomal aberrations may be numerical, such as monosomy and trisomy in different chromosomes, and may be associated with a prognosis in AML (5). These include monosomy that means loss of all one copy of a chromosome or deletions part of a chromosome (10), which leads to loss of specific genes, or alter the dosage of some genes resulting in inactivation of tumor suppressor genes or activation of

Corresponding Address:
Amal Mohammed Ali
Department of Medical Genetics, Iraqi Center for Cancer and Medical Genetics Research, Mustansiriyah University, Baghdad, Iraq
Email: amal.ali@iccmgr.org

the oncogene and defective DNA repair gene (10,11). In addition to numerical chromosomal abnormalities in some genetic syndromes associated with cancer development (12). It is also a complex karyotype, detected in myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML), and is associated with a reduced median survival (9). These abnormalities were observed more prominently in elderly patients with AML, with links found between chromosomal aberrations and mortality rates in this age group (11).At the molecular level, the disease manifests itself as changes in both epigenetic and genetic signatures, involved in hematopoietic proliferation and differentiation, which result in the accumulation of poorly differentiated myeloid cells through the development of AML (13), resulting in the accumulation of large numbers of immature myeloid cells in the bone marrow and peripheral blood (14). These cells are capable of dividing and proliferating, but cannot differentiate into mature hematopoietic cells, which are characterized by myeloblasts that cannot function as normal blood cells (15,16), bone marrow, and peripheral blood accumulate with leukemic blasts resulting in a reduction in the production of normal and functional white blood cells (17,18). Most AML patients tend to be relapsed with low survival rates, with a relapse leading to the death of the majority of patients (19). Genetic alterations may involve oncogenes or lead to the lack of suppressor genes (20). So, AML characterized by events of genetic alterations resulting in aberrations of the chromosome, which are important prognostic and predictive markers of AML , and serve to use new therapies targeting these molecular changes (21). The aim of this study was to investigate and record the role of chromosome 5 and 8 aberrations in the development of newly diagnosed, on treatment and relapsed cases of AML in Iraqi patients.

Materials and Methods

Samples Collection

The total number of participants in the study was thirty Iraqi patients diagnosed with AML collected from the Baghdad Teaching Hospital, the Al-Imammin Teaching Hospital and the National Center for Hematology, Mustansiriyah University, Baghdad, Iraq. Their ages ranged from 14 to 90 years, 15 male and 15 female, divided into three subgroups, 9 newly diagnosed cases, 13 cases on treatment (incomplete remission) and 8 relapsed cases. Peripheral blood samples were collected from all patients.

Chromosomal analysis

For chromosomal analysis using the G banding technique, a 5 ml peripheral blood sample was collected from all participants, patients, and controls, stored in heparinized test tubes. Chromosomal analysis was performed by cultured lymphocytes in RPMI medium with PHA in an incubator at 37 ° C for 72 hours. Metaphase arrest was performed by adding colcemid for 25 min followed by hypotonic KCl (0.075M) treatment for 45 min and later fixation with a 3:1 methanol-acetic acid mix. Cells treated with trypsin enzyme for G-banded metaphases to perform chromosomal analysis, cells were treated with trypsin followed by Giemsa staining. Chromosomal aberrations were examined and analyzed by cytovision, (Leica) according to the International System for Human Cytogenetic Nomenclature (ISCN) (22) and the Iraqi Center for Cancer and Medical Genetic Research method.

Results

The results of chromosomal analysis for patients with AML revealed the presence of abnormalities on chromosomes 5 and 8 within the complex karyotype. Regarding chromosome 5 abnormalities were detected in 16 (53.33%) of patients with AML out of 30, 13 male and 3 female, as shown in Table 1. Different types of abnormalities were diagnosed, including deletion 5q and monosomy 5 in 6 patients (20%) for each and trisomy 5 in 4 patients (13.33%). Deletion 5q was detected with high frequency in newly diagnosed patients with AML followed by the on treatment and relapsed patients' groups (3, 2 and 1 respectively) as depicted in figure1. Monosomy 5 was found with high frequency in AML patients on treatment followed by newly diagnosed and relapsed AML patients, (3,2 and 1 respectively). Figure 2 shows a complex karyotype of male AML involving monosomy 5. Chromosome 5 Trisomy was detected in one patient for each group of AML patients. Figure 3 shows the G-banding complex karyotype for peripheral blood lymphocytes in male AML patient with trisomy 5.

