ZANCO Journal of Pure and Applied Sciences
The official scientific journal of Salahaddin University-Erbil
https://zancojournals.su.edu.krd/index.php/JPAS

ISSN (print):2218-0230**, ISSN (online):** 2412-3986**, DOI: http://dx.doi.org/10.21271/zjpas**

RESEARCH PAPER

Molecular Cytogenetic Study in Patients with Acute Lymphoblastic Leukemia (ALL) in Erbil Province

*Heveen Omar Hassan & *Nadhum Jalal Esmael
* Department of Biology , College of Education Salahaddin University -Erbil , Kurdistan Region, Iraq.

A B S T R A C T:

Background: Acute lymphoblastic leukemia (ALL) is the most common malignancy in children and is also important in older adults. Chromosome number or structure abnormalities are seen in approximately 90% of children and 70% of adult patients with ALL. The aim of this study was to determine the prevalence of these chromosomal abnormalities in ALL patients using Fluorescence *in situ* Hybridization (FISH) technique and to define the frequency of chromosomal abnormalities of ALL patients in adults and children in Erbil Province.

Methods: In this cross-sectional study, we evaluated karyotype results in blood samples, that collected from 55 patients with ALL (in both sexes) in Nanakali Hospital in Erbil Province. Thirty healthy individuals were selected as the control group. Patients ages ranged between 1 to 63 years old. The samples were centrifuged to extract nucleated cells. The cells were then subjected to hypotonic shock, fixed with methanol and acetic acid. A cell suspension was then prepared for FISH technique. After examining the samples with fluorescent microscope, the obtained data along with demographic and baseline characteristics of patients were entered in SPSS software, then statistically analyzed.

Results: The prevalence of chromosomal abnormalities among ALL group was 52.7% (n = 29). Of these, 31¬% (9 people) had abnormalities in chromosome number and 69¬% (20 people) had abnormalities in chromosome structure. The most common chromosomal abnormality was translocation t (9; 22), which accounted for 31¬% of all abnormalities and its prevalence among ALL patients was 16.4¬%. Clonal trisomy and t (12; 21) also accounted for 13.8% and 10.3% of abnormalities, respectively. Clonal trisomy was the most common abnormality in chromosome number, accounting for 44.4% (n = 4) of abnormalities. Only one patient with single chromosome X (X0) pattern was observed in patients. There was no significant (P> 0.05) relationship between the incidence of chromosomal abnormalities with gender, family history, history of surgery and bacterial infection, occupation, place of residence, smoking and blood type as stated from the questionnaire form.

Conclusion

In the current study, concluded that at least one chromosomal abnormality was found in more than half of all patients with ALL. Structural abnormalities were more common than chromosome number abnormalities. Awareness of the magnitude of the problem demands implementation of preventive, diagnostic and therapeutic strategies for leukemia's in the Kurdistan region as well as planning epidemiologic studies and research programs.

KEY WORDS: Acute Lymphoblastic Leukemia,Cytogenetic Analysis,Molecular Cytogenetic Analysis, Chromosomal Abnormalities, Fluorescence in situ Hybridization(FISH), Erbil, Iraq.
DOI: http://dx.doi.org/10.21271/ZJPAS.33.3.11
ZJPAS (2021), 33(3);107-116.

1. INTRODUCTION:

Acute lymphoblastic leukemia is characterized by excessive production of immature lymphocytes (lymphoblast) in the bone marrow preventing normal hematopoiesis.

If untreated ALL can cause death due to crowding out normal cells in the bone marrow and by metastasizing to other essential organs through the peripheral blood, the lymph nodes, spleen, liver, central nervous system (CNS), and skin are the most commonly diagnosed organs. Symptoms are caused by varying degrees of anemia, neutropenia, and thrombocytopenia, or the penetration of ALL cells into tissues. Acute lymphoblastic leukemia is the most common malignant disease in children

* **Corresponding Author:**
Heveen Omar
E-mail: heveenhassan8@gmail.com
Article History:
Received: 18/02/2021
Accepted: 13/04/2021
Published: 20/06 /2021

with a peak incidence between the ages of 2 -5 (Terwilliger & Abdul-Hay, 2017). It also is associated with about 20% of acute adult leukemia patients. Although the peak incidence of ALL is early in life, 45% of patient are nonetheless diagnosed in adulthood (over 20 years). The global prevalence of ALL varies between 1 and 5 per 100,000 individuals and is slightly higher in men than women (Katz et al., 2015). The prevalence of this disease is higher in Latin countries and Spain and generally industrialized countries and urban areas. It is more common in Caucasians than African Americans (Kakaje et al., 2020).

