Cytogenetic studies of Acute Myeloid Leukemia

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Abstract

Acute myeloid leukemia (AML) describes as a group of hematopoietic stem cell disorders characterized by expansion of undifferentiated myeloid progenitors. Acquired chromosomal anomaly particularly reciprocal translocations constitute one of the major events contribute to leukemogenesis.

Patient and Methods:
45 untreated, newly diagnosed patients with de novo AML were enrolled in the present study and subjected to cytogenetic analysis. Four ml of heparinized peripheral blood were collected for 72 hours synchronized culture, and then chromosome G- banding analysis was performed using standard methods. The karyotypes were designated according to the International System for Human Cytogenetic Nomenclature (ISCN). The collected data were analyzed statistically.

Result:
Cytogenetic analysis and karyotype results were obtained in 45 patients with de novo AML. Males constituted 33.3%, and females constituted 66.7% of this group. The patients' age ranged from 17-60 years. Chromosomal anomalies have been detected in 21 out of 45 patients (46.7%). However five different types of chromosome anomalies have been detected; where seven cases (33.3%) carrying t(15;17)(q22;q21); six cases (28.5%) carrying t(8;21)(q22;q22); three cases (14.3%) had trisomy 8; three cases (14.3%) had monosomy 7; and lastly two cases (9.5%) carrying inv(3)(q21q26).

Conclusion:
Conventional cytogenetic analysis reliability detects chromosomal abnormalities in AML patients at the time of diagnosis. Chromosomal anomalies detected in Egyptian AML patients, are similar to some extent to those recorded in other areas of the world.
Key words: cytogenetic, myeloid leukemia, karyotypes , trisomy, monosomy

Introduction:

Acute myeloid leukemia (AML) represents a hematopoietic malignancy characterized by abnormal cell proliferation and stalled differentiation leading to the accumulation of immature cells in bone marrow, in peripheral blood and eventually in other tissues (Schlenk, *et al*., 2008). AML are greatly variable in clinical course and response to therapy as well as in the genetic and molecular basis of their pathology (Mrozek & Bloomfield, 2006).

The FAB (French-American-British) doctors have classified AML into subtypes (M0 through M7) based on the predominant line of differentiation, and the cell maturity.

FAB classification has limited prognostic value, whereas certain recurrent chromosomal abnormalities, prior to drug exposure and a history of a myelodysplastic syndrome are predictive of outcome (Kumar *et al*., 2007). One the other hand, the world health organization (WHO) classification of AML incorporates clinical, morphologic, immunophenotypic and genetic features in an attempt to define entities that are biologically homogeneous and have clinical relevance. The classification encompasses four major categories; AML with recurrent genetic abnormalities, AML with multilineage dysplasia, AML that is therapy related and

Assessment of

AML not otherwise categorized (Schnittger *et al.*, 2005).

At the cytogenetic level, clonal chromosome aberrations are identified in approximately 50% of all patients with de novo adult acute myeloid leukemia (Akagi *et al.*, 2009). These chromosomal abnormalities provide important insights into the genes involved in the pathogenesis of leukemia. Clinically, chromosome aberrations are one of the most important prognostic factors in AML. This prognostic information is increasingly being used for treatment decisions, eg, whether a patient is assigned to an intense therapy such as bone marrow or blood stem-cell transplantation or to a less intense regimen. The remaining half of the patients lacks microscopically visible chromosome aberrations. For this clinically heterogeneous group of patients, prognostic markers are warranted for establishing risk-adapted therapy (Do¨hner, *et al*. 2000).

