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Chromosomes Of Children With Acute Lymphocytic Leukemia In Al Kut

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Abstract

Background and Objectives: analyze the chromosomes of children with acute lymphocytic leukemia (cALL) in Kut to identify chromosomal abnormalities and study the chromosomal changes in patients.

Patients and Methods: Clinical notes and cytogenetic analysis were studied for all patients. Cases were collected from oncology unit at Al Karama Hospital in Al Kut.

Results: Chromosomal analysis of all patients showed chromosomal aberrations, and most of the aberrations were numericalabnormalities, were divided according to their karyotype into 4 groups: hyperdiploidy(47%), hypodiploidy(29.4%), pseudodiploidy(5.9%), and normal karyotype(17.5%).

Conclusion: Chromosomal abnormalities, particularly numerical abnormalities, are associated with the development of acute leukemia and the high complexity of karyotyping of patients.

Keywords: Childhood Acute Lymphoblastic Leukemia, Chromosomal Changes.

Introduction

Acute lymphoblastic leukemia (ALL).Is also known as acute lymphocytic leukemia. The term "acute" found in acute lymphocytic leukemia derives from the fact that the disease progresses very rapidly, producing immature blood cells rather than mature cells, the term "lymphoblastic" means that it originates from early immature lymphocytes, which are a type of white blood cell, that invade the blood and spread throughout the body (Hoffman,et al. 2013).The disease can spread to other organs, including the liver, spleen, lymph nodes, and the central nervous system. It can be lethal within a few months if not treated. ALL is characterized by malignant transformation, lymphoid progenitor cell proliferation in the bone marrow (BM), blood, and extramedullary hematopoietic sites, and replacement of normal blood cells (Alvarnas, et al. 2015). ALL is one of the most common types of cancer in the world, especially in Iraq, where the symptoms of leukemia are similar to those of many diseases, making it difficult to diagnose leukemia because there are no specific symptoms (AL-Hashimi, and Wang , 2013). It is the most common form of hematoma in children. About 20% of adult leukemia is caused by ALL, and affects 80 percent ofchildren, in Asia and Eastern Europe, the prevalence of ALL is predicted to be lower than in North America and Oceania (Solomon, et al . 2017 ; Katz , et al . 2015).The role of cytogenetics in biological decisions is the basis of acute lymphoblastic leukemia (ALL), where chromosomal abnormalities play a critical role in ALL growth and have a

significant impact on disease prognosis (Short, et al. 2016), It has a clinical effect and distinct phenotypic properties (De Lorenzo , et al. 2014).

Chromosomal research has been used for diagnostic classification, risk stratification, and disease progression control for many decades (Nizzamani , et al. 2016).Cytogenetic research has a high success rate in detecting chromosomal anomalies (Ayatollahi , et al.2015).Most studies have shown that in addition to structural changes, there are types of chromosomal changes that are either increased or decreased numbersor both (Campos, et al. 2015). Despite substantial progress in treating adult ALL, the average survival for adults aged 18 to 60 years old is only 35%, compared to more than 80% for childhood ALL (Bassan, and Hoelzer , 2011).

The aim of the study is to analyze the chromosomes of children with acute lymphocytic leukemia and to study the chromosomal changes of the patients.

Patients and Methods

This study was conducted during a period of one year from March 2020 to March 2021 on children attending the oncology unit at Al Karama Hospital in Kut who was diagnosed with acute lymphoblastic leukemia .The study included 16 males (59.2%) and 11 females (40.7%). Complete clinical data and blood samples were taken from the patients. Cytogenetic analysis of peripheral blood samples was performed using traditional cytogenetic methods according (short time culture), and the use of culture media (Chromosome medium P) and colchicine solution, reagents, and stains (KCL, PBS, fixative solution, trypsin solution, and others), many laboratory equipment and tools. Cellular proliferation and chromosomal analyses were performed according to (Geleick, et al.1990 ;Yassen , 1990) with some modifications.

