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CYTOGENETIC STUDY OF ACUTE LYMPHOCYTIC LEUKEMIA WITH T (9;22)(Q34;Q11)

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ABSTRACT

Study samples were collected from (Nanakali Hospital) for hematology in Erbil / Iraq during the period from June 2018 until (March 2019). Including (60) patients (40 males and 20 females) with acute lymphoblastic leukemia (ALL), the study included 20 people from the control group (10 males and 10 females). The group of patients and the control age groups ranged between (1-15) years. 5 ml of blood was placed in tubes (lithium heparin) to make blood cell cultures for cytogenetics. Acute lymphocytic leukemia (ALL) is the most common type of malignancy in children. This type is more common in children. It is responsible for 75% of cases of leukemia in children, as well as affects adults, especially those over the age of 60 years, and the survival rate for children is more than adults and the cure rate reaches 90%. Males suffer from this disease more than females, the chromosomal aberrations that have been identified in childhood and have an important role in diagnosing, predicting and managing the disease. The aim of this study is to diagnose and detect the chromosomal abnormalities of patients with acute lymphocytic leukemia before receiving any treatment using Karyotype and FISH. In this study, the spatial transition between chromosome 9 and chromosome 22 [t (9, 22) (q34; q11)] was determined in 2 patients (3.3%). The number and percentages of patients with acute lymphocytic leukemia were also distributed according to gender, where the percentage of male patients was (66.6%), while the percentage of affected females was (33.3%). Therefore, the ratio of males to females in the study sample was (2).

***Keywords*:** Acute lymphoblastic leukemia, Chromosomal abnormalities, Karyotype, Fluorescence in situ hybridization.

*Research from Ph.D. thesis of the First author

Acute lymphocytic leukemia (ALL) is a neoplastic disease characterized by abnormal proliferation of immature lymphocytes. It is the most common type of hematoma ever diagnosed in children, and accounts for about 25% of cancer diagnoses among children under the age of 15 (Howlander *et al*., 2015). The karyotype is an important factor in the diagnosis of ALL; it is an independent predictor index with a direct influence on treatment choice. Chromosome transfers are often observed in leukemia and lymphoma, and are among the important cellular genetic factors involved in tumor formation. Chromosomal abnormalities have been detected by conventional cellular genetics as well as FISH, which is an important element in assessing the classification and predicting the results of all patients (De Braekeleer *et al.,* 2010).

Chromosome transfers play an important role in human cancer, especially in hematopoietic and lymphatic tumors (Mitelman *et al*., 2007). Chromosome transfers are a cause of tumors. Rearranging genes may alter the original locations of the tumor gene to generate pathological effects in two main ways (Nambiar *et al*., 2008). The first is the synthesis of a tumor-causing protein resulting from fusion of genes and is called a Fusion gene. The best example of this type is the BCR / ABL gene, which results from the transmission of the ABL gene on chromosome 9 to the BCR gene on chromosome 22 [t (9; 22)] also called the Philadelphia chromosome (Wang *et al*., 2016). which is common in chronic myeloid leukemia (CML) patients, and the formation of BCR-ABL protein has been shown to have an abnormal effect on the activity of tyrosine kinase (TK) associated with tumor growth in both myeloid leukemia. CML) and acute lymphocytic leukemia (ALL) (Aber *et al*., 2016; Nambiar *et al*., 2008).

The second method is to place the tumor-causing genes close to new Cis-regulatory elements. An example of this type is the increase in the gene expression of c-MYC in lymphoma burkitt lymphoma due to (t (8; 14). This results in c-MYC, along with the heavy chain regulatory elements in the Immunoglobulin (IGH) (Zheng, 2013). And on the mechanics of the process of transition between chromosomes, it is a complex biological process and there are two basic steps that this process depends on. First, a break or separation of the double DNA strip occurs at two different locations simultaneously. Second, the ends of these sites that have been broken or separated must come close to one another and be coherent (Wang *et al*., 2016). Regardless of these basic steps, increasing evidence shows that there are still many factors affecting the formation of Chromosomal processes, such as nuclear engineering, gene expression, and other unknown mechanisms (Hakim *et al*., 2012). Therefore, understanding the mechanisms of chromosome transmission may help us to develop new methods for early diagnosis and treatment of leukemia.

