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The Vital Role of Karyotyping Techniques in Diagnosing and Subtyping Leukemia Disorders: Insights and Implications

Maati. M. Elghuol [a,b], Haloom Abdelsalam ELhashmi[c] ,Saad Salem Alakkari[a ,b] , Khalifa Khalil khalifa[a,b] , Omar Habouni [a,b]

[a] Molecular Diagnostics Department, Faculty of Biomedical Science, University of Benghazi, Benghazi, Libya.
[b] Research and Consulting Department, Faculty of Biomedical Science, University of Benghazi, Benghazi, Libya.
[c] Pediatric department, Faculty of Medicine, University of Benghazi, Benghazi, Libya.

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ABSTRACT

Leukaemia is a group of malignant neoplasms that arise from altered hematopoietic cells, leading to a diverse range of complex and heterogeneous diseases. Acquired chromosomal aberrations, including deletions, translocations, and amplifications, contribute to the emergence of various subtypes of leukaemia. As a result, karyotyping has become a crucial tool for diagnosing and classifying different forms of leukaemia. This study utilized the chromosomal G-banding method, a cross-sectional approach, to examine the karyotype of peripheral blood samples from five Libyan patients with leukaemia at the Benghazi Pediatric Hospital. The analysis successfully revealed several chromosomal abnormalities, and the patients were classified into the subclasses of B-ALL and AML leukaemia disorders. These findings highlight the significance of karyotyping in diagnosing and predicting leukaemia. Furthermore, this research illustrated how precise karyotyping analysis can provide invaluable information that can ultimately improve patient outcomes and treatment strategies.

الدور الحيوي لتقنيات Karyotyping في تشخيص وتصنيف امراض سرطان الدم: رؤى و آثار

معاطي مصباح الغول[1.2] و حلوم عبد السلام الهاشمي [3] و سعد سالم العكاري[1.2] و خليفة خليل خليفة[1.2] د. عمر الحبوني[1.2]

[1] كلية العلوم الطبية الحيوية, جامعة بنغازي, بنغازي - ليبيا
[2] قسم البحوث والاستشارات ، كلية العلوم الطبية ب الحيوية ، جامعة بنغازي – ليبيا
[3] قسم طب الأطفال، كلية الطب البشري ، جامعة بنغازي – ليبيا

الكلمات المفتاحية:
تقنيات التنميط النووي
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مستشفى بنغازي للأطفال

الملخص

سرطان الدم هو مجموعة من الأورام الخبيثة التي تنشأ من الخلايا المكونة للدم المتغيرة، مما يؤدي إلى مجموعة متنوعة من الأمراض المعقدة وغير المتجانسة. تساهم الانحرافات الكروموسومية المكتسبة، بما في ذلك عمليات الحذف والانتقال والتضخيم، في ظهور أنواع فرعية مختلفة من سرطان الدم. ونتيجة لذلك، أصبحت تقنية Karyotyping أداة حاسمة لتشخيص وتصنيف الأشكال المختلفة من سرطان الدم. استخدمت هذه الدراسة طريقة النطاق G الكروموسومية، وهي طريقة مقطعية، لفحص Karyotyping عينات الدم المحيطية من خمسة مرضى ليبيين مصابين بسرطان الدم في مستشفى بنغازي للأطفال. كشف التحليل بنجاح عن العديد من التشوهات الكروموسومية، وتم تصنيف المرضى إلى فئات فرعية من اضطرابات سرطان الدم B-ALL وAML. تسلط هذه النتائج الضوء على أهمية تقنية Karyotyping في تشخيص سرطان الدم والتنبؤ به. علاوة على ذلك، أوضح هذا البحث كيف يمكن لنتائج تقنية Karyotyping الدقيقة أن توفر معلومات لا تقدر بثمن، يمكنها في النهاية ان تؤدي الي تحسين نتائج التشخيص وايضا اختبار استراتيجيات العلاج المناسب للأنواع الفرعية لسرطان الدم.