For the correct diagnosis and classification of ALL, the morphologic recognition and phenotypic characteristics of lymphoblast's in the blood and bone marrow are also essential. These cases require accurate evaluation of peripheral blood and bone marrow samples with appropriate preparation and phenotypic analysis of blasts through flow cytometry and immunohistochemistry methods, using an appropriate plate and cytoplasmic markers (Gurbuxani et al., 2020). The results of peripheral blood and bone marrow lymphoblast surveys can be varied. A definitive diagnosis of ALL is based on bone marrow biopsy and identification of malignant clones in flow cytometry with a distinction between B and T cell cancers. ALL B cells account are identified by the expression of CD19, CD22, and CD79a (Raponi et al., 2011).

This invasive form shows a survival rate of 80-90% with chemo immunotherapy. There are some specific recurrent chromosomal abnormalities in ALL patients that are important in determining the prognosis of the disease for treatment planning. Abnormalities in chromosome number or structure are encountered in approximately 90% of children and 70% of adult ALL patients (Gurbuxani et al., 2020). The most common chromosomal shifts in ALL are t (9; 22) (190p) in adults and t (12; 21) in children, respectively. The most common numerical disorder in ALL is hyperdiploidy with chromosome numbers from 51 to 63, which occurs with trisomy of chromosomes X, 4, 6, 10, 14, 17, and 18 and chromosome 21 of four copies. These cytogenetic abnormalities are caused by somatic cell mutations (instead of germ cell mutations), which are often caused by chromosomal DNA translocation, resulting in new (abnormal) protein products from the combined genes.

Cytogenetic analysis is the standard tool for initial evaluation, diagnosis, management of hematological malignancy of a patient that suspected to cancer (Goh et al., 2006) and used as a prognostic indicator for monitoring therapy (Parikh and Tefferi, 2012). Also, it provides evidence of the progression of disease at an earlier phase than hematological marker by detecting various chromosomal aberrations.

The advent of the FISH technique in the 1980s revolutionized cytogenetic analysis. The FISH method was introduced as a technique for identifying trisomy's and displacements in metaphase and interphase nuclei using DNA libraries. This technique is a very good tool for studying the structure and function of chromosomes, polyploidy, aneuploidy, foreign gene penetration, and genome evolution, and for physical mapping of genes. In many applications, in situ hybridization (ISH) requires effective methods for chromosome preparation where chromosomes have to be well-preserved and properly distributed (Liehr, 2017). FISH uses fluorescently labeled DNA probes to determine chromosomal positions within the nucleus. Fluorescent materials generate color signals and are detected using a fluorescent microscope.

Recent changes in the WHO classification identify specific types of B-ALL with recurrent cytogenetic abnormalities (Swerdlow et al., 2017). Translocation (13p; 23q) t (1; 19) is one of the most common translocations that take place in both adult and juvenile populations with an overall frequency of 6%. This shift is observed in the B-ALL field Hyperploid, which is associated with a poor prognosis. The shift can also occur in balanced forms - (13p; 23q) t (1; 19) or unbalanced - (13p; 23q) t (1; 19) der (19) and can lead to integration (Shago, 2017). The current study was aimed to evaluate and the cytogenetic and molecular evaluation of patients with acute lymphocytic leukemia (ALL) using *in situ* fluorescence hybridization (FISH), prevalence of chromosomal translocations in patients with acute lymphocytic leukemia and to determine the frequency of different translocations and comparing their frequency and relationship between chromosomal displacement and risk factors in patients with acute lymphocytic leukemia (Alghasi A, et al., 2019).