Chromosome 8 abnormalities were detected with lower frequencies in patients with AML compared to chromosome 5 abnormalities, which were diagnosed in 12 (40%) of 30 patients, 9 males and 3 females, as shown in Table 2. Types of abnormalities diagnosed, including deletion 8q with the highest frequency detected in 6 patients (20%) followed by derivative 8 in 4 patients (13.33%) and trisomy 8 in 2 patients (6.66%). Deletion 8q was detected with high frequency in relapsed patients with AML followed by on treatment and newly diagnosed patient groups (3, 2 and 1 respectively) as shown in figure 4. Regarding derivative chromosome 8, it was diagnosed in 4 patients, 3 relapsed, and 1 AML on treatment patient (Figure 5). The trisomy of chromosome 8 was detected in one patient in both relapsed and on treatment patient (Figure 6).

Table (1): Chromosomal abnormalities of Chromosome 5 in groups of patients with AML.

Chromosome5 Abnormality type n (%)	AML Patients Groups N=30			Total n (%)	Sex n (%)	
	Newly diagnosed n (%)	Treated n (%)	Relapsed n (%)		Male	Female
Deletion 5q n=6 (20%)	3 (10%)	2 (6.66%)	1 (3.33%)	16 (53.33%)	13 (81.25%)	3 (18.75%)
Monosomy 5 n= 6 (20%)	2 (6.66%)	3 (10%)	1 (3.33%)			
Trisomy 5 n= 4 (13.33%)	1 (3.33%)	2 (6.66%)	1 (3.33%)			

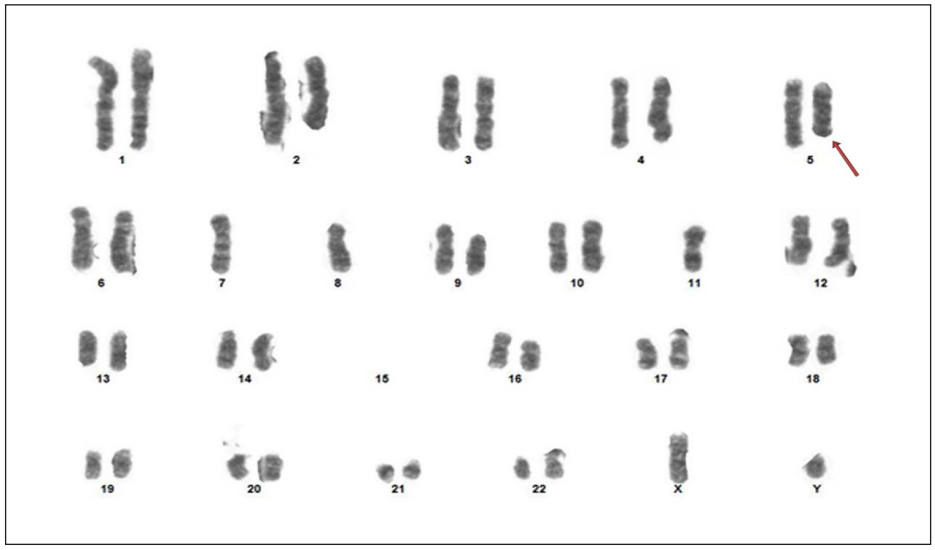

Figure 1: The G-banding karyotype of peripheral blood lymphocyte in male patient with AML revealed a karyotype of : del(5q), -7, -8, -15, the red arrow pointed to the deletion of 5q , (100X) magnification .