Materials and Methods

Study samples were collected from (Nanakali Hospital) for hematology in Erbil / Iraq during the period from June 2018 until (March 2019). Including (60) patients (40 males and 20 females) with acute lymphoblastic leukemia (ALL) who were diagnosed based on clinical examination by a specialist physician and morphological evaluation of peripheral blood cells and bone marrow image, as well as on blood flow patterns of cellular immune blood vessels (Flow cytometry). And make sure that all patients have not received any treatment and have type B cells (CD22, CD20, CD19, CD10) indicating acute lymphocytic leukemia of B cells, as well as the study included 20 people from the control group (10 males and 10 females). The group of patients and the control age groups ranged between (1-15) years. 5 ml of blood was placed in tubes (lithium heparin) to make blood cell cultures for cytogenetics.

Cytogenetic Analysis

Special farms were made from peripheral blood samples for patients and healthy subjects. Then add phytohaemagglutinin to speed up and stimulate the mitosis of lymphocytes. Then the mitosis of cells was stopped by adding colchicine's at the metaphase phase, as this substance works to stop the formation of spindle strands in cells. After adding a low-concentration aqueous solution, hypotonic solution, to enter the water inside the cells and swell, then they are installed and spread on a special glass slide. The slides were left at 37 ° C for 24 hours and then the chromosomes were stained using a special pigment usually Giemsa, which illustrates the genotype patterns on the chromosomes. The chromosomes are then examined microscopically to identify distortions such as deletion, duplication, or acquisition of the entire chromosome, or the transfer of all or part of the chromosome arm to another chromosome, or any other abnormalities (Wan, 2014).

Fluorescence in Situ Hybridization (FISH) Analysis

This technique is fast and accurate and enables us to examine the chromosomes present in all stages of the metaphase and in the interphase cells. In it, the implanted cells are fixed to glass slides, and they are allowed to interact with a group of DNA probes called fluorochrome. These sensors are attached to the regions supplementing them on chromosomes. Then they are stimulated by lamps that emit light at a specific wavelength, which results in a fluorescent emission, which works to color them, so they are different from all chromosomes, and can also be studied under a fluorescent microscope**.**

Statistical analysis

The frequencies of recurrent were estimated based on the total number of cases tested within each corresponding group**.**

Results

Table (1) shows the numbers and percentages of patients with acute lymphocytic leukemia distributed by sex, as the percentage of male patients was (66.6%), while the percentage of infected females was (33.3%), thus the male to female ratio in the study sample (2). Cytogenetically the ABL- BCR fusion gene was detected in all patients with acute lymphocytic leukemia and the chromosome positive rate was 3.3% and the negative rate was 96.6%.

Table 1 : Characteristics of pediatric patients with ALL.

Characteristics		N(60)	Frequency/%
Gender	**Male**	**40**	**66.6**
	Female	20	33.3
Ph	Positive	2	3.3
	Negative	58	96.6

The results shown in the present study as in Fig (1) showed the presence of genetic material transfer between the long arms of chromosomes 9 and 22 tons (9,22) (q34; q11) using karyotype technique. FISH technique was also used in fluorescent in situ hybridization with a probe complementing the specific sequence, and the sequence was examined using a specific (fluorescent) microscope according to the probe mark as in Fig (2) ABL (chromosome 9) in red and green BCR (chromosome 22) producing a known gene As ABL-BCR, and it is known as the Philadelphia chromosome, while the results showed in healthy people, two red parts and two green parts were found in the nucleus, and in patients there was a red part and another green and a yellow particle in the nucleus. Where this chromosomal anomaly was observed in two cases (male and female), with a percentage of (3.3)% .