Chromosome translocations constitute large proportion of the chromosomal anomalies, while other rearrangements i.e .inversions, deletions occur in less than 3%of cases (Godley, 2009). Frohling *et al*. (2002) has reported that in most cases of AML, conventional cytogenetic study reliably detects chromosomal abnormalities and this method should not be replaced by fluorescent *in situ* hybridization (FISH). FISH should be used as a complementary method for the detection of more subtle abnormalities. Furthermore, molecular

Patient and Methods:

45 patients; diagnosed as *de novo* AML, with no previous treatment, were subjected to this study. Patients have been collected, with formal consent, from the following sites: Clinical Pathology Department, Sews Canal University, Kasr El-Einy Center of Radiation, Oncology and Nuclear Medicine, Cairo University and Clinical Hematology Unit in El Maady Military Hospital, Cairo - Egypt

cytogenetic using this comprehensive set of DNA probes provides a valuable diagnostic tool for patients with poor chromosome morphology, low or no yields of metaphase cells. Cytogenetic aberrations have been recognized as the most important prognostic variable in AML. In recent years, there has been growing interest in moving beyond cytogenetic to subsets of AML with molecular aberrations (i.e., mutated or abnormally expressed genes) that can be used for further stratification of AML patients (Renneville *et al.*, 2008).

On the other hand, AML with no chromosomal abnormalities that are detected by conventional karyotyping and FISH techniques, are classified as AML not otherwise categorized. This patient group falls into the intermediate prognostic group, but individual patients can have substantially different clinical courses. Identification of prognostic markers suitable for further subdivision of this group will help to optimize their treatment. Molecular analysis has yielded novel gene mutations that serve as markers important for prognostic stratification of this group of AM (Löwenberg, 2008).

Major advances in the understanding of leukemogenesis have been made by the characterization and the study of acquired cytogenetic abnormalities particularly reciprocal translocations, as well as some gene mutations observed in AML (Renneville *et al.*, 2008).

Methods:
- **Data collection:**
  Patient personal data, the results of hematological examinations (complete blood count; hemoglobin level, white cell count (WBCs), platelets count, peripheral blast cells), immunophenotyping of myeloid cells by flow cytometry (CD13/DR, CD14, CD15, CD33, CD34, CD64), and the cytochemical analysis of the WBCs have been collected.

- **Cytogenetic analysis**

    Four ml of heparinized peripheral blood were collected for 72 hours synchronized culture, and then chromosome G- banding analysis was performed using standard methods. The karyotypes were designated according to the International System for Human Cytogenetic Nomenclature (ISCN) (Mitelman, 1995). The collected data were analyzed statistically.

Result:

Cytogenetic analysis and karyotype results were obtained in 45 patients. However, our study population revealed that 24 patients of 45 (53.3%) had normal karyotypes and 21 patients of 45 (46.7%) had abnormal karyotypes. Males constituted 33.3% of this group and females constituted 66.7%. Patients' age ranged from 17-60 years. Age and sex distribution of our studied population with normal karyotype are shown in (table 1).

However, five different types of chromosomal abnormalities among the 21 cases have been detected; and are classified as follow: seven cases (33.3%) carrying t(15;17)(q22;q21) (figure 1); six cases (28.5%) carrying t(8;21)(q22;q22) (figure 2); three cases (14.3%) had trisomy 8 (figure 3); three cases (14.3%) had monosomy 7 (figure 4); and lastly two cases (9.5%) carrying inv(3)(q21;q26) (figure 5).

		Number	Percentage
Sex	**Male**	8	38.1%
	Female	13	61.9%
Age	**Mean ± SD**	36.21 ± 12.4	
	Range	17 - 60	