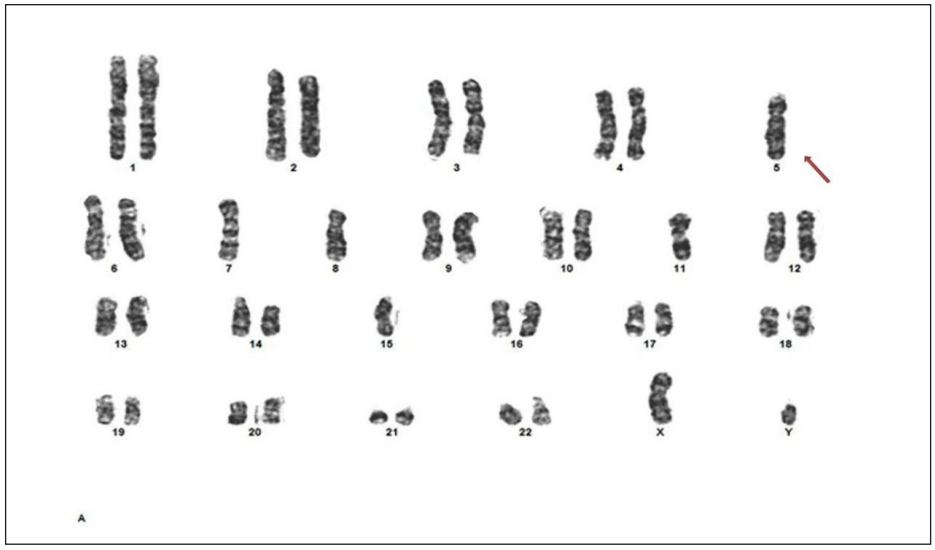

Figure. 2: The G-banding karyotype of peripheral blood lymphocytes in a male patient with AML revealed -5,-7,-8,-11,-15, the red arrow pointed - 5, (100X) magnification.

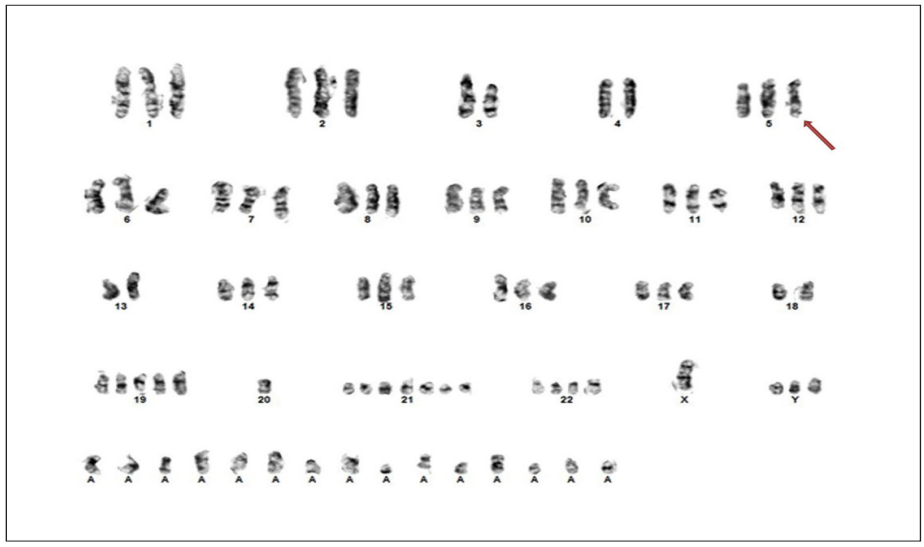

Figure 3 : G-banding karyotype of peripheral blood lymphocyte of male patient with AML revealed trisomy 1,2, 5,6,7,8,9,10,11,12,14, 15,16,17,Y, tetrasomy 19,22 and heptasomy 21, deletion of a copy of chromosome 20 , in addition to unknown markers . The red arrow pointed to trisomy 5 , (100X) magnification.

Table (2): Chromosomal abnormalities of Chromosome 8 in the groups of patients with AML.

Type of chromosome 8 abnormality n (%)	AML patients' groups n=30			Total n (%)	Sex n (%)	
	Newly diagnosed n (%)	Treated n (%)	Relapsed n (%)		Male	Female
Deletion 8q n=6 (20%)	1 (3.33%)	2(6.66%)	3 (10%)	12 (40%)	9 (75%)	3(25%)
Derivative 8 n=4 (13.33%)	-	1 (3.33%)	3 (10%)			
Trisomy 8 n=2 (6.66)	-	1(3.33%)	1(3.33%)			