1. Introduction

Cytogenetic analysis, such as karyotyping, is a specialized area of genetics that is critical in diagnosing different leukaemia subtypes. This powerful technique involves a deep study of the structure and function of chromosomes, providing an intricate understanding of the

*Corresponding author:
E-mail addresses: maati.elghool@uob.edu.ly , (H. ELhashmi) haloomelhashmi22@gmail.com , (S. Alakkari) Saadakire@gmail.com , (K. Musmari), Kelmusmari@gmail.com , (O. Habouni) RoyHabouni@gmail.com
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complex molecular genetic fundamentals and shedding light on the pathogenic mechanisms associated with leukaemia disorders. [2], [14] Leukaemia comprises a range of neoplasms originating from malignant transformation of bone marrow cells, resulting in diverse complications affecting hematopoiesis and the immune system. [9] ,[15] These types of cancer impact the peripheral blood, bone marrow, liver, spleen, and lymph nodes. The World Health Organization (WHO) classification (2008), developed by haematologists worldwide, is the most widely accepted classification system for leukaemia disorders. This classification categorizes leukaemia into various subtypes based on distinct molecular genetic characteristics. [3] Therefore, cytogenetic methods are utilized for accurate diagnosis and identification of different subtypes of leukaemia.

A. Acute lymphoblastic leukaemia (ALL).

Acute lymphocytic leukaemia (ALL) is a malignant disease that arises when a lymphoid progenitor cell undergoes genetic alterations and uncontrolled proliferation. ALL can develop at any stage of life, with approximately 75% of cases occurring in children under 6 years old. It is slightly more common in males. [24] The precise cause of acute lymphocytic leukaemia (ALL) remains incompletely elucidated. Several contributory factors, encompassing genetic, environmental, maternal nutritional status, viral infections, history of malignancy, and exposure to chemotherapy, have been noted to influence the pathogenesis of ALL subtypes. [4], [18] and [23]

From the clinical perspective, the most prevalent cytogenetic abnormality observed in adult acute lymphoblastic leukaemia (ALL) is the chromosomal translocation t(9;22) (q34;q11), giving rise to the BCR/ABL1 fusion gene, known as the Philadelphia chromosome. Patients with this genetic anomaly often present with aggressive disease characteristics resistant to conventional chemotherapeutic interventions. [7], [8]

B. Acute Myeloid Leukemia (AML)

Acute myeloid leukaemia (AML) constitutes a perilous hematologic malignancy characterized by the uncontrolled proliferation of myeloid blasts within the bone marrow, peripheral blood, or other tissue compartments. The clinical diagnosis is established upon the identification of ≥20% blast cells within the bone marrow or peripheral blood. [19, 29] Certain specific clonal cytogenetic abnormalities, such as t(8;21) (q22;q22), inv(16) (p13;q22), t(16;16) (p13;q22), and t(15;17) (q22;q12), serve as diagnostic markers for AML, irrespective of the blast count. AML may manifest spontaneously or as a progression from a pre-existing hematologic disorder, such as myelodysplastic syndrome (MDS). [12], [13] However, the following factors are considered to pose a risk: exposure to radiation, benzene, or chemotherapy drugs (such as alkylating agents); previous haematological disorders (such as aplastic anaemia); inherited genetic conditions; and chromosomal abnormalities associated with certain syndromes, such as Down syndrome (trisomy 21). [14], [20]

C. Chronic myeloid leukaemia (CML)

Chronic myeloid leukaemia (CML) is a malignant disorder affecting hematopoietic stem cells, resulting in pronounced myeloid hyperplasia within the bone marrow. [16] Disruption of hematopoiesis stems from the BCR-ABL fusion gene, a consequence of translocation t(9;22), representing a well-established pathogenic determinant in CML. Despite treatment with a tyrosine kinase inhibitor, the 'chronic phase' of CML may evolve into an 'accelerated' or 'blast' phase in 5% - 10% of patients, provoking the onset of acute myeloid or acute lymphoblastic leukaemia (ALL). [28]

Chronic myeloid leukaemia (CML) is anticipated to constitute approximately 15% of new leukaemia cases in the United States in 2021. The incidence of CML is highest among individuals aged 65 to 74, although it can affect individuals of all age groups, with a slight male predominance. Exposure to ionizing radiation, particularly at higher doses, stands as the sole established risk factor for the development of CML. [26]

D. Chronic lymphocytic leukaemia (CLL)

Chronic lymphocytic leukaemia (CLL) stands as the most prevalent form of leukaemia in the Western world, typically afflicting elderly patients with a clinically diverse progression. [6] This disease is characterized by the clonal expansion and aggregation of mature B-cells, generally CD5-positive, within the blood, bone marrow, lymph nodes, and spleen. The genesis of leukaemia stems from specific genetic alterations that impede the programmed cell death of clonal B-cells. [19] Approximately 80% of chronic lymphocytic leukaemia (CLL) patients exhibit acquired chromosomal abnormalities, with the predominant anomaly being deletions on the long arm of chromosome 13, particularly involving band 13q14 (del[13q14]), evident in roughly 55% of cases. An isolated del(13q14) is associated with a benign disease course. Furthermore, deletions of the long arm of chromosome 11 (del[11q]) are present in about 25% of chemotherapy-naïve patients with advanced disease stages and 10% of patients with early-stage disease. [5], [6] and [30]