Table 1: *Distribution of sex and age in patients with AML-NK (n=24).*

Assessment of
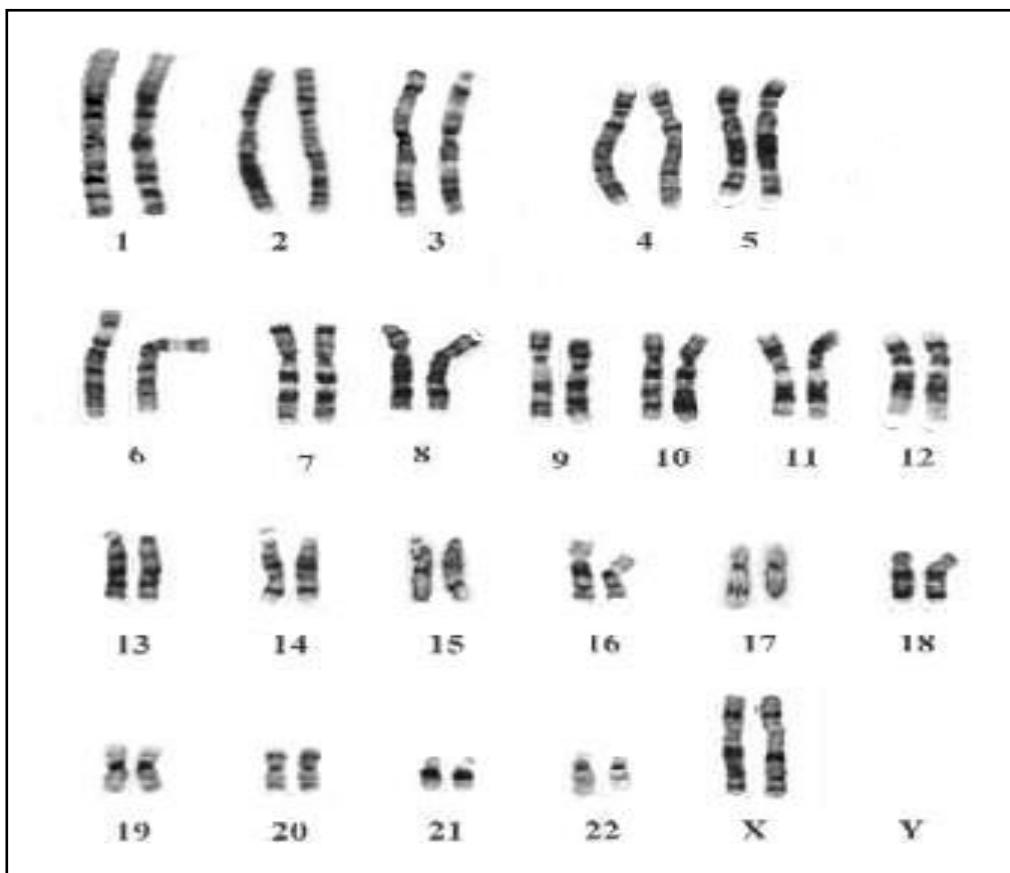
(Fig. 1): 46,XX,t(15;17)(q22;q21)