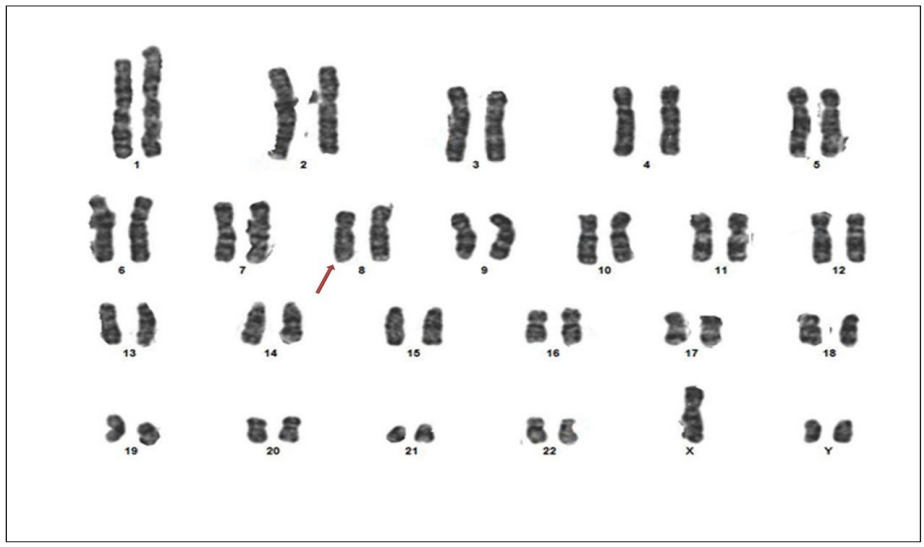

Figure.4: G-banding karyotype of peripheral blood lymphocyte of male patient with AML revealed to the deletion of 8q (red arrow), (100X) magnification.

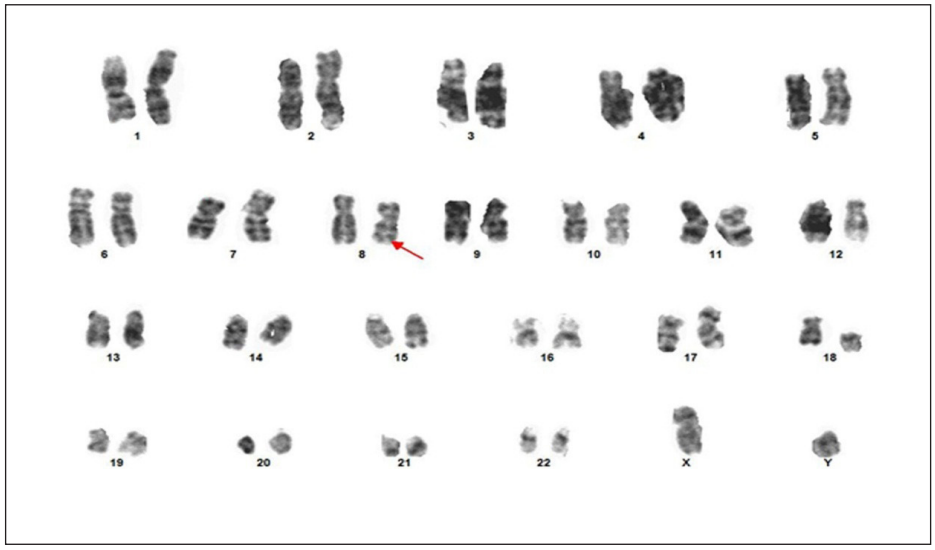

Figure .5: G-banding karyotype of peripheral blood lymphocyte of a male patient with AML revealed derivative 8 (red arrow), (100X) magnification.

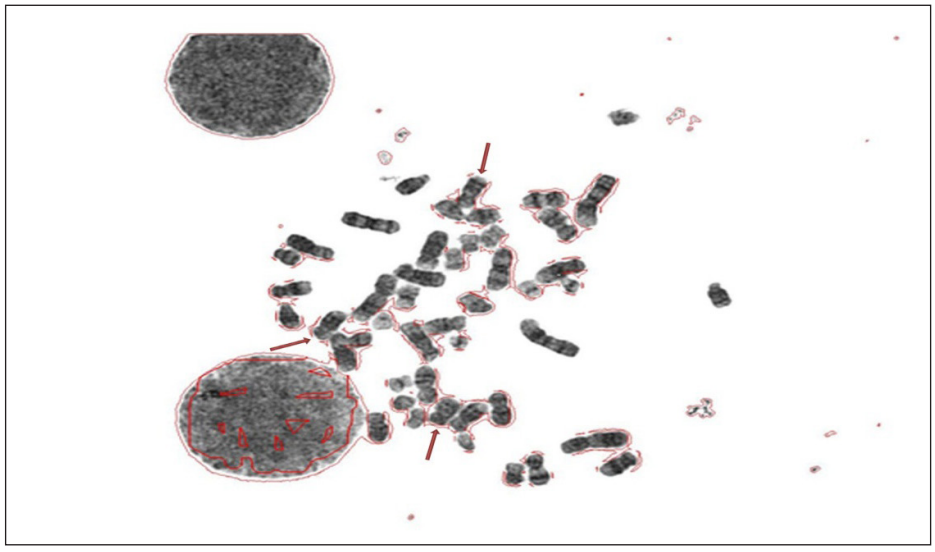

Figure. 6 : G-banding metaphase of peripheral blood lymphocyte of a male patient with AML revealed trisomy chromosome 8 (red arrows), (100X) magnification.