2.MATERIALS AND METHODS

2.1Participants

This current retrospective cohort study was conducted on 55 patients & 30 healthy individuals & it current out from January 2020 to August 2020, in Nanakali hospital for blood diseases and oncology the current study cases were reviewed with a final diagnosis of ALL. Definite diagnosis in all cases was established based on morphology, cytochemistry, histochemistry, and flow cytometric analysis. All the cases were referred from affiliated hospitals in Nanakali Hospital for Blood Diseases and Cancer, Erbil, Iraq. Fesh peripheral blood samples were collected.

Briefly, peripheral blood samples were cultured in RPMI 1640 basal medium, containing 10% fetal calf serum (Gibco Invitrogen-USA), for 72 hours at 37°C, at 45□ angle for an increased growth surface with frequent smooth shaking every 24 hours. Then treated with 100 micrograms/ ml of colcemid (Gibco-Invitrogen-USA) to stop the cells in the metaphase of mitosis. After harvesting with hypotonic solution (0.075 M KCL) and fixation with acetic acid /methanol (1/3), the chromosomes were spread and stained using the standard G-banding technique was performed by treating the prepared slides with trypsin working solution and Giemsa stain solution (Moorhead *et al*., 1960). For each case, a minimum of 20 metaphases was analyzed by using the CytoVision chromosomal karyotyping automatic system (Genetix CompanyUSA). Karyotype was written according to the International System Chromosome Nomenclature (ISCN). A successful cytogenetic analysis required the detection of at least 2 or more cells with the same structural change or chromosomal gain, 3 or more cells with the same chromosomal loss, in at least 20 metaphases (Qureshi, 2008). The patients' karyotypes were thereafter subdivided into groups based on the WHO classification.

For the molecular cytogenetic study, a total of (3-5) ml of venous blood was collected from 15 patients, ages ranged between 4 to 52 years with ALL and 5 healthy individuals were selected as the control group. The FISH technique was performed according to (Llobet-Brossa et al., 1998) where the FISH probes were reversibly connected to a glass device and then contacted with the hybridization buffer hardened from the saline-sodium citrate (SSC) solution. After dissolving the probes in the solution, denaturation of the probes and target DNAs was performed by heating the solution at 78 ° C for 5 minutes. Hybridization conditions were provided by placing the samples at 42 ° C for 14-16 hours. After hybridization, the samples were combined with Diamidino-2-phenylindole (DAPI) and allowed to produce color in the dark. Finally, the samples were placed on a slide and examined under a fluorescent microscope.

A multi-probe panel was designed for ALL to identify FISH probes including BCR / ABL translocation, mixed cell line leukemia rearrangement (MLL), TEL-AML1 gene fusion, and trisomy 4.10. The probes were located in regions 11.2q22 34q9, 23q11, 13p12 22q21 and 11.1q -11.1p10 / 11.1q -11.1p4, which represent chromosomal abnormalities (11q; 34q) t (9; 22), 23q11, (22q; 13q) t (12; 21), respectively, and trisomy were 4.10.

Demographic characteristics are examined in two groups of control and patients with ALL. Demographic and baseline information inspected includes age, gender, occupation, family history, maternal and paternal kinship, place of residence, history of bacterial infection and surgery, cytogenetic assessment method, smoking, and blood type as came from the questionnaire form.

3.RESULTS

The current study conducted a cytogenetic analysis on 55 ALL patients with the age ranged between 1-63 years. The prevalence of chromosomal abnormalities in the control and patient groups were investigated according to their type and the prevalence of each type was reported separately as shown in (Table 1).

The gender distribution of subjects in the control and experimental groups of males and females are 60% and 40% respectively, as represented in (figure 1). In terms of gender, 60% of participants were male and the remainders were females, 69.4% of the participants were live in Erbil. In terms of genetic abnormality, karyotyping was used in 76.5% of cases, and FISH were used in 23.5%.

The mean age of the whole population was 15.42. The mean age of the control group was15.93, and the mean age of the experimental group was 15.14. These findings indicate that both groups were well adjusted together in terms of age

(Table 2). In both groups, the most common age groups were 1 to 10 years, 11 to 20 years and 21 to 30 years, and their percentage values were 4.11% and 47.3% respectively. No one in the present study was between 41 and 50 years old.

While according to (Figure 2), karyotyping in both groups was 16.7% and 27.3%, respectively. While the percentages of Karyotyping using the FISH were 83.3 and 72.7 in both control and patient groups respectively.

