

Karyotype in Pediatric Acute Lymphoblastic Leukemia: Impact On Clinical Presentation and Duration of First Remission

ASHRAF KHAIRY, M.D. and AZZA EL-SISSY, M.D.*

Teh Department of Pediatric Oncology Unit, *Clinical Pathology Department, National Cancer Institute, Cairo University.

ABSTRACT

Purpose: In this study we are aiming at investigating the correlation between karyotype and the clinicopathologic features of pediatric acute lymphoblastic leukemia, duration of first remission and outcome of patients.

Material and Methods: A total of 40 pediatric patients with the diagnosis of acute lymphoblastic leukemia (ALL) were included in this study. The patients were treated according to ALL P.NCI III/98 protocol used at the Pediatric Oncology Unit, National Cancer Institute, Cairo University.

Results: Analyzing the patients with respect to their chromosomal pattern; the majority of patients (17/40, 42.5%) showed a pseudodiploid karyotype. Their mean age was 10.2 ± 4.8 years, M/F ratio 2.4:1. Massive hepatosplenomegaly (HSM) was encountered in 64.7%. The mean total leucocyte count (TLC) was 66.53 ± 5.2 cells per μl . Their mean first complete remission (CR1) was 11.05 ± 2.3 months, EFS was 40% at 12 months and 17.78% at 24 months. Patients with normal karyotype came next, representing 13/40 (32.5%). Their mean age was 8.4 ± 1.8 years, M/F 0.8:1. Massive HSM was found in 62.5%. The mean TLC was 78.74 ± 3.8 cells per μl . Their mean CR1 was 11.62 ± 1.2 months, EFS was 41.67% at 12 months and 33.33% at 24 months. The third group represented patients with hyperdiploidy (8/40; 20%). Their mean age was 8.8 ± 3.1 years, M/F 7:1. Massive HSM was found in 50%. The mean TLC was 45.16 ± 3.1 cells per μl , their mean CR1 was 18.10 ± 3.4 months, EFS was 75% at 12 months and 62.5% at 24 months. The least group showed a hypodiploid pattern (5/40; 12.5%). Their mean age was 13 ± 2.6 years, all were males. Massive HSM was encountered in 100%. The mean TLC was 20.00 ± 2.9 cells per μl . Their mean CR1 was 10 ± 2.8 months.

Conclusion: Egyptian patients with childhood ALL who have hyperdiploid karyotype, specially those having >50 chromosomes carry a better prognosis than patients with other chromosomal abnormalities. Pseudodiploid karyotype is the most frequent among Egyptian ALL cases and this could be the reason for our overall poor treatment results. Normal karyotype cannot be used as a prognostic parameter in ALL cases. Hypodiploid karyotype carries the worst prognosis.

Key Words: Acute lymphoblastic leukemia - Chromosomal pattern - Karyotype - Prognosis.

INTRODUCTION

The acute leukaemias are heterogeneous group of neoplasms affecting uncommitted or partially committed hematopoietic stem cells.

Leukemia, both lymphoid and myeloid, can be characterized by morphologic assessment, cytochemical, immunological, cytogenetic, ultra- structural and molecular genetic analysis with respect to biologic features and more specific therapeutic requirements [1].

Traditionally recognized risk groups defined by age, sex, presenting WBC count have been shown to contain subgroups of patients with different outcome that are predicted by blast karyotype, early response to therapy, immunophenotype, and molecular genetic abnormalities. Clinically body mass index, liver, spleen size, presence of anterior mediastinal masses have been variably reported to confer prognostic significance.

Cytogenetic studies in ALL from various centers have proven and confirmed their independent prognostic significance.

Ploidy distribution and recurrent translocations associated with specific morphology and immunophenotype are well recognized in ALL and their prognostic value was confirmed by several studies [2].

Some of these studies have also recognized the correlation between cytogenetic findings and some clinical and hematological features as well as the stage of leukaemic cell maturation.

This contributes significantly in designing the potential therapeutic strategy.

In this study our primary goal was to find out the impact of cytogenetics on the clinical presentation and the duration of first remission among Egyptian pediatric patients with ALL.

MATERIAL AND METHODS

A total of 40 pediatric patients with a documented diagnosis of acute lymphoblastic leukemia (ALL) treated at the pediatric oncology unit, National Cancer Institute, Cairo University were included in this study.

All patients were subjected to the diagnostic work-up which included: history, clinical, and radiological (chest X-ray) examination. Laboratory evaluation including complete blood picture, blood chemistry, bone marrow analysis, CSF examination, determination of leukemic cell surface markers (immunophenotyping) using flowcytometry and chromosomal pattern detection (karyotyping). Cytogenetic analysis of the cultured bone marrow aspirates or the peripheral blood samples was carried out using conventional cytogenetic methods including banding and karyotyping techniques according to the basic techniques of Moorhead et al. [3].

Culture: Growth medium was prepared by mixing the following: 1-RPMI 1640-Earle's base (Gibco) 100ml, 2- Foetal bovine serum (Gibco) 25ml, 3- Penicillin 10.000 U/ml and Streptomycin 10mg /ml (Gibco) 1.3ml.

Procedure: Cultures were set in a disinfected laminar air flow. Culture medium was prepared by placing 5ml of growth medium. The sample was added (5 drops of peripheral blood or 2-3 drops of bone marrow) in each tube. Three cultures were prepared for each sample, mixed gently and then incubated for 24, 48, and 72 hours at 37°C in slanting position.