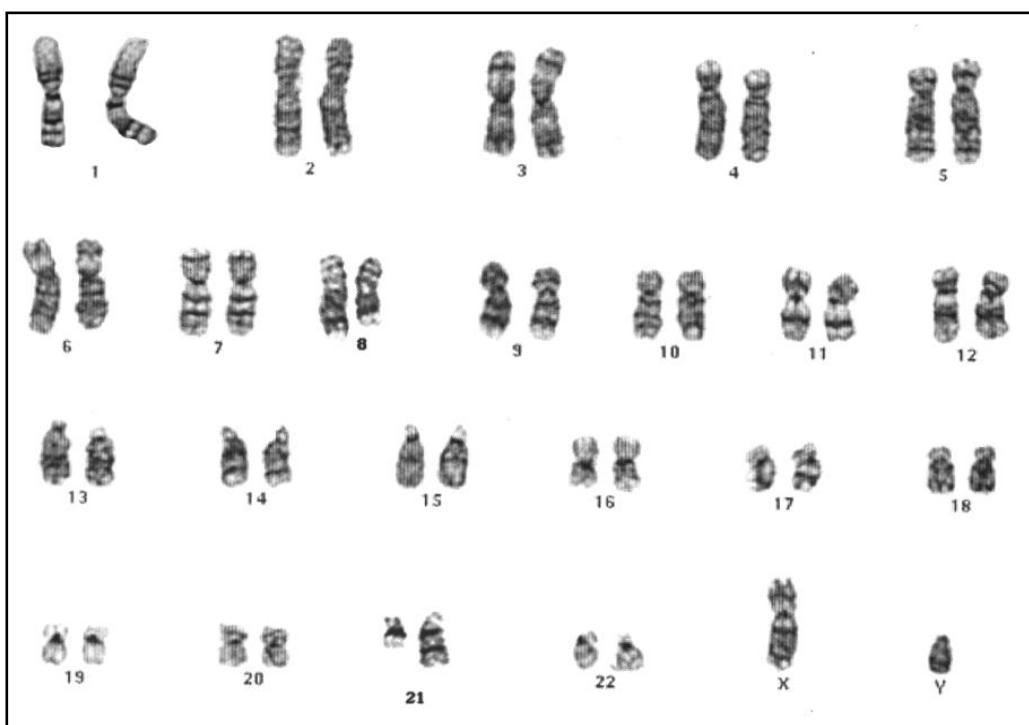
(Fig. 2): 46,XY,t(8;21)(q22;q22)
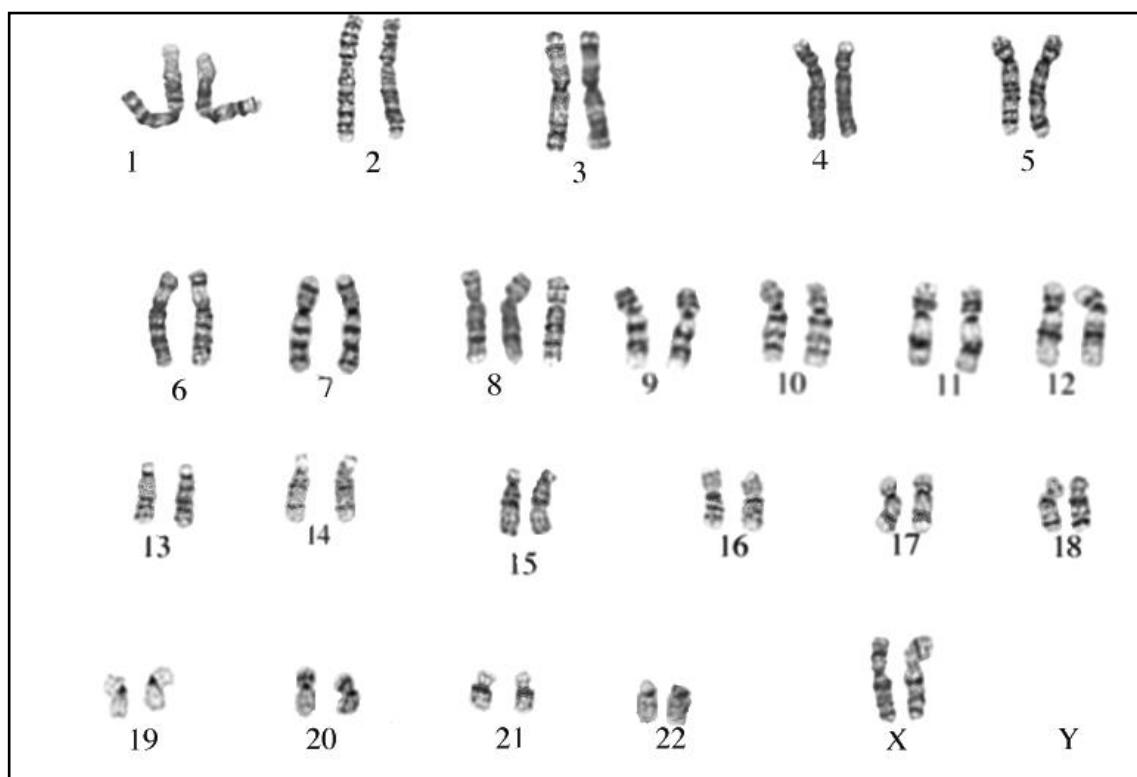
(Fig. 3): 47,XX,+8
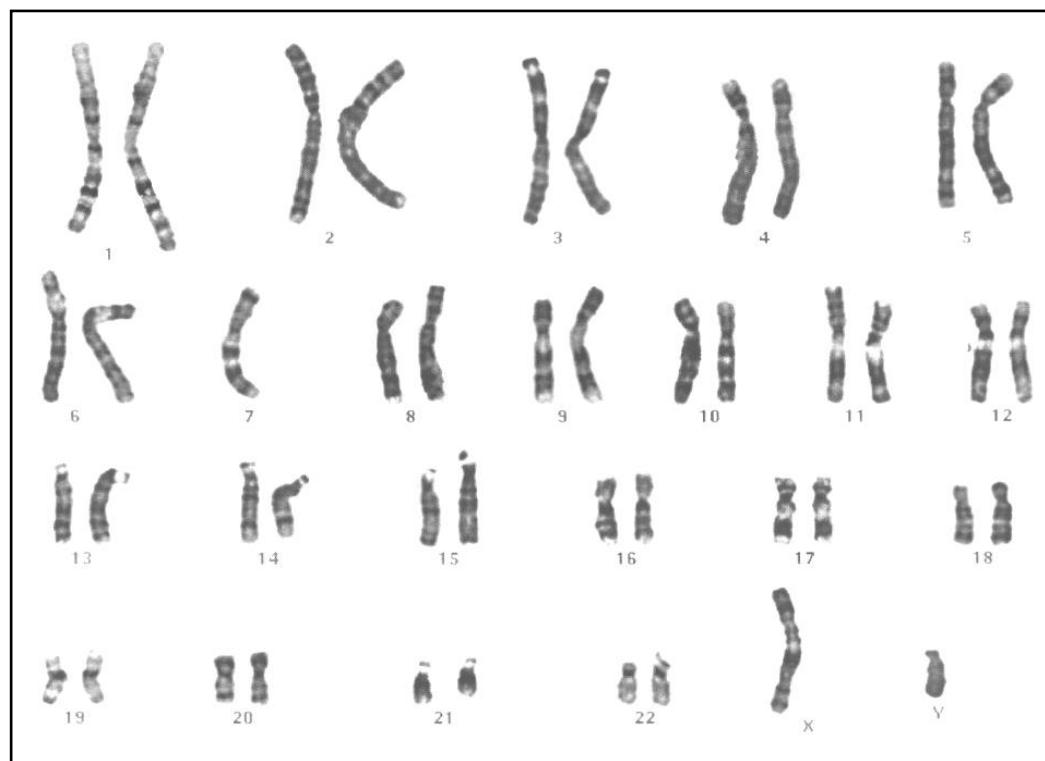
(Fig. 4): 45,XY,-7