Genetic abnormalities were assessed in 5 control group members and 15 experimental group members, using the FISH method. 25 members in the control group and 40 members in the experimental group were also tested with karyotyping. Examination of the results of FISH and karyotyping revealed no chromosomal abnormalities in the control group, and therefore all cases belong to the experimental group (ALL patients).

The prevalence of chromosomal abnormalities among people with ALL was 52.7% (n = 29), which means that more than half of the patients showed at least one chromosomal abnormality. Of these, 31% (9 individuals) had abnormalities in chromosome count and 69% (20 individuals) had abnormalities in chromosome structure (Table 3). 36.43% of patients with ALL showed structural abnormalities and 16.4% showed abnormal chromosome counts (Figure 3).

The most common chromosomal abnormality was translocation t (9; 22), which accounted for 31% of all abnormalities. Its prevalence among ALL patients was 16.4.%. “Clonal trisomy” and “t (12; 21)” accounted for 13.8% and 10.3%, respectively (Table 4). Table 5 showed the prevalence of chromosomal abnormalities in patients with ALL by type of abnormality. Clonal trisomy was the most common abnormality in chromosome number, accounting for 44.4% (n = 4) of abnormalities. Only one case of a single X (XO) pattern was observed in patients. The most common chromosome structure abnormalities were “t (9; 22)” and then “t (12; 21)”, which accounted for 45% and 15% of structural abnormalities, respectively. The prevalence of structural abnormalities was recognized in patients with ALL by type of mutation. A total of 20 structural abnormalities were reported, of which 70% were displacement, 25% were omission, and 5% were doubling (Table 6).

Table 1: Demographic characteristic, basic information, abundance and frequency of the subjects

	Measures	**Abundance**	**Frequency**
Gender	Male	51	60
	Female	34	40
Cytogenetic method	FISH	20	23.5
	Karyotype	65	76.5
Group	Control	30	35.3
	ALL test	55	64.7
Job	Jobless	29	34.1
	Student	39	45.9
	Freelance	14	16.5
	Employee	3	3.5
Location	Erbil	59	69.4
	Outside of Erbil	26	30.6

family history	Yes	8	13.3
	No	52	86.7
Affected relatives	Yes	24	28.2
	No	61	71.8
Bacterial infection	Yes	15	27.3
	No	40	72.7
Smoking	Yes	13	16.2
	No	67	83.8
History of surgery	Yes	7	8.2
	No	78	91.8
Blood group	A	16	18.8
	B	27	31.8
	O	31	36.5
	AB	11	12.9

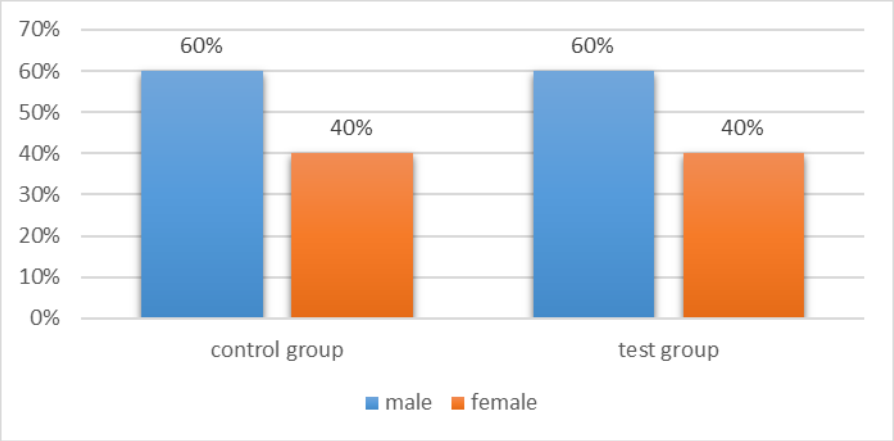

Figure 1: Gender distribution of subjects in the control and experimental groups.