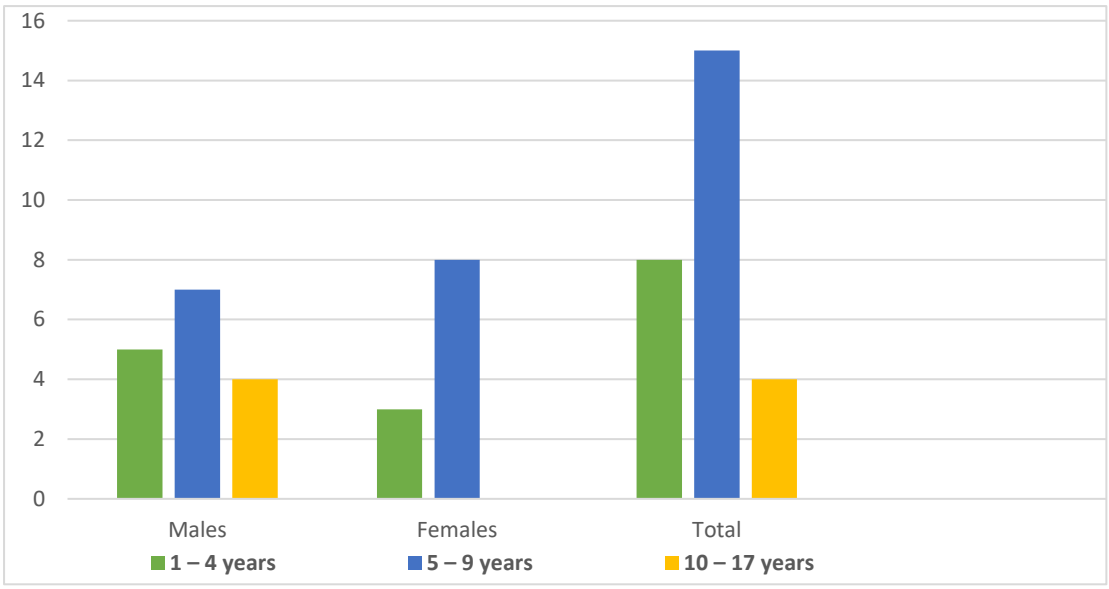
Statistical Analysis

The tables and data were analyzed by test (Chi square test), average, and percentage. Statistical analysis was performed using SPSS v.26 (Statistical Package for Social Science, Chicago, IL, USA).

Results

The results of the first studied group showed that the minimum age for starting the research was from the age of one year, while the maximum-recorded age was 17 years, making the age groups ranged between 1-17 years, with an average age of 7 years. As shown in figure(1).In this study, 27samples of peripheral blood of children under treatment chemotherapy were received for cytogenetic preparation. Shown 4 cases (14.8 %) no cell and no metaphase, 3 cases (11.1 %) clumped metaphases, 3 cases (11.1 %) short chromosomes we can countable but not analyzed, and only 17 cases (62.9 %) good growth and good metaphase.The obtained chromosomal analysis results are the result of data analysis from 17 cases of acute lymphoblastic leukemia, and to illustrate the assessment of chromosome number, they are summarized in Table (1)

Fig (1) Age and sex distribution of patients ALL



	1 – 4 years	5 - 9 years	10 - 17 years
Males	5	7	4
Females	3	8	0
Total	8	15	4

No. of patient	Countable metaphase range (5-20)	Chromosomal numerical aberration
7	10	43,XX,-13,-14,-15
8	10	43,XX,-13,-14,-15
9	15	46,XX
11	10	47,XX,+6
12	11	67,XY aneuploidy
16	10	46,XX
18	7	47,XY,+13
19	10	67,XX aneuploidy
20	17	47,XY,+6
22	20	67,XY aneuploidy
25	8	47,XX,+6
26	10	44,XY,-6,-7
27	16	44,XY,-13,-14
28	5	46,XX
29	20	43,XY,-13,-14,-15
30	15	48,XX,+6,+7
34	20	46,XY / 47,XY,+6

Discussion

The infection rate in the age group (5-9) years is the highest in this study, although most previous research indicates that the most vulnerable age group is (1-5), and the reason is the Corona pandemic and the rejection of the patient family because of his young age and anemia problems.