Discussion

The results of this study revealed numerical and structural abnormalities of both chromosomes: 5 and 8 in 16 (53.33%) and 12 (40%), respectively, within a complex karyotype and high frequencies in numerical chromosome abnormalities for chromosome 5 and structural abnormalities for chromosome 8. The highest frequency of abnormalities of chromosomes 5 was detected in both newly diagnosed and on treatment AML patients' groups followed by relapsed patients, while abnormalities of chromosomes 8 were detected in relapsed AML patients followed by on treatment patients and finally newly diagnosed AML patients. Chromosomal abnormalities involving chromosomes 5 and 8 were reported in previous studies, as a sole abnormality or within the complex karyotype (23- 26).

Chromosome 5 contains an important gene involved in leukemogenesis and may be associated with rapid disease progression and poor outcome. Among these genes: transcriptional regulator(s) of telomerase reverse transcriptase (TERT), where reactivation is a critical stage in carcinogenesis (20, 27). The deletion of 5q leads to the loss of a single allele of more than one gene on 5q that can act in concert to alter hematopoiesis, promote self-renewal of hematopoietic stem and progenitor cells (HSPCs), induce apoptosis of hematopoietic cells, and disrupt differentiation (27,28). Cell division cycle 25c (*CDC25C*), early growth response protein 1(*EGR1*), heat shock protein 9(*HSPA9*),catenin

alpha1(*CTNNA1*), and diaphanous homology1 (*DIAPH1*) , genes located on the chromosome 5q, can be implicated and contributed in high-risk myelodysplastic syndrome (MDS)/AML, and the tumor suppressor *CNNTA1* on the long arm of chromosome 5, was demonstrated in primary osteosarcoma and breast cancer cells and it was documented that patients who have del 5q showed higher mortality rates compared to loss of a chromosome 5 (28, 29) .

Trisomy of chromosome 8, among the most common trisomies in AML which may be found as a sole aberration or with other karyotype abnormalities (30, 31). AML with trisomy 8 is linked to mutations in DNA methylation genes, spliceosome complex genes, and myeloid transcription factor genes. These changes likely have a more significant impact on the development, management, and prognosis of leukemia than the presence of trisomy 8 alone (32). As many oncogenes required for leukemogenesis may be amplified and cause an increasing dose of some oncogenes, such as *c-myc* (proto-oncogenes) (8q24), *c-mos* (8q22) and *ETO* (8q22) with a significant role in leukemogenesis (30,32). Trisomy 8 leads to gene mutations, such as CCAAT enhancer binding protein alpha (*CEBPA*) and nucleophosmin 1 (NPM1) (30, 31), which act as class II mutations in collaboration with class I alterations occurring as a result of the chromosomal abnormality (30, 31). In addition, a previous study showed that *NPM1* and *FLT3* (members of receptor tyrosine kinase) mutations were negative in the majority of patients with trisomy 8 (30). Chromosome 8 abnormalities associated with *RUNX1*, *ASXL1*, *JAK2*, and *TET2* genes, which mutated at high frequencies, resulting in leukemic pathogenesis, thus AML with trisomy 8 is classified as intermediate-risk AML (33, 34). The results of our study show the importance of complete karyotype analysis as a means of elucidating the AML biological entities.

Conclusions

Chromosomal aberrations involving chromosomes 5 and 8 are linked to the development and prognosis of AML and the presence within a complex karyotype may lead to disease progression and the worst prognosis, especially for abnormalities of chromosome 5 that were detected at highest frequencies compared to abnormalities of chromosome 8. Future molecular genetic testing that accompanies complete karyotype analysis should be conducted to improve the diagnosis and management of patients with AML.

Ethical approval

The study followed the ethical standards mentioned in the Declaration of Helsinki. The Ethics Committee of the Iraqi Ministry of Health and Environment accepted the study protocol (document No.1416, dated 19/5/2019). Patients provided their verbal and written consent to participate in the study before samples and clinical data were collected.

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Authors contribution

All authors have made equal contributions to this work.

Conflict of interest

The author has no conflicts of interest.

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