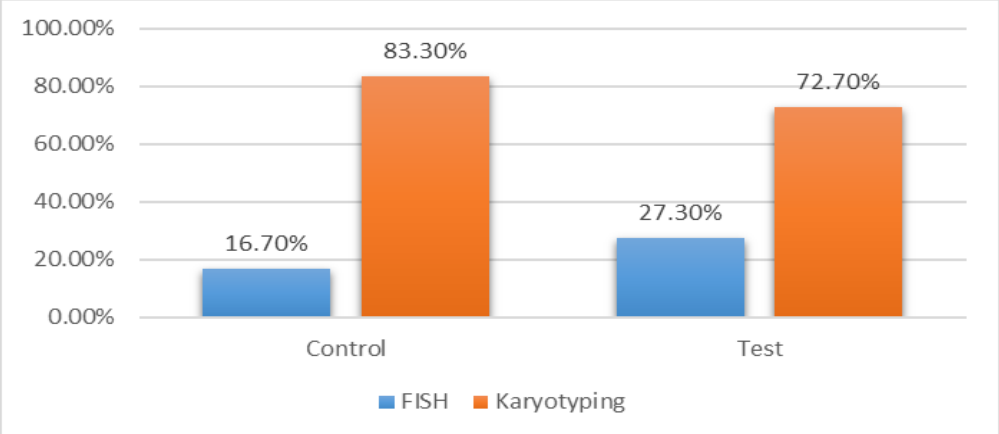

Figure 2: Type of cytogenetic method used to determine chromosomal abnormalities in control and experimental groups.

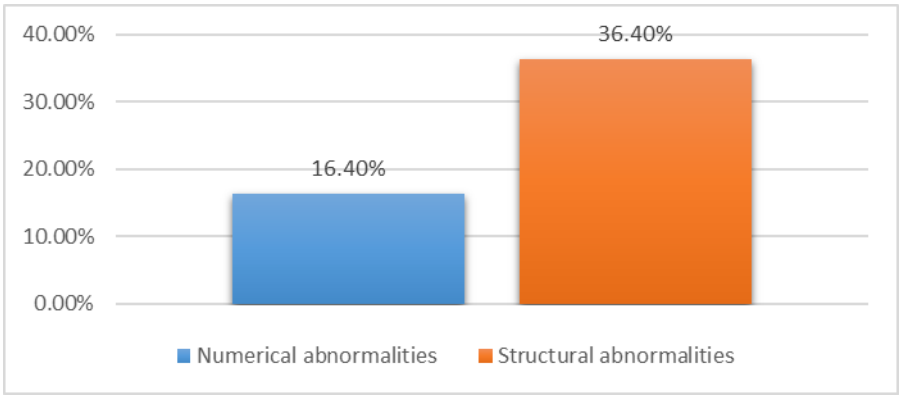

Figure 3: Prevalence of chromosomal abnormalities and type of abnormalities in ALL patients