In the world, studies were found Leukemia is the most common type of childhood malignancy. It accounts for 30% of all cancers diagnosed in children under the age of 15 in industrialized countries. ALL is characterized by immature lymphocytes that accumulate in the bone marrow and is the most common in childhood, with a high incidence at ages (2-5) and 25% of all cancers in this age group (Cabral, et al. 2012).

ALL accounts for over 75% of all new cases in children, and the risk of injury is highest in children under 5 years of age (American Cancer Society, 2021).

Another study was (ALL) with an incidence peak between the ages of 2 and 6 years (William, and Teena . 2016 ; Bispo, et al. 2020).A local study showed that the most common type in children (0-14) years, is ALL, which occurs in about 80% of cases of leukemia (Alrudainy, et al. 2011).Through the above studies, we conclude that there is a clear and influential correlation between age and disease, as the age factor in this study showed a significant relationship with the disease through the results of the statistical analysis, (P <0.05), as in table (2).ALL is reportedly slightly more common in males than females for unknown reasons

(Terwilliger, and Abdul-Hay . 2017 ; Santiago , et al. 2017 ; Matloub , et al. 2016).

In this study, the distribution of acute lymphoblastic leukemia patients by sex did not show a significant difference between male and female patients, of the 27,he was 16 (59.2%) male, and 11 (40.7%) female. The statistical analysis of this study showed that the sex factor does not significantly affect the disease, and (P>0.05),as in table (3), where this result agreed with the study (Rafieemehr ,et al. 2019).The overall male / female ratio was (1.4:1),this percentage agreed with the result of a study conducted in Karbala governorate in Iraq, and ALL was the most common with an average age of 10 years and the ratio of males to females was 1.4:1 (Mjali A, et al. 2019). A study (Jalal, et al. 2017) reported that the male/female ratio is (1.7:1), the reason for the difference here may be the low number of samples collectedduring the sampling period.

According to the table (1), 17 children with acute lymphoblastic leukemia have been studied. Numerical abnormalities of chromosomes for patients were divided according to their karyotype into 4 groups: hyperdiploidy, hypodiploidy, pseudodiploidy, and normal karyotype as shown in table (4).

Table (2) Relationship of age with ALL

Age with disease		Patient	
		N	%
Age group (year)	1 - 4	8	29.6
	5 - 9	15	55.6
	10 - 17	4	14.8
Total		27	100
Chi-Square Tests		P-value	0.001

Table (3) Relationship of sex with ALL

Sex with disease		Patient	
		N	%
Sex	Male	16	59.3%
	Female	11	40.7%
Total		27	100%
Chi-Square Tests		P-value	0.217 N.S

Table(4) Summary of Cytogenetic analysis of patient group of ALL

groups	Number of patients	Frequency %	Sex M/F	Median age
Normal(46)	3	17.5	0:3	7
Hypodiploid< 46	5	29.4	3:2	7.2
Hyperdiploid> 46	8	47.0	4:4	8
Pseudodiploid <46>	1	5.9	1:0	8

