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Original article

Molecular Cytogenetic Study of Chronic Lymphocytic Leukemia Patients Diagnosed in Erbil City Using Fluorescence in Situ Hybridization (FISH) Technique

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Abstract
Abnormalities in chromosomes were assessed using cytogenetic and molecular cytogenetic analyses utilizing the FISH technique on blood samples from patients diagnosed with chronic lymphocytic leukemia. The study included the selection of 50 patients (32 males and 18 females) for both the early diagnosis phase (before therapy) and the treatment phase, examining various factors such as sex, age, and occupation. The results revealed that most patients in the over-70 age demographic are male. The majority lacked a familial history of this condition. Patients with chronic lymphocytic leukemia exhibited a higher prevalence of the chromosomal defect deletion (13) (q14) at 43.8%, followed by deletion (11) (q23) at 18.8%. A trisomy 12 alteration was observed at 12.5%, along with a loss on chromosome 17, also present at 12.5%. Tetraploidy occurred seldom (6.2%), notwithstanding the existence of chromosomal defects, specifically deletions (6) at q25-q27. The current study indicates that structural chromosomal modifications were more prevalent than numerical changes regarding chromosomal aberrations, with both types associated with chronic lymphocytic leukemia.
Keywords. *Cytogenetic study, FISH Technique, Chronic Lymphocytic Leukemia, Deletion, Trisomy 12.*

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Introduction

Cancer is one of the main causes of death in the world, as it ranks second after cardiovascular disease. The World Health Organization (2008) estimated the existence of 12.4 million new cancer cases, and 7.6 million cancer deaths were recorded in the world. Leukemia ranked ninth out of the ten common cancers in males in terms of incidence and death rate. The tenth out of the ten common cancers in females worldwide. [1] In Iraq, the incidence of cancerous diseases increased with a clear increase in the rates of leukemia, as this disease represented the second rank among the ten common cancer diseases in Iraq in 2004 (Ministry of Health, Iraqi Cancer Board, 2008) [2]. Chronic lymphocytic leukemia is characterized by the occurrence of a series of changes in the genetic material of cancer cells that play an important role in the occurrence and development of CLL. These changes include numerical changes of the chromosomes and structural changes of the chromosomes [3]. Less than 40% of people have chronic lymphocytic leukemia (CLL), yet as people age, their chance of developing the disease rises significantly. Approximately 65 years old is the median age of patients at diagnosis. Thirty percent of leukemias are CLL-related, and its frequency is highest in Western countries (1.5 to 2.5 per 100,000 person/year) [4].

Studies have focused on the cytogenetic and molecular aspects of chronic lymphocytic leukemia to determine the chromosomal changes associated with the disease [5]. Fluorescence in situ hybridization (FISH) may detect approximately 80% of the relevant genetic abnormalities, but cytogenetic banding analysis can only identify fewer than 50% of these abnormalities. Fluorescence in situ hybridization (FISH) can detect specific sites of specific DNA sequences in metaphase or interphase cells [6]. The aims of the present study include determining the prevalence of chromosomal aberrations in patients with chronic lymphocytic leukemia and comparing the frequency of different aberrations. Relationship between chromosomal abnormalities in patients with chronic lymphocytic leukemia. Cytogenetic evaluation of patients with chronic lymphocytic leukemia (CLL) using fluorescence in situ hybridization (FISH).

Methods

Collection of samples

50 patients (32 men and 18 women) with chronic lymphocytic leukemia were included in the study samples. The patients were taken from the NanaKeli Hospital for Hematology and Cancer in Erbil City following their diagnosis through clinical and laboratory examinations. The patients were in the early stages of the disease (before treatment) and

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during treatment. After receiving formal authorization and with the help of the medical professionals overseeing patients in these facilities, from October 2019 through October 2020. Eight age group samples (four males and four females) were selected for the control group, which consisted of healthy individuals.

Blood culture

Heparinized whole blood (0.5 ml) was combined with 4.5 ml of PB-Max culture medium (which includes fetal bovine serum, RPMI-1640, L-glutamine, and antibiotics to promote cell growth), and 0.4 ml of PHA was added as a supplement. For 72 hours, culture tubes were incubated at 37 °C; the tubes were then stored at 45 °C for a larger growing surface and regular, gentle shaking of each for a whole day.