Due to the high incidence of leukaemia in the Benghazi population and the crucial role of cytogenetics in diagnosing and predicting leukaemia subtypes, an opportunity arises to highlight the positive outcomes associated with diagnosis and differentiations of leukaemia subtypes. Currently, Benghazi City requires the critical use of accurate techniques for leukaemia diagnosis, such as the chromosome mapping technique (Karyotyping), which is accepted in many nations, for the initial diagnosis of different subtypes of leukaemia and to make this test available for the early diagnosis of infants or those who suspect or exhibit leukaemia-related symptoms. The similar features and associated leukaemia diseases may overlap between the various types of leukaemia disorders, making diagnoses based on clinical features inaccurate. Thus, diagnosis of leukaemia diseases, using a suitable technique such as the chromosomal mapping technique (karyotyping analysis), is crucial for managing leukaemia disorders effectively. This study emphasised the importance of understanding chromosomal abnormalities and explained how chromosomal banding techniques (Karyotyping) play a critical role in diagnosing and predicting the prognosis of various leukaemia subtypes.

2. Methodology

The study used specific cytogenetic techniques (chromosome mapping analysis) to diagnose and distinguish different subtypes of leukemia in patients at Benghazi Pediatric Hospital.

2.1 Materials and chemical reagents

Peripheral blood samples of leukemia patients from the Benghazi Pediatric Hospital

2.2 Chemical reagents

2.3 Procedure of Karyotyping

A. Media preparation
B. Blood Sample Preparation
C. Samples of culture and fixation

2.4 Samples Stanning

The procedure for karyotyping a leukaemia sample is as follows: after preparing the media and blood sample, the cells were cultured in RPMI-1640 medium for 3 days at 5% CO2. Next, the samples were exposed to colchicine and incubated for 30- 60 minutes at 37°C to arrest cell division at metaphase for visualizing chromosomes. Following this, the cells were treated with a hypotonic solution (KCl) and then a fresh cold fixative. The cell suspension was then dropped onto a clean slide and left to dry overnight. The slides were subsequently stained with Giemsa stain solution, washed with a buffer solution, and left to air dry. Finally, the chromosomes can be observed using a phase contrast lab microscope.

3. Results

In the current study, chromosomal aberrations (structural and/or numerical) were identified using chromosome banding analysis (CBA) with smart type software of karyotype. In addition, the ATLAS of GENETICS AND CYTOGENETICS website in oncology and haematology was used as a reference to compare the control (normal) karyotype with patient samples.

The findings of this study demonstrate the importance of karyotyping in the diagnosis and subtyping of leukaemia. The successful visualization of chromosomal abnormalities allowed for the accurate classification of leukaemia patients into the subclasses of B-ALL Figure (5). and AML leukaemia disorders Figure (2). These results emphasize the significance of precise karyotyping in providing essential information that aids in predicting disease, treatment strategies, and improving patient outcomes.

3.1 G-banded karyotype analysis of samples.

Figure (1): G-banded karyotype of the control sample

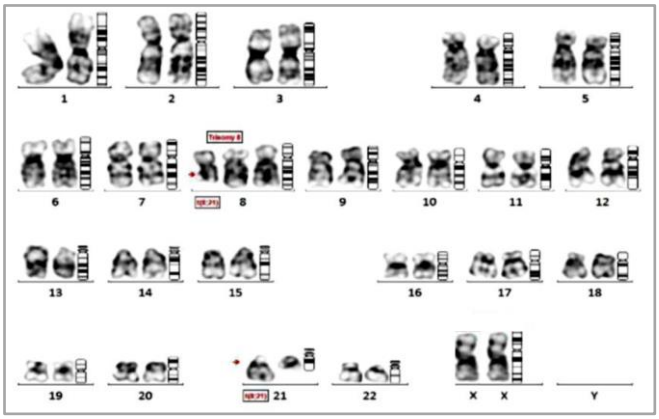

Figure (2): G-banded karyotype of female AML patient sample (Abnormal)