(Fig. 5): 46,XY,inv(3)(q21;q26)

Discussion:

Acute myelogenous leukemia represents a hematopoietic malignancy characterized by abnormal cell proliferation and stalled differentiation leading to the accumulation of immature cells in the marrow itself, in peripheral blood and eventually in other tissues. Karyotype is generally an important prognostic factor in AML; a favorable prognosis being associated with minor karyotypic changes, low frequency of abnormal bone marrow cells and changes specifically involving the *CBF* gene. While a poor prognosis is associated with a monosomy 5, monosomy 7, trisomy 8, abnormalities in the 3q and changes specifically involving the *MLL* gene. Atypical cytogenetic findings have been sporadically reported in AMLM4 but the scarcity of these abnormalities poses a challenge of using such changes as prognostic factors, reinforcing the need for the collection of clinical data on rare events

(Strout *et al.*, 1999; Chen and Sandberg, 2002).

Primary chromosomal abnormalities in AML are highly specific and considered to be associated with leukemic transformation, whereas secondary changes are less specific and probably contribute to disease progression. The common chromosomal abnormalities that have been recognized in the acute myelomonocytic leukemia include monosomy 5 or del(5q), monosomy 7 or del(7q), trisomy 8, t(6;9)(p23;q34), and rearrangements involving the Mixed Lineage Leukemia (MLL) gene mapped at 11q23 [del(11)(q23); t(9;11)(p22;q23), t(11;19)(q23;p13)], and Core Binding Factor B (CBF) mapped at 16q22 [del(16)(q22), inv(16)(p13q22), t(16;16)(p13;q22)]. Less frequently, trisomy 4, trisomy 22, t(8;21)(q22;q22) and rearrangements with breakpoints in 3q21, 3q26, 8p11, 11p15 and 11q13 have also been reported in FAB type M4 patients (Fett-Conte *et al*, 2007).