Harvesting the blood culture

After three days of incubation, 0.1 mL of colcemid was added to the culture tubes were exposed to colcemid for 60 min at 37°C. The tubes were centrifuged at 1500 rpm for 8 minutes, then most of the supernatant medium was removed carefully. The remaining cell pellet was gently resuspended in 8 mL hypotonic solution (KCL) and incubated at 37°C for 20 min. Then it was centrifuged for 8 min at 1500 rpm, and the hypotonic supernatant was discarded, resuspended, and mixed thoroughly, leaving 0.5 ml of pellet. 5 mL of freshly prepared fixative (3 volumes of methanol and one volume of glacial Acetic acid (3:1) was progressively applied to each tube with continuous shaking of 5 mL per tube, and the tubes were chilled for 20 minutes. Centrifuging the tubes at a speed of 1500 rpm for 8 minutes. Remove the supernatant and apply the fixative solution, washing with the fixative solution until a clear filtrate is obtained. A 5 mL fixative solution was used to suspend the cells. The final cell suspension may be used to prepare glass slides right away or frozen for later use.

Slide Preparation

5 to 6 drops of cell suspension were dropped uniformly from an appropriate distance (typically 30 cm) onto a wet, chilled, and grease-free slide using a Pasture pipette. The slide was dried at room temperature. The prepared slides were kept in a sterile container at 37°C for 1-3 days before staining.

Banding

After being distilled, the glass slides were left to dry at room temperature for 24 - 48 hours before the trypsin packing. The glass slide was placed in a horizontal position on a stand. Trypsin solution was poured onto the slide for 50 - 60 seconds at room temperature, then the slide was washed with PBS solution cooled in the refrigerator, and the strip was dyed directly with Giemsa dye and left to dry at room temperature.

Microscopic Examination

For chromosomal analysis, an Olympus Microscope was used with an ocular lens of 10X and an objective lens of 100X.

Results and Discussion

The study samples included 50 patients (32 males, 18 females) with chronic lymphocytic leukemia, CLL, and 8 controls (4 males,4 females), their ages ranged between (40-85) years, with an average age of 60 years, from Erbil city and outside of the Erbil city. The study was done to determine the chromosomal aberrations in the peripheral blood of chronic lymphocytic leukemia patients. The research was also carried out to see how certain disease-related factors can influence the rate of results.

Figure 1 reveals that the majority of patients with chronic lymphocytic leukemia (CLL) lived in Erbil City, where they made up 58% of the patient population. Males made up 62% of the patient population, which was higher than females' 38%. In contrast, 42% of CLL patients lived outside of Erbil City, with males making up 67% of the patient population and females 33%. The enormous quantity of environmental toxins and dense population that characterize cities have a substantial effect on people's health. The high rate of disease diagnosis among men in the city, as reported in published studies, may be related to the buildup of environmental pollution elements [7].

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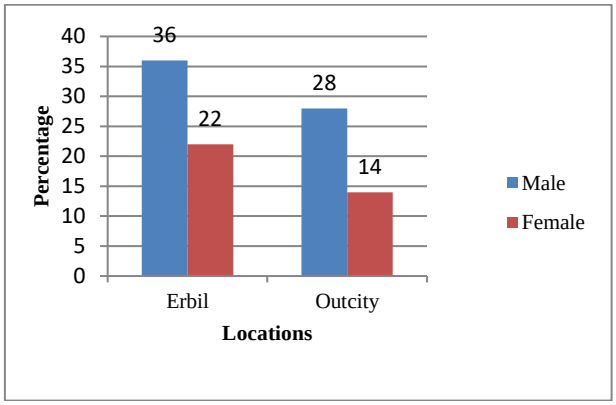

Figure 1. Demographic and baseline information of the subjects.