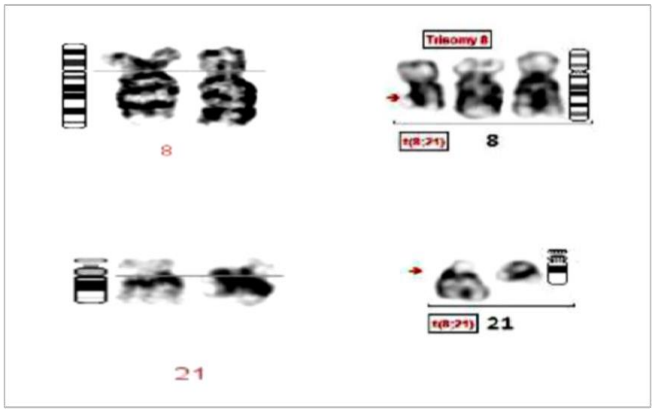

Figure (3): G-banded karyotype of AML alteration showing t(8;21) (q22;q22) with trisomy 8.

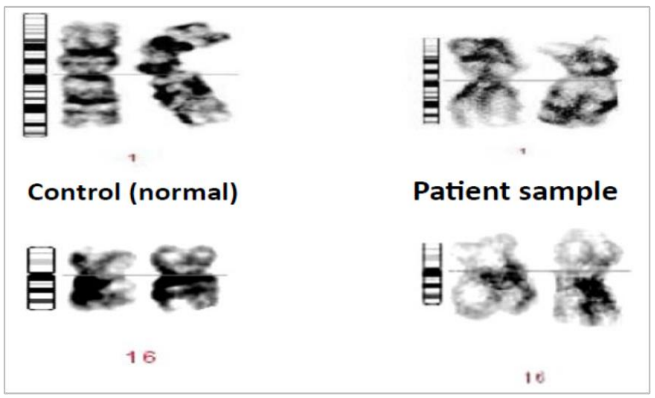

Figure (4): G-banded karyotype of AML alteration showing t(1;16)

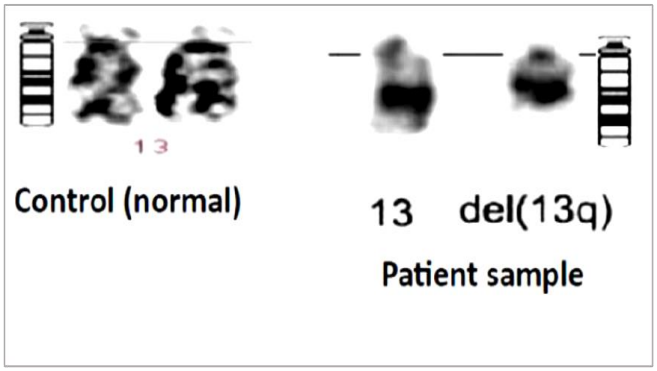

Figure (5): G-banded karyotype of leukaemia subtype ALL, showing del(13q) alteration

Acquired chromosomal aberrations are leading to the development of leukemia disorders. Deletions, translocations, and amplifications which are clonal changes in karyotype can be detected in most hematological malignant disorders.

The obtained results show that the patient sample, when compared to the normal control, has a number and structural abnormalities. The comparison revealed chromosomal alterations, including the detection of t(8;21) with trisomy 8, which is indicative of a subtype of leukemia (AML), as shown in Figures (2, 3). Additionally, an alteration in t(1;16) was also observed, as shown in Figure (4). Furthermore, another patient sample when compared to the normal control, has numerically normal chromosomes but exhibits structural abnormalities. The comparison revealed chromosomal alterations, including the deletion of 13 del(13q). This alteration indicates subtype ALL of the leukemia disease. A del(13q) chromosome is depicted in Figure (5).

4. Discussion

Leukaemia is a broad term for cancers of the blood cells, characterized by increased proliferation of leukocytes and their precursors. Various cellular and genetic abnormalities underlie leukaemia development, and cytogenetic analysis has been vital in uncovering these alterations. Among the common cytogenetic techniques used is karyotyping, which offers valuable insights into the genetic makeup of leukaemia-affected cells. By visualizing and analyzing the chromosomal composition of these cells, karyotyping provides essential information for diagnosing and classifying leukaemia subtypes.