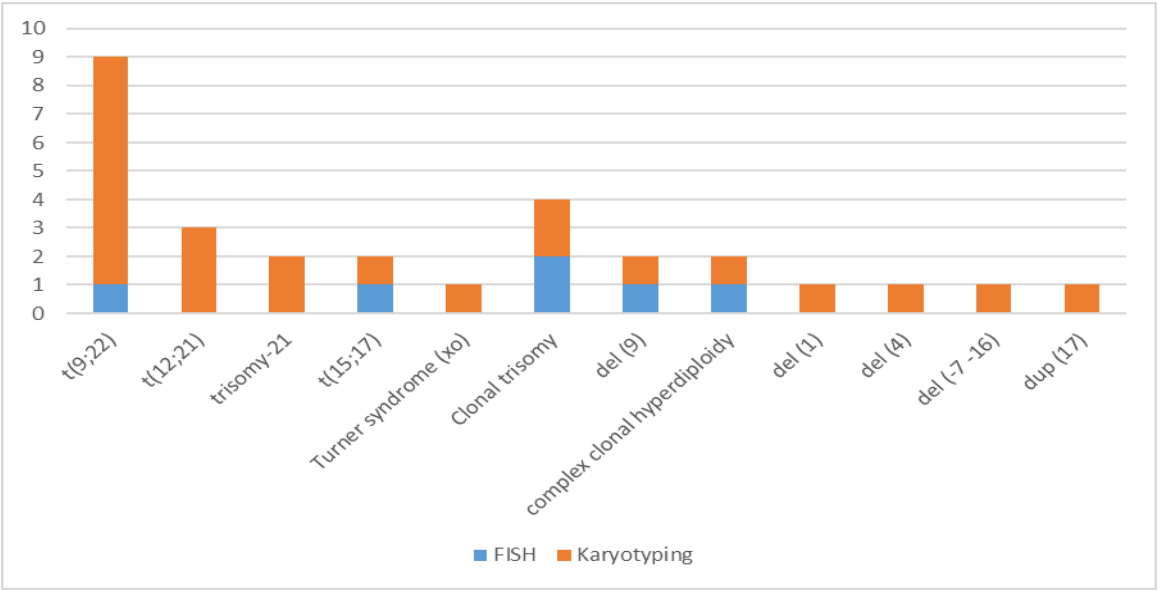

Figure 4: Frequency of chromosomal abnormalities in patients with ALL by cytogenetic studies.

Table 2: The average age of the subjects by groups

	Number(n)	**Average (Mean)**	**S. D**	**Minimum (Year)**	**Maximum (Year)**
Control	30	15.93	13.61	1	63
Test	55	15.14	13.20	1	63
Total	85	15.42	13.27	1	63

S.D = Standard Deviation

Table 3: Prevalence of chromosomal abnormalities and type of abnormalities in ALL patients with cytogenetic studies

		Abundance	Frequency	Prevalence in patients
Chromosomal abnormalities	Yes	29	52.7	52.7
	No	26	47.3	47.3
Type of chromosomal abnormality	No.	9	31	16.4
	Structure	20	69	36.4

Table 4: Prevalence of chromosomal abnormalities in patients with ALL with cytogenetic studies

Chromosomal abnormalities	Abundance	Frequency	Prevalence in patients
t(9;22)	9	31	16.4
t(12;21)	3	10.3	5.5
Trisomy 21	2	6.9	3.6
t(15;17)	2	6.9	3.6
Turner syndrome (xo)	1	3.4	1.8
Clonal trisomy	4	13.8	7.3
del(9)	2	6.9	3.6
complex hyperdiploidy cancer clonal	2	6.9	3.6
del(1)	1	3.4	1.8
del(4)	1	3.4	1.8
del(16- 7-)	1	3.4	1.8
dup(17)	1	3.4	1.8

Table 5: Prevalence of chromosomal abnormalities in patients with ALL by type of abnormality

Type of chromosomal abnormality		Abundance	Frequency
Anomalies Number of chromosomes	Trisomy 21	2	22.2
	Single X (xo)	1	11.1
	Clonal trisomy	4	44.4
	complex hyperdiploidy cancer clonal	2	22.2
	Total	9	100
Anomalies Chromosome structure	t(9;22)	9	45
	t(12;21)	3	15
	t(15;17)	2	10

	del(9)	2	10
	del(1)	1	5
	del(4)	1	5
	del(16- 7-)	1	5
	dup(17)	1	5
	Total	20	100

Table 6: Prevalence of structural abnormalities in patients with ALL by type of mutation

Type of mutation	**Abundance**	**Frequency**	**Prevalence in patients**
displacement	14	70	25.5
Delete	5	25	9.1
To be doubled	1	5	1.8
Total	20	100	36.4

4.DISCUSSION

The current study findings revealed that the prevalence of chromosomal abnormalities among people with ALL was 52.7% (n = 29), which means that more than half of the patients showed at least one chromosomal abnormality. Of these, 31% (9 individuals) had abnormalities in chromosome number and 69% (20 individuals) had abnormalities in chromosome structure, the

prevalence of this anomaly among ALL patients was reported to be 16.4% and the prevalence of chromosome structure abnormalities was 36.4%, which was consistent to the findings reported by (Moorman et al.,2010), and his prevalence of genetic abnormalities was 74%. While in another study done by Shaikh et al. (2014) investigated chromosomal abnormalities in children under 15 years of age with ALL. In this study, which examined a total of 153 children with ALL, the prevalence of chromosomal abnormalities was reported to be 48.8%. This may belong to the difference in the sample size of the study.