Distribution of chronic lymphocytic leukemia patients, according to age groups and sex

The study samples were divided into four age groups, as shown in Figure 2. An increase in the number of patients diagnosed with chronic lymphocytic leukemia was observed at the age of more than 60 years [8], revealing that the incidence of CLL rises with age and strikes persons under 50 only slightly. The age group that was most common at ages equal to or exceeding 70 years was found to be 50–59, followed by the age group (40–49), and the age group (60–69) was found to be the most frequent at ages under 60. While from the statistics of the Cancer Council in Iraq for the year 2004 (Ministry of Health, Iraqi Cancer Board,2008) show the most frequent age group was more than 50 years was the age group (50-54), followed by Age groups (60-64), (55-59), (<70), (65-69). The high incidence of chronic lymphocytic leukemia in males compared to females may be due to differences like work, the influence of hormones, and genetic factors related to sex [9]. It was also found that the number of male patients is 32, the number of females is 18, and the proportion of males (64 %) is higher than that of females (36%) [10]. provided support for these findings, indicating that the average ratio of CLL in males compared to females is 2, with a range of 1.0 to 4.7. The results showed that chronic lymphocytic leukemia (CLL), an increase in the incidence rate has been observed in people over 50 years of age.

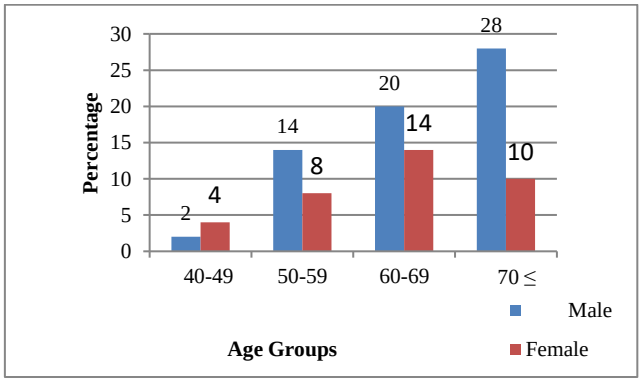

Figure 2. The distribution of CLL patients according to age group and sex

Distribution of CLL patients, according to the family history

Figure 3 shows that the percentage of absence of the family history of the disease (60%) is higher than the percentage of patients who have a family history (40%), and this may indicate a greater role for other factors (non-genetic). In chronic lymphocytic leukemia events, such as exposure to pollutants in the environment. It was noted that the highest incidence of a history of cancer in the family was to have cancer (Leukemia or solid tumors). Chronic lymphocytic leukemia (CLL) shows one of the strongest familial tendencies in disease compared to the rest of cancer diseases [11]. Genetic factors may play an important role, in addition to the role of environmental factors, in causing CLL, and the presence of more than one case of cancerous diseases (leukemia or solid tumors) in the same family may be due to the presence of common genetic factors or exposure to Similar environmental factors make them more vulnerable to infection Cancer diseases, including CLL [12,13].

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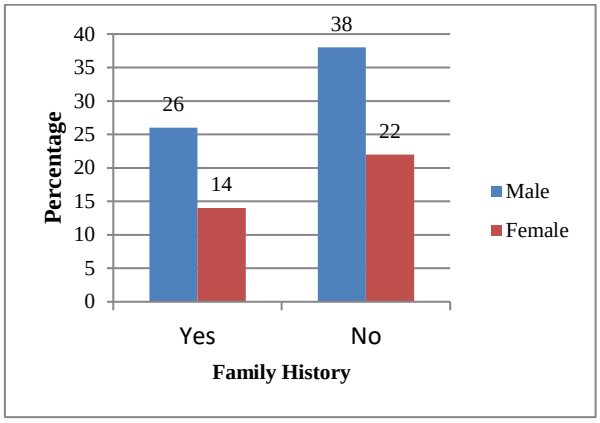

Figure 3. The Distribution of CLL Patients according to Family History.