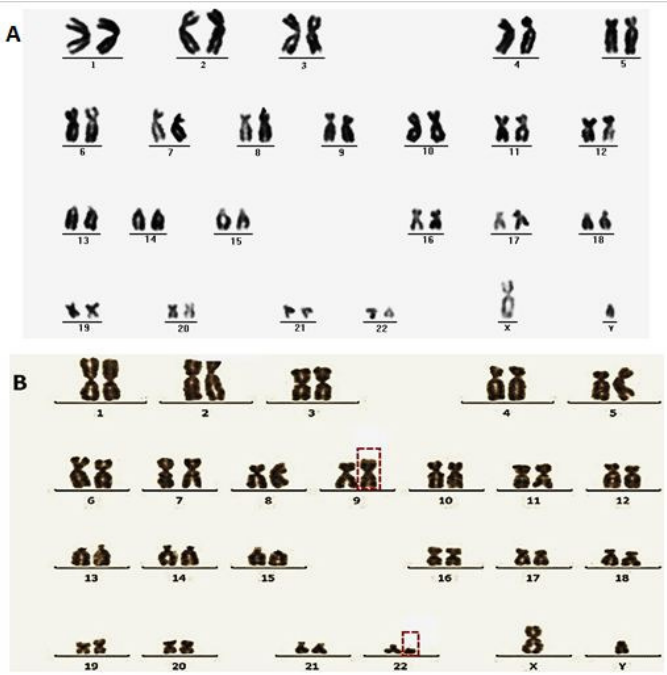
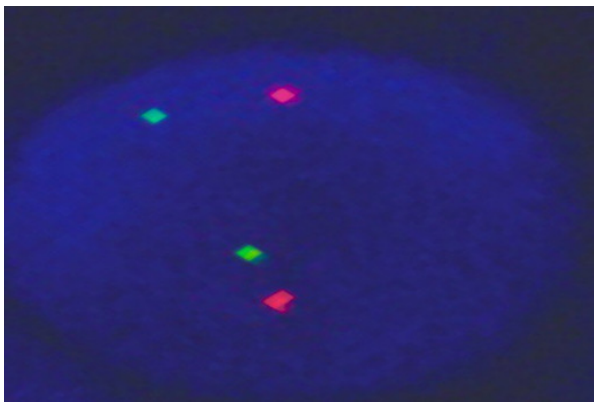
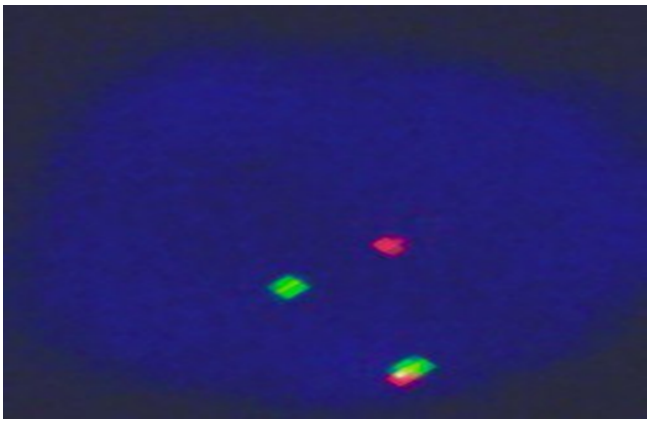


Fig. 1: (A) the karyotype of healthy chromosomes and (B) the chromosomal transition t (9,22). Chromosomes were analyzed at the Metaphase Mitotic cell division stage using a special Cariotype analysis program under 100 X magnification power.



(A)



(B)

Fig. 2: FISH assay to determine the transition between chromosome 9 and 22 (t (9; 22). (A) represents healthy (two red, two green) and (B) patients with acute lymphocytic leukemia (red, green, yellow). Chromosomes were analyzed at Anaphase by a Fluorescent Microscope under a magnification force of 1000 X.