Despite the promising of new molecular methods, classical cytogenetic methods along with FISH remain the reference test for many hematological neoplasms. Therefore, cytogenetic testing should be initially performed in all cases of leukaemia disorders. [4, 18]. These routine test helps differentiate between different types of leukaemia disorders. Karyotyping is a crucial diagnostic tool, that examines the chromosomes in the cells. Additionally, can help to determine the subtypes of leukaemia and the patient's prognosis. [10[, [21]

Based on the data from Benghazi Pediatric Hospital still doesn't have good information about the cytogenetic technique for early detection and diagnosis of different leukaemia subtypes. Additionally, how precise karyotyping provides essential information that aids in predicting disease behaviour, modifying treatment strategies, and improving patient outcomes in the Benghazi population.

In this context, the G-banding technique was used to examine the karyotypes of peripheral blood samples from five Libyan patients with leukaemia at the Benghazi Pediatric Hospital. This cross-sectional analysis aimed to study the chromosomal abnormalities in these patients and categorize them into specific leukaemia subtypes.

Many previous studies about haematological malignancy concluded that the chromosome translocation abnormality is considered a marker of the Leukemia (AML) subtype. [17[, [21] and [22]

Another important aspect of chromosomal abnormalities in leukaemia is the role of translocation alterations in the AML subtype. Many previous studies about haematological malignancy concluded that the chromosome translocation abnormalities are considered a marker of the Leukemia (AML) subtype. [2], [11]

The present study revealed that translocations involving chromosomal bands including t(8;21) are observed in one of the leukemia samples. Similar results were reported by Katsuya Yamamoto, et al (2015), who

demonstrated that the t(8; 21) translocation is specifically observed in the acute myeloid leukaemia (AML) subtype. as shown in Figure (3).

Further changes were identified as indicative of the subtypes of leukaemia (AML) in the samples under study. The patient's chromosomes showed structural abnormalities, including the presence of t(1;16). as shown in Figure (4).

The findings by Safaei, A., and Shahryari (2013). clearly shows that karyotyping can indeed detect multiple mutations in chromosomes. Hence, this continues to build and add to the importance of karyotyping in the diagnosis of different types of leukaemia disorders. In the present study, del(13q) in ALL is also detected. as shown in Figure (5). A del(13q) chromosome is found in approximately 2% of cases in both adult and pediatric disease at presentation. Up to 4% of cases may have some loss of 13q material, either due to full monosomy or unbalanced rearrangements. The frequency of chromosome 13 deletions is higher during relapse. [23], [27]

In Libyan society, leukaemia affects physical health, as well as psychological and social well-being. This work is the first of its kind in Benghazi City. It demonstrates the potential for applying karyotyping analysis to gain crucial insights for diagnosing, predicting, and intervening in therapy for leukaemia patients in Benghazi and beyond.

5. Conclusion

Despite the promising of new molecular methods, classical cytogenetic methods along with FISH remain the reference test for many hematological neoplasms. Therefore, cytogenetic testing should be initially performed in all cases of leukaemia disorders. Overall, the present study illustrates the applicability and effectiveness of karyotyping in the analysis of leukaemia-related chromosomal abnormalities. This information not only aids in understanding the underlying pathogenesis of leukaemia but also contributes to improved management and treatment approaches for these complex hematologic malignancies.

6. Limitation of study

A. The Libyan community's limited understanding of the importance of cytogenetic analysis has hindered the cooperation of patients, caregivers, and even doctors in providing blood samples.

B. Cytogenetic analysis can be difficult because it is difficult to obtain good quality and viable cells for analysis, especially in patients with low cellularity or poor sample quality. Inconsistent results can also be obtained when cultivating cells and obtaining Low-quality metaphase spreads.

C. Conventional cytogenetic analysis, such as karyotyping, has a limited ability to detect small-scale genetic alterations. As a result, subtle chromosomal abnormalities or genetic mutations may be missed. However, some techniques offer higher resolution and can help overcome this limitation, such as Fluorescence In Situ Hybridization (FISH) or array Comparative Genomic Hybridization (aCGH).

7. Recommendation of study

a. Cytogenetics is a basic diagnostic tool that is easy to learn and has a significant impact on early diagnosis and treatment decisions. Thus, People should be made aware of the importance of the karyotyping technique in the early detection of many cancers and genetic diseases, particularly leukaemia disorders.

b. Establishing a specialized cytogenetics laboratory in Benghazi city hospitals, with the necessary infrastructure, equipment, and trained personnel to perform cytogenetic analysis. This presents an opportunity to play a crucial role in demonstrating the positive impact of early leukaemia diagnosis and management.

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