Distribution of Chronic Lymphocytic Leukemia Patients, according to Occupations

Figure 4 shows the distribution of patients with Chronic lymphocytic leukemia according to occupation. The patients with Chronic lymphocytic leukemia were more common among employees (34 %). Chronic lymphocytic leukemia, like other diseases that lead the environment with its various factors surrounding the individual, has an important role in causing the disease, including the chemicals that the individual deals with because of the nature of his work, studies [14,15] have indicated an increased risk of CLL in agricultural workers, chemists, workers in gas stations, and army workers because of their exposure to chemicals that make them more vulnerable to chronic lymphocytic leukemia. These results indicate exposure to undiagnosed pollutants that have contributed to the development of chronic lymphocytic leukemia.

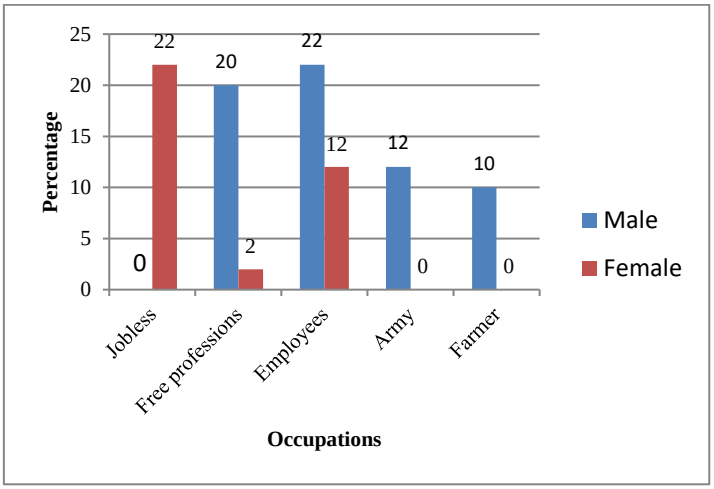

Figure 4. The Distribution of Chronic Lymphocytic Leukemia Patients, According to Their Occupations

Study of chromosomes in CLL patients

The chromosomal aberrations were successfully studied in 40 cases (25 males, 15 females); 5 cases (3 males, 2 females) were in the stage of early diagnosis (before treatment), and 35 cases (22 males, 13 females) were during the treatment phase. 16 cases showed abnormal chromosomes, and 24 cases of normal chromosomes, which shows the results of the chromosomal analysis for patients. Three of them were numerical chromosomal alterations, and 13 were structural chromosomal alterations. The findings demonstrated that karyotype (G-banding) and the FISH technique can be used to identify chromosomal abnormalities in CLL patients. Del (13) (q14) aberration was more prevalent in patients by 43.8 %, while del (11) (q23) aberration was second (18.8%). 12.5% of the sample had a del (17) chromosomal alteration, and 12.5% of the sample had a trisomy 12 change. Although chromosomal abnormalities del (6) (q25-q27) were present, tetraploidy was less common by 6.2%. These findings were confirmed by John et al. (2005), who reported that chromosomal abnormalities were found in 37 out of 46 (80.4%) CLL patients using cytogenetic G-banding analysis and molecular cytogenetics (FISH). The most frequent finding was the deletion of 13q14, which was followed by trisomy 12 and 17p13 deletions, as well as a few less frequent chromosomal abnormalities. These results were also supported by

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[16] found that the most common anomaly was 13q deletion, occurring in 55 % of cases. Other anomalies were 11q deletions (18%), Trisomy 12 (16%),17p deletions (7%), and 6q deletions (6%) in order of frequency (6 %).
Previous studies [17,18] indicated that the deletion in the long arm of chromosome 13 from the chromosomal changes that accompany the development of CLL is associated with the final fate of patients if it is accompanied by other chromosomal changes. The genes affected by this deletion may play to significant role in the generation of CLL [19]. The deletion at 11q22 was related to the deletion of the gene for mutated telangiectasia (ATM). In a process involving a variety of factors, including p53, the ATM protein plays an important role in cellular responses to double DNA breakage [20]. Deletion of 11q22-q23 is associated with a more severe poor prognosis in patients younger than 55 years of age with CLL [21]. Trisomy 12 is one of the chromosomal changes that accompany the development of CLL is found in patients in the advanced stages of the disease and is associated with the patient's ultimate fate. Deletion (6) (q25) is one of the chromosomal aberrations that are associated with infection with different types of lymphoid malignancies, including chronic lymphocytic leukemia (CLL) as it is associated with a good final fate of the patient, whose presence may indicate the role of a tumor suppressor gene located in the region. Deleted the long arm of chromosome 6, which performs an important role in the pathogenesis of CLL [22]. In the molecular cytogenetics part, by use of the fluorescence in situ hybridization (FISH) technique to detect chromosomal aberrations (deletion 13, deletion 17, deletion 11, trisomy 12) is shown in Figure 5 (a, b, c, d).