Through the results obtained from the cytogenetics study of the chromosomes of patients with acute lymphocytic leukemia, the numerical increase (hyprediploid) in the most common chromosomes was 47% and the ratio of males to females was 4:4 with an average age of 8 years, followed by the numerical decrease (hypodiploid) by 29.4 % and the ratio of males to females 3:2 with an average age of 7 years, in addition to the presence of 17% of cases Where the chromosome number is normal (and the probability of the chromosome number is normal, but there is a change in the chromosomal structure that we could not diagnose through G-banding or it needs other techniques such as fluorescence hybridization technique FISH), and we got one case in which the number of chromosomes is variable (pseudodiploid) cells Between the increase and decrease, examples of all these cases are found in figures (2), (3), (4). This study agreed with the studies (Shams, et al.2019 ;Safaei , et al. 2013), where there are more numerical changes than structural changes. Many studies pointed to this result, especially the increase in the number of chromosomes like (Heim, and Mitelman , 2009 ;Pui, et al. 2011; Paulsson, et al. 2010), were found the Chromosome growth is not random, and the eight chromosomesthat make up 80% of all growth are +4 (78%), +6 (85%), +10 (63%), +14 (84%), + It is 17. (68%), + 18 (76%), + 21 (99%), and + X (89%). Trisomy 4, 10, and 17 are associated with favorable results for children, high diploid (>50 chromosomes) are the most common cytogenetic subgroup of BCP-ALL in childhood and are associated with long-term survival. High diploidy is more common in children (15%) than in adults (6%) (Reismuller, et al. 2017).

Through what we obtained from chromosomal changes, we found that each of the following chromosomes is frequently missing and duplicated in most of the study cases (6, 7, 13, 14, and 15)

and that these chromosomes are very important for their presence in the cell because most of them carry genes that have an important role in regulating the cell cycle.

An example ofretinoblastoma protein (pRB) carried on chromosome 13 is the tumor suppressor protein pRB, one of the functions of pRB is to prevent excessive growth of cells by inhibiting cell cycle progression until the cell is ready to divide so that any defect in this gene causes it to lose its function, and the cell rushes into excessive division (MacDonald, and Dick. 2012).

In addition to the HLA complex, which is located on the short arm of human chromosome 6, it is chains that encode about 100 genes (Trowsdale, and Knight . 2013), most of which are involved in the regulation of the immune response (Busch, et al. 2019).

BCL2L2, encodes the anti-apoptotic protein Bcl-w of the Bcl-2 family. This gene is carried on chromosome 14 (Adams, and Cory. 2018), and since the cases studied were subject to treatment, it is likely that this imbalance is the result of the patient receiving chemotherapy, hence the importance of studying chromosomes or conducting a cytogenetic examination of the patient during treatment to follow up the stages of treatment and determine Direction of treatment in the right direction.

The authors also found that loss of the entire chromosome 6 with remaining chromosome duplications is common in ALL, with a tumor suppressor located on the arm of chromosome 6p (HLA locus) suggested being caused by genes on chromosome 6 (Christopher, et al. 2003).

Other researchers and their results indicated that the loss of an important region of chromosome 7 in children may be on the two arms of the chromosome (Heerema, et al. 2004).

This results is useful in following up on the patient undergoing treatment by studying chromosomal changes at all stages of treatment and how to respond to treatment also.

Figure (2) Chromosomal aberration (hyperdiploid) of patient No.19 (67XX) **(1000 X)**

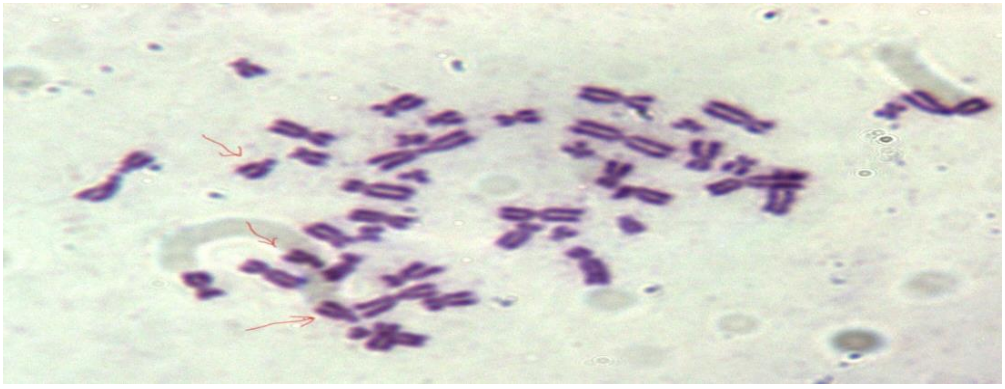


Figure (3) Chromosomal aberration (hypodiploid) of patient No.29 (43XY**)**(43,XY-13,-14,-15) **(1000 X)**