In the current study, the most common chromosomal abnormality was translocation t (9; 22), which accounted for 31% of all abnormalities. Clonal trisomy and t (12; 21) accounted for 13.8% and 10.3%, respectively. Clonal trisomy was the most common abnormality in chromosome number, accounting for 44.4% (n = 4) of abnormalities. Only one case of a single X (X0) pattern was observed in patients. The most common chromosome structure abnormalities were t (9; 22) and then t (12; 21), which accounted for 45% and 15% of structural abnormalities, respectively. A total of 20 structural abnormalities were reported, of which 70% were displacement, 25% were omission and 5% were doubling. 25.5% of the subjects had chromosomal abnormalities of displacement type, 9.1% of deletion type, and 1.8% of duplication, t-displacement (9; 22) was the most common structural chromosomal abnormality and accounted for 16.4% of cases.

According to a study evaluated the cytogenetics of patients with ALL, the prevalence of t-shift (9; 22) among large sample size (236 patients) was 15%. This translocation was the most common chromosomal disorder in ALL patients (Moorman et al., 2010).

Although, in another study done by Shaikh et al. (2014), 14.2% of chromosomal abnormalities were of the displacement type, 4.72% of the deletion type, and 7.87% of the duplication type performed on children under 15 years of age. The most common chromosomal abnormality was hyperploidy (13.4%) followed by displacement t (9; 22) (7.08%) was the most common structural disorder This difference in the prevalence of

structural disorders is probably due to differences in the study population.

A study was performed by Roberts et al. (2017) on 798 patients with ALL ranging in age from 21 to 86 years. This study showed that the Philadelphia chromosome abnormality t (9; 22) is about 20% among these individuals, which was in agreement with the current study results. The difference observed in the results can be due to differences in sample size and age group of the subjects.

A study by Reddy et al. (2019), evaluated genetic abnormalities in 204 patients with ALL. The most common karyotypes observed include normal karyotype in 39.7% (n = 81), hyperdiploidy in 12.7% (n = 26), t (9; 22) in 4.4% (n = 9), and t (1; 19) in 3.9%. (8 people), and normal karyotype was observed in 47.3% of patients, and t (9; 22) abnormality was reported in a larger population. This discrepancy in the results may be due to differences in the geographical area of the subjects or the sample size.

A study by Chennamaneni et al. (2018) on the cytogenetic effect on treatment outcomes and survival of children with ALL. A total of 240 patients under the age of 18. Out of 240 patients, 125 (52%) were cytogenetically evaluable. Of these, 77 patients (61.6%) had normal cytogenetics, 19 patients (15.2%) had undesirable t (9; 22), 10 patients (8%) had unfavorable cytogenetics, including t (9; 11), hypodiploidy and the karyotype was complex, 10 patients (8%) had favorable cytogenetics including t (12; 21), t (1; 19) and hyperdiploidy, 9 patients (7.2%) had different cytogenetics (Chennamaneni et al., 2018). In the above study, more than half of the subjects had a normal karyotype, while in the present study, less than half of the subjects had a normal karyotype, and the results are not consistent. The difference might be because the above study was performed on individuals under 18 years of age and the present study included adults.

5.CONCLUSION

the current, study concluded that at least one chromosomal abnormality was found in more than half of the patients with ALL. There was no relationship between demographic and baseline information such as gender, age, blood type, family history, etc. with the occurrence of chromosomal abnormalities. Structural abnormalities were more common than chromosome number. Awareness of the magnitude of the problem demands implementation of preventive, diagnostic and therapeutic strategies for leukemia's in the Kurdistan region as well as planning epidemiologic studies and research programs. Extensive studies with larger sample sizes are required in this area.

REFERENCES

ALGHASI A, JASEB K, PEDRAM M, KEIKHAEI B, REZAEEYAN H, GALEHDARI H, MALEKASKAR AM, RAHIM F, NASAB MA. 11q23 translocation in children with acute lymphocytic leukemia following primary response to chemotherapy: prognostic significance and diagnostic accuracy. Clin Cancer Investig J 2019; 8:79-83

CHENNAMANENI, R., GUNDETI, S., KONATAM, M. L., BALA, S., KUMAR, A., & SRINIVAS, L. (2018). Impact of cytogenetics on outcomes in pediatric acute lymphoblastic leukemia. *South Asian journal of cancer*, 7(4), 263.