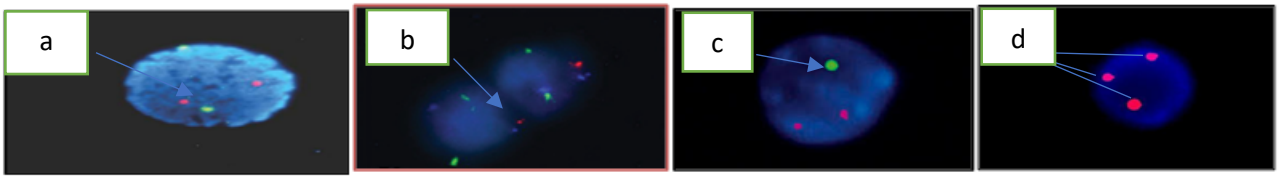

Fig. 5. (a) An interphase of peripheral blood cell with del(13)(q14-q22) (deletion) of a patient (male) with chronic lymphocytic leukemia. The DNA probe specific for 13q14 (one green signal\cell) .

Fig. 5. (b) Two interphase nuclei with del(17)(p13) (deletion) of a patient (male) with chronic lymphocytic leukemia.The DNA probe specific for 17p13 (one red signal\cell) .

Fig. 5. (c) An interphase of peripheral blood cell with del(11)(q23) (deletion) of a patient (male) with chronic lymphocytic leukemia. The DNA probe specific for 11q23(one green signal\cell)

Fig .5. (d)An interphase of peripheral blood cell with Trisomy 12 of a patient (male) with chronic lymphocytic leukemia. The DNA probe specific for Trisomy +12 (three red signal\cell).

Conclusion

The current study indicates that the incidence of CLL is greater in males than in females and is more prevalent among older individuals. The questionnaire results about family history, smoking, and exposure to chemical substances suggest that individuals were exposed to undiscovered agents or pollutants that contributed to the onset of chronic lymphocytic leukemia. the existence of numerical and structural chromosomal alterations. Structural chromosomal changes were more prevalent than numerical ones, with some associated with chronic lymphocytic leukemia. The study indicated that the frequency of chromosomal abnormalities del(13) (q14-q22) is more prevalent in CLL patients.

Conflict of interest. Nil

References

1. International Agency for Research on Cancer (IARC). In: Encyclopedia of global health. Thousand Oaks (CA): SAGE Publications; 2008.
2. Ministry of Health, Iraqi Cancer Board. Results of Iraqi Cancer Registry 2004. Baghdad: Ministry of Health; 2008.
3. Schwab M. An introduction to human cancers. In: Molecular biology of human cancers. Dordrecht: Springer; 2005. p. 25-46.
4. Linet MS. Incidence of haematopoietic malignancies in US radiologic technologists. Occup Environ Med. 2005 Dec;62(12):861-7.
5. Baliakas P, Iskas M, Gardiner A, Stilgenbauer S, Ghia P, Rosenquist R. Chromosomal translocations and karyotype complexity in chronic lymphocytic leukemia: a systematic reappraisal of classic cytogenetic data. Am J Hematol. 2014 Feb;89(3):249-55.