A

B

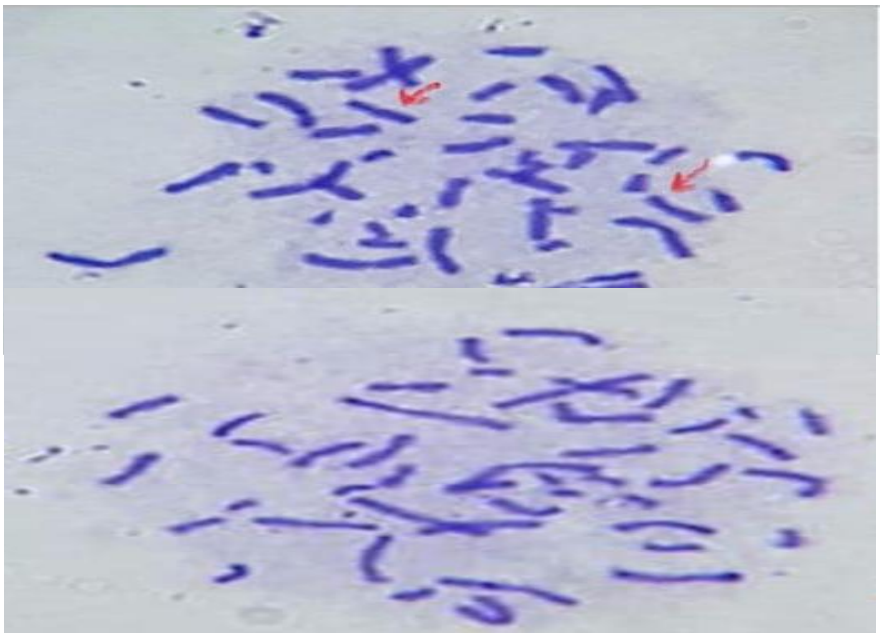


Figure (4) Chromosomal aberration (Pseudodiploid) of patient No.34 A: metaphase (47XY) B: metaphase (46XY)**(1000 X)**

Conclusion

This first study in Wasit included a study of the chromosomes of patients among children less than 17 years of age, as it increases in this age group and may be related to environmental factors, malnutrition, and lack of awareness. The results of the chromosomal analysis were:

Hyperdiploid is most common in this study of patients undergoing chemotherapy, followed by hypodiploid. This finding may be useful in the diagnosis of disease as well as in the monitoring of chemotherapy.