Harvesting and slide preparation: The solutions used were: 1-Colcemid solution (Gibco) 10µg/ml, 2-Hypotonic solution 0.56% KCl, 3-Fixative 75ml absolute methanol + 25ml glacial acetic acid.

Procedure: Two drops of Colcemid (0.02ml) were added to each culture tube with gentle shaking and were then incubated for 45-60 minutes at 37°C. Tubes were then centrifuged

at 1000 rpm for 10 minutes. The supernatant fluid was discarded leaving as little medium as possible over the cell pellet. The hypotonic solution was pre-warmed to 37°C. Five [5] ml of the hypotonic solution were added drop by drop to each culture tube with shaking. The cultures were then incubated at 37°C for 15 minutes, centrifuged for 10 minutes, and the supernatant was discarded. Five drops of freshly prepared fixative were added to each tube. The tubes were then centrifuged at 1000 rpm for 10 minutes and the supernatant was discarded. The cells were re-suspended in a small volume of fixative. Three to four drops were put on a cold wet slide. The slide was then dried on a hot plate for 15-30 seconds at 40°C.

G-Banding: Slides were aged for one hour in a 90°C oven, then they were cooled to room temperature in a covered box. Slides were then immersed vertically in a Coplin jar containing trypsin solution (0.3%) for 30 seconds to 3 minutes and then immersed in a jar filled with saline. Slides were then stained in Giemsa stain solution for 1-4 minutes. They were then rinsed in diluted water, air-dried and were examined using a research binocular high microscope (Olympus, PM-10AK).

Chromosomal analysis and karyotyping: The chosen metaphase spread was then photographed and analyzed using a computer image analyzer (Vysis Quips XL =Genetics work station) according to the Paris Conference recommendations [4] and the International System of Human Cytogenetic Nomenclature (ISCN) recommendations [5]. For each 20 metaphases spreads were analyzed to detect any chromosomal aberrations.

Treatment plan:

The 40 ALL patients received the standard pediatric ALL chemotherapy protocol applied at the NCI. The protocol composed of three phases, omitting the use of radiation therapy for CNS leukemia prophylaxis. The first induction phase composed of the administration of the basic 4 drugs. Vincristin (VCR) IV, 1.5mg/m² and Daunorubicin: IV, 25mg/m² were given on days 1,8,15. Prednisolone (PO) 40mg/m² started on day 1-28 then was tapered over 10 days. L-asparaginase IM, 6000 U/m² was given on alternating days, 3 times a week, for 9 doses, Triple intrathecal Methotrexate,

Cytarabine and Hydrocortisone were given on days 1 and 43. Etoposide (VP16) and cytarabine (Ara-C): 300mg/m² IV, each was given on days 22, 25, and 29.

Bone marrow examination for re-evaluation was done on day 43 to determine the remission status. Patients who achieved complete remission were promoted to the second phase of therapy (consolidation) and were offered high dose Methotrexate (HD-MTX) IV, 500mg/m² over 1 hour followed by 1500mg/m² over 23 hours given on days 44 and 51.

The third continuation phase, based on using different drug combinations, was given on weekly bases for a total of 120 weeks. This phase consisted of VP16 and Cytosin each 300 mg/m² IV on weeks 1,5,9,13,25,29,33,37,41,45,49,53, 57,61. 6 Mercaptopurin (6MP) 75 mg/m² PO, for 7 days + Cytosin 300 mg/m² IV. On weeks 65,69,73,77,81,85,89,93,97,101,105,109,113, 117. 6 MP 75 mg/m² PO, for 7 days + MTX 40 mg/m² IM on weeks 2,10,26,34,42,50,58,62, 66,70,74,78,82,86,90,94,98,102,106,110,114, 118. MTX: 40 mg/m², IM + Ara-C: 300 mg/m² IV, on weeks 3,11,27,35,43,51,59,67,75,83,91, 99,107,115. VCR IV, 1.5mg/m²+L-asparaginase: IM, 10000 u/m² once + Prednisolone: PO, 40mg/m² for 7 days given on weeks 4,8,12,24, 28,32,36, the coming weeks only VCR + Prednisolone were given on weeks 40,44,48,52,56, 60,64,68,72,76,80,84,88,92,96,100,104,108, 112,116,120. HD-MTX: IV, 500mg/m² over 1 hour followed by 1500mg/m² over 23 hours + 6MP: 75 mg/m² PO, for 7 days on weeks 6,14, 21,22,30,38,46,54. VP16 + Ara-C each 300 mg/m² IV on weeks 7,15,23,31,39,47,55. 6 MP: 75 mg/m² PO, for 7 days + Ara-C: 300 mg/m² IV on weeks, 63,71,79,87,95,103,111,119.

Follow-up:

By the end of the 120 weeks of continuation therapy, complete re-evaluation was performed which included bone marrow analysis, CSF examination and bilateral testicular biopsy. Patients were then put under follow up once monthly by clinical examination + CBC.

Statistical Methods:

An IBM compatible PC was used to store and analyze the data. Software package namely SPSS was used for data management and calculation of mean and standard deviation to describe quantitative data, to compare propor-

tion, to analyze survival data and to compare survival curves.

RESULTS

A total of 40 patients within the pediatric age group with a diagnosis of ALL were included in our study