Discussion

The results of the study indicates that the infection rate is at Males are two times higher than females (2)%. This corresponds to what Novak et al (2012) stated when studying a sample of patients with acute lymphocytic leukemia, as the ratio of males to females was (1.7)%. The results are also consistent with the findings of Snodgrass et al (2018) that the ratio of males to infected females was (1.59) %. The incidence in males is higher than that of females by (1.86)%. The difference in the risk of this disease between males and females may be attributed to the genetic and molecular differences between them, and sex hormones contribute to the difference in the rate of disease between them (Do *et al*., 2010; Dorak and Karpuzoglu, 2012).

The diagnosis of ALL depends on the cellular form, immune profiling, cell genetics, and molecular genetic analysis of leukemia burst cells in the peripheral blood and bone marrow. Through the use of Cytogenetic analysis Karyotype technology for leukemia cells, a translocation location between Chromosome 9 and 22 has been observed. The presence of two cases (male and female), at a rate of (3**.**3%)**.**

Cytogenetic analysis (Karyotype) technology was used to detect chromosomal aberrations, regardless of whether these deviations are numerical or structural (Norppa, 2004). That all acute lymphocytic leukemia is characterized by Chromosome Aberration, either in terms of Numerical or Structural. Numerical Abnormalities include an increase in the number of chromosomes (Hyperdiploidy) or lack of a number (Hypodiploidy)**.** As for the structural abnormalities, Abnormalities Structural includes the transfer between translocations, the most important of which are t [12; 21], [1; 19], [9; 22], [11;4] and also rearrangements as in (MYC, MLL) (Zuckerman and Rowe, 2014)**.** And previous studies have shown that patients who show a significant frequency of loss or acquisition resulting from increased chromosome or in cases of Deletion and Duplication and Inversion coupled with other changes in chromosomes can contribute significantly to the development of the disease (Demirhana *et al*., 2019).

Also, the Fluorescent in situ Hybridization (FISH) technique was used, which is a cellular genetic technique used to investigate the presence of specific DNA or RNA sequences for disease diagnosis or treatment follow-up (Everitt *et al*., 2012). It is used to detect genetic abnormalities such as the transfer of chromosomes and chromosome abnormalities. This technique may reveal the rearrangement of rare chromosomes and this technique exists in the form of a group of materials or reagents, providing rapid and accurate detection systems (Robinson *et al*; 2005). This was used to note any cellular disorders such as chromosomal inversion, relocation or deletion of part of it. Specifically, the spatial transfer between chromosome 9 and chromosome 22 t (9,22) (q34.1; q11.2) has been identified in this study. The results showed the presence of this chromosomal anomaly in the number of 2 patients (3.3%).

The results of previous studies showed the presence of this chromosomal anomaly at a rate of 6.4% (Udayakumar *et al*., 2007). Another study discovered the Philadelphia chromosome in (24%) of ALL cases of childhood (Bhutani *et al*; 2004). Another study was found in India, and it was 8.3% with the combined gene BCR-ABL (Sugapriya *et al*; 2011). These results are largely consistent with our current study. Cellular genetic analyzes are very useful for determining chromosomal aberrations, and approximately 75% of acute lymphocytic leukemia in children that contains abnormalities in chromosomes has been detected by conventional cellular genetics and FISH (Coccé *et al*; 2015)**.** And the prediction of the course of the disease (Prognosis) is a very important factor for determining the strategy of treatment, and among the most important of these factors determining the course of the disease is the study of chromosomes and the diagnosis of cellular genetic disorders**.**

Research has found that patients with cellular genetic disorders of type spatial transmission between chromosome 12 and 21 and chromosome 15 and 17 and chromosome 16 inversion are good pathways and there is a high probability that the disease will be treated, while cellular genetic disorders of type inversion in chromosome 3 and spatial transmission between Chromosomes 4 and 11 and chromosomes 9 and 22 indicate that the disease has a poor course (Coccé *et al*; 2015). Hence, it is necessary to further our understanding of the role that deviations and instability in chromosomes play not only in tumor growth but also in response to treatment**.** Previous studies have proven that 80% of children treated in all medical centers are alive and disease-free within 5 years of treatment (Pui *et al*; 2008). A major contributor to long-term survival is the progression in anti-cancer treatments (Byrne, 1999)**.**

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