References

1. **Adams JM,** Cory S. (2018). The BCL-2 arbiters of apoptosis and their growing role as cancer targets. Cell Death Differ. 25:27–36
2. **Al -Hashimi MY,** Wang X. (2013). Trend of Leukemia in Ninawa/Iraq. Clinical and Experimental Medical Sciences. 1(8): 353-362.
3. **SalimOudahMezan,** SalihMeri Al Absi, Abdullah HasanJabbar et al., Synthesis and characterization of enhanced silica nanoparticle (SiO2) prepared from rice husk ash immobilized of 3-(chloropropyl) triethoxysilanea, Materials Today: Proceedings, https://doi.org/10.1016/j.matpr.2020.12.564
4. **Alvarnas JC,** Brown PA, Aoun P, Ballen KK, Barta SK, Borate U, et al. (2015) Acute lymphoblastic leukemia, version 2.2015. Journal of the National Comprehensive Cancer Network. 13:1240-1279
5. **American Cancer Society**. (2021). Cancer Facts & Figures. Atlanta, Ga: American Cancer. Society; 2021
6. **Ayatollahi H,**Keramati MR, Sadeghian MH, Ghayoorkarimiani E, Mirzaee F, Shakeri S. (2015). De novo chromosomal abnormalities in acute leukemia patients in northeast of Iran. International Journal of Biology Pharmacy and Allied Science (IJBPAS).;4(11):6312-26
7. **Bassan, R.**&Hoelzer, D. (2011) Modern therapy of acute lymphoblastic leukemia. J ClinOncol, 29, 532-543
8. **Bispo, J.A.B.;**Pinheiro, P.S.; Kobetz, E.K. (2020). Epidemiology and Etiology of Leukemia and Lymphoma. Cold Spring Harb. Perspect. Med., 10, a034819.
9. **Busch R,**Kollnberger S, Mellins ED. (2019). HLA associations in in-flammatory arthritis: emerging mechanisms and clinical im-plications. Nat Rev Rheumatol.; 15(6):364381.
10. **Cabral SNS,** Santos SL, Beltrão AB, Augusto LGS. (2012). Baseline of acute lymphocytic leukemia for surveillance of environmental health on the oil refinery area of the State of Pernambuco, Brazil, 2004 to 2008. EpidemiolServSaúde Dez;21(4):601-8
11. **Campos CB,**MeloCPdS, Duarte SM, Neto JCA, Dutra ÁP, Atalla Â, et al. (2015). Molecular genetics and cytogenetics data of 317 patients with de novo acute leukemia. Open J Hematol.;6(1):3-6
12. **Christopher** R.E. McEvoy, Alexander A Morley, Frank Firgaira: (2003) Evidence for whole chromosome 6 loss and duplication of the remaining chromosome in acute lymphoblastic leukemia Genes Chromosomes and Cancer 37(3):321-5
13. **De Lorenzo P,** Moorman A, Pieters R, Dreyer Z, Heerema NA, Carroll A, et al.(2014). Cytogenetics and outcome of infants with acute lymphoblastic leukemia and absence of MLL rearrangements. Leukemia.;28(2):428-30. doi: 10.1038/leu.2013.280.
14. **Geleick D,** Mu¨ller H, Matter A, Torhorst J, Regenass U: (1990). Cytogeneticsof breast cancer. Cancer Genet Cytogenet, 46:217–22
15. **Heerema N.A ,** J B Nachman, H N Sather, M K La, R Hutchinson, B J Lange, B Bostrom, P G Steinherz, P S Gaynon, F M Uckun, Children's Cancer Group :(2004) Deletion of 7p or monosomy 7 in pediatric acute lymphoblastic leukemia is an adverse prognostic factor: a report from the Children's Cancer Group : Leukemia. May;18(5):939-47
16. **Heim, S.** and F. Mitelman. (2009). Cancer cytogenetics. Third ed. Acute Lymphoblastic Leukemia., New Jersey: John Wiley & Sons, Inc. Hoboken
17. **Hoffman R,**E. J. Benz Jr, L. E. Silberstein, H. Heslop, J. Anastasi, and J. Weitz, (2013). Hematology: basic principles and practice. Elsevier Health Sciences.