Our chromosomal analysis result showed that 53.3% of patients had normal karyotype. However, great variation have been detected in other studies; where, Garzon *et al*. (2007) has detected 50%, but Alcalay *et al*. (2005) has detected 89.6%, and lastly Frohling *et al*. (2005) has detected 45% of cases in their studies with normal karyotype. The discrepancy between the different results could be due to difference in the study population number, and/or due to geographical gene variation among normal population.

The prevalence of chromosomal abnormalities was estimated as 46.7% in the rest of patients. Translocation (15;17) has been frequently observed (33.3%) in our results; that in turn is more or less similar to the result that was observed by . Gutiérrez-Angulo *et al*. (2004) among AML patients in his pilot study. Translocation (15;17) results in fusion of parts of the PML gene on chromosome 15q22 and the RARA gene on chromosome 17q12. The resulting fusion protein blocks differentiation at the promyelocyte stage. According to the two hit model hypothesis of leukemogenesis, t(15;17) is one of class 2 mutations which blocks differentiation. Mrozek and Bloomfield (2006) have reported that all AML patients with t(15;17)(q22;12-21) have excellent prognosis.

On the other hand, t(8;21) translocat the AML1 gene on chromosome 21 and fused it to the ETO gene on chromosome 8, results in an AL1-ETO fusion protein which in turn will inhibit the expression of CBF target genes resulting in blockage of myeloid differentiation. So that, t(8;21) can consider as one of the class 2 mutations involved in leukemogenesis. Additionally, trisomy 8 is frequently seen with AML. Association between trisomy 8 and leukemia may be due to higher expression of ETO gene due to increased copies of chromosome 8 (Gutiérrez-Angulo *et al.*, 2004).

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طارق عبد الله عطيه

قسم الهستولوجيا- كلية الطب-جامعة –الازهر- القاهرة

تعد سرطانات الدم الميلودية الحادة مجموعة من اضطرابات الخلايا الجزعية لمكونات الدم والتي تتسم بتوسع في الأسلاف الغير مميزة للخلايا النخاعية. تمثل حالات الشذوذ الكروموسومى المكتسبة وخاصة حالات تبادل المواقع عبر الجينات واحدة من أكبر الأسباب المؤدية إلى سرطانات الدم. بجانب هذه الأسباب نجد طفرات الجينات تمثل عامل هام في نشوء مرض سرطان الدم و قد أثبتت الدراسات أن نسبة 45-50% من مرضى سرطان الدم الميلودي الحاد. الميلودي الحاد لم يكتشف لديهم شذوذ كروموسومى.

المرضى و طرق العمل:

قد أجريت هذه الدراسة على 45مريضا بسرطان الدم الميلودي (ممن لم يسبق علاجهم)، تم سحب عينة دم (4 مل) لعمل تحليل للكروموسومات. تم عمل تحليل الكروموسومات بعد 72 ساعة من زراعة الخلايا, وتم تحليل النتائج احصائيا

النتائج:

تم الحصول على التحليل الكروموسومى من زراعة خلايا الدم لخمس وأربعين مريضا. أسفرت النتائج عن وجود 24 مريضا من 45 (53.3%) يحملون نمط كروموسومى طبيعي, و 21 مريضا من 45 (46.7%) يحملون شذوذ النمط الكروموسومى، حيث تم اكتشاف 5 أنواع لشذوذ النمط الكروموسومى.

الاستنتاج:

نستخلص من هذه الدراسة أن الوسائل التقليدية لدراسة الكروموسومات توضح أنماط الشذوذ الكروموسومى في حالات سرطان الدم الميلودي الحاد.