6. Carney DA, Wierda WG. Genetics and molecular biology of chronic lymphocytic leukemia. Curr Treat Options Oncol. 2005 May;6(3):215-25.
7. Lien SY, Young L, Gau BS, Shiao SP. Meta-prediction of MTHFR gene polymorphism-mutations, air pollution, and risks of leukemia among world populations. Oncotarget. 2016 Dec;8(3):4387-98.
8. Wierda WG. Molecular biology of chronic lymphocytic leukemia. In: Chronic lymphocytic leukemia. Totowa (NJ): Humana Press; 2004. p. 77-94.
9. Linet MS, Wacholder S, Zahm SH. Interpreting epidemiologic research: lessons from studies of childhood cancer. Pediatrics. 2003 Jul;112(Suppl 1):218-32.
10. Talab F, Allen JC, Thompson V, Lin K, Slupsky JR. Lck is an important mediator of B-cell receptor signaling in chronic lymphocytic leukemia cells. Mol Cancer Res. 2013 May;11(5):541-54.
11. Caporaso N, Goldin L, Plass C, Calin G, Marti G, Bauer S, Rassenti L, Croce CM, Negrini M. Chronic lymphocytic leukaemia genetics overview. Br J Haematol. 2007 Nov;139(5):630-4.
12. Linet MS, Devesa SS, Morgan GJ. Chronic lymphocytic leukaemia: an overview of aetiology in light of recent developments in classification and pathogenesis. Br J Haematol. 2007 Nov;139(5):672-86.
13. Rauscher GH. Family history of cancer and incidence of acute leukemia in adults. Am J Epidemiol. 2002 Sep;156(6):517-26.
14. Paz-y-Miño C, Bustamante G, Sánchez ME, Leone PE. Cytogenetic monitoring in a population occupationally exposed to pesticides in Ecuador. Environ Health Perspect. 2002 Sep;110(11):1077-80.
15. Lien SY, Young L, Gau BS, Shiao SP. Meta-prediction of MTHFR gene polymorphism-mutations, air pollution, and risks of leukemia among world populations. Oncotarget. 2016 Dec;8(3):4387-98.
16. Döhner H, Stilgenbauer S, Benner A, Leupolt E, Kröber A, Bullinger L, Döhner K, Bentz M, Lichter P. Genomic aberrations and survival in chronic lymphocytic leukemia. N Engl J Med. 2000 Dec;343(26):1910-6.
17. Struski S, Dastugue N, Demur C, Dobbelstein S, Prade N, Bousquet M, Rubie H. 13q deletions in B-cell lymphoproliferative disorders: frequent association with translocation. Cancer Genet Cytogenet. 2007 Apr;174(2):151-60.
18. Chena C, Arrossagaray G, Scolnik M, Palacios MF, Slavutsky I. Interphase cytogenetic analysis in Argentinean B-cell chronic lymphocytic leukemia patients: association of trisomy 12 and del(13q14). Cancer Genet Cytogenet. 2003 Oct;146(2):154-60.
19. Ng D, Toure O, Wei MH, Arthur DC, Abbasi F, Fontaine L, Marti GE, Fraumeni JF Jr, Goldin LR, Caporaso NE. Identification of a novel chromosome region, 13q21.33-q22.2, for susceptibility genes in familial chronic lymphocytic leukemia. Blood. 2006 Oct;109(3):916-25.
20. Stankovic T, Weber P, Stewart G, Bedenham T, Murray J, Byrd PJ, Moss PA, Taylor AM. Ataxia telangiectasia mutated-deficient B-cell chronic lymphocytic leukemia occurs in pregerminal center cells and results in defective damage response and unrepaired chromosome damage. Blood. 2002 Jan;99(1):300-9.
21. Dewald GW, Brockman SR, Paternoster SF, Bone ND, O'Fallon JR, Allmer C, James CD, Jelinek DF, Tschumper RC, Hanson CA, Pruthi RK, Witzig TE, Call TG, Kay NE. Chromosome anomalies detected by interphase fluorescence in situ hybridization: correlation with significant biological features of B-cell chronic lymphocytic leukemia. Br J Haematol. 2003 Apr;121(2):287-95.
22. Amiel A, Fejgin M, Goldberg-Bittman L, Sharoni R, Hadary R, Kitay-Cohen Y, Lishner M. Deletion of 6q27 in chronic lymphocytic leukemia and multiple myeloma detected by fluorescence in situ hybridization. Cancer Genet Cytogenet. 1999 Jul;112(1):53-6.