18. **Jalal S.D,** Al-Allawi N.A, Al Doski A.A. (2017). Immunophynotypic aberrancies in acute lymphoblastic leukemia from 282 Iraqi patients. Int. J. Lab. Hematol ; 39(6):625-32
19. **Katz AJ,** Chia VM, Schoonen WM, Kelsh MA (2015). Acute lymphoblastic leukemia: an assessment of international incidence, survival, and disease burden. Cancer Causes Control 26(11):1627–1642
20. **MacDonald JI,**Dick FA. (2012). Posttranslational modifications of the retinoblastoma tumor suppressor protein as determinants of function. Genes Cancer. 3:619-633
21. **Matloub Y,** Stork L, Asselin B, Hunger SP, Borowitz M, Jones T, et al. (2016). Outcome of children with standard-risk T-lineage acute lymphoblastic leukemia-comparison among different treatment strategies. Pediatr Blood Cancer;63(2):255-61.
22. **Mjali A,** Al-Shammari HH, Abbas NT, Azeez ZD, Abbas SK. (2019). Leukemia Epidemiology in Karbala province of Iraq. Asian Pac J Cancer Care, Jul 17
23. **Nizzamani GS,**Nizamani ZA, Fahim A, Ujjan IU. (2016). Acute lymphoblastic leukemia: Chromosomal abnormalities in childhood reporting at a tertiary care hospital of Sindh. Professional Med J.;23(3): 312-6.
24. **Paulsson K,**Forestier E, Lilljebjörn H, Heldrup J, Behrendtz M, Young BD, et al. (2010). Genetic landscape of high hyperdiploid childhood acute lymphoblastic leukemia. Proc Natl. Acad. Sci. U.S.A. Dec 14; 107(50):21719–24
25. **Pui C-H.** Acute lymphoblastic leukemia. Springer.(2011)
26. **Rafieemehr H,**Calhor F, Esfahani H, Ghorbani GS. (2019). Risk of acute lymphoblastic leukemia: results of a case–control study. Asian Pac J Cancer Prev.
27. **Reismuller B,** Steiner M, Pichler H, Dworzak M, Urban C, Meister B, et al. (2017). High hyperdiploid acute lymphoblastic leukemia (ALL): a 25-year population-based survey of the Austrian ALL-BFM (Berlin-Frankfurt-Munster) Study Group. Pediatr Blood Cancer.;64:e26327
28. **Safaei A,**ShahryariJ, Farzaneh MR, Tabibi N, Hosseini M. (2013). Cytogenetic findings of patients with acute lymphoblastic leukemia in Fars Province. Iran J Med Sci.;38(4):301-7.
29. **Santiago R,**Vairy S, Sinnett D, Krajinovic M, Bittencourt H. (2017). Novel therapy for childhood acute lymphoblastic leukemia. Expert OpinPharmacother; 18: 1081-99.
30. **Shams SF ,**Ayatollahi H , M Sadeghian, et al. (2019). A Retrospective Survey of Molecular, Cytogenetic, and Immunophenotype Data of Patients with Acute Lymphoblastic Leukemia in Northeast Iran. Middle East Journal of Cancer; July 2019 10 ; (3) 175-182.
31. **Short NJ,**Kantarjian HM, Jabbour EJ, O'brien SM, Faderl S, Burger JA, et al. (2016). Prognostic impact of persistent cytogenetic abnormalities at complete remission in adult patients with acute lymphoblastic leukemia. Am J Hematol.;91(4):385-9. doi: 10.1002/ajh.24296.
32. **Solomon B,**Parihar N, Ayodele L, Hughes M (2017). Global incidence and prevalence of acute lymphoblastic leukemia: 10 years forecast. J Blood DisordTransfus 8:5 (suppl)
33. **Terwilliger T,** Abdul-Hay M. (2017). Acute lymphoblastic leukemia: A comprehensive review and 2017 update. Blood Cancer J 2017;7(6):e577. https://doi.org/10.1038/bcj..53
34. **Trowsdale J,** Knight JC. (2013). Major histocompatibility complex genomics and human disease. Annu Rev Genomics Hum Genet. ;14:301323
35. **William L.** Carroll, TeenaBhatla, (2016). In Lanzkowsky's Manual of Pediatric Hematology and Oncology (Sixth Edition)
36. **Yassen N.Y.** (1990). Cytogenetic study on human colorectal cancer cells. PhD. Thesis submitted University of Sheffield Medical School