GOH, H., HWANG, J., KIM, S., LEE, Y., KIM, Y. & KIM, D. (2006). Comprehensive analysis of BCR-ABL transcript types in Korean CML patients using a newly developed multiplex RT-PCR.

GURBUXANI, S., WYNNE, J. P., & LARSON, R. A. (2020). Acute Lymphoblastic Leukemia: Clinical Presentation, Diagnosis, and Classification. In Acute Leukemias (pp. 157-167): Springer.

KAKAJE, A., ALHALABI, M. M., GHAREEB, A., KARAM, B., MANSOUR, B., ZAHRA, B., & HAMDAN, O. (2020). Rates and trends of childhood acute lymphoblastic leukaemia: an epidemiology study. Scientific Reports, 10(1), 1-12.

KATZ, A. J., CHIA, V. M., SCHOONEN, W. M., & KELSH, M. A. (2015). Acute lymphoblastic leukemia: an assessment of international incidence, survival, and disease burden. Cancer Causes & Control, 26(11), 1627-1642.

LLOBET-BROSSA, E., ROSSELLO´-MORA, R. AND AMANN, R. (1998) Microbial community composition of Wadden Sea sediments as revealed by fluorescence in situ hybridization. Appl. Environ. Microbiol. 64, 2691–2696.

LIEHR, T. (2017). *Fluorescence in situ hybridization (FISH)*: Springer.

MOORMAN, A. V., CHILTON, L., WILKINSON, J., ENSOR, H. M., BOWN, N., & PROCTOR, S. J. (2010). A population-based cytogenetic study of adults with acute lymphoblastic leukemia. Blood, *The Journal of the American Society of Hematology*, 115(2), 206-214.

PARIKH, S. A., & TEFFERI, A. (2012). Chronic myelomonocytic leukemia: update on diagnosis, risk stratification, and management. *American Journal Hematology*, 87(6), 610-619.

QURESHI, F. (2008). Clinical and Cytogenetic Analyses in Pakistani Leukemia Patients. *Pakistan Journal Zoology*, 40(3),147-157.

RAPONI, S., STEFANIA DE PROPRIS, M., INTOPPA, S., LAURA MILANI, M., VITALE, A., ELIA, L., GUARINI, A. (2011). Flow cytometric study of potential target antigens (CD19, CD20, CD22, CD33) for antibody-based immunotherapy in acute lymphoblastic leukemia: analysis of 552 cases. Leukemia & lymphoma, 52(6), 1098-1107.

REDDY, P., SHANKAR, R., KOSHY, T., RADHAKRISHNAN, V., GANESAN, P., JAYACHANDRAN, P., . . . MANASA, P. (2019). Evaluation of Cytogenetic Abnormalities in Patients with Acute Lymphoblastic Leukemia. *Indian Journal of Hematology and Blood Transfusion*, 35(4), 640-648.

ROBERTS, K.G., GU, Z., PAYNE-TURNER, D., MCCASTLAIN, K., HARVEY, R.C., CHEN, I.M., PEI, D., IACOBUCCI, I., VALENTINE, M., POUNDS, S.B. AND SHI, L., 2017. High frequency and poor outcome of Philadelphia chromosome–like acute lymphoblastic leukemia in adults. *Journal of Clinical Oncology*, 35(4), p.394.

SHAIKH, M. S., ALI, S. S., KHURSHID, M., & FADOO, Z. (2014). Chromosomal Abnormalities in Pakistani Children with Acute Lymphoblastic Leukemia. *Asian pacific Journal of cancer Prevention*, 15(9), 3907-3909.

SHAGO, M. (2017). Recurrent cytogenetic abnormalities in acute lymphoblastic leukemia. In Cancer Cytogenetics (pp. 257-278): Springer.

SWERDLOW, S., CAMPO, E., HARRIS, N. L., JAFFE, E., PILERI, S., STEIN, H., . . . LE BEAU, M. (2017). WHO classification of tumours of haematopoietic and lymphoid tissues (Revised 4th edition). IARC: Lyon, 421.

TERWILLIGER, T., & ABDUL-HAY, M. (2017). Acute lymphoblastic leukemia: a comprehensive review and 2017 update. *Blood cancer journal*, 7(6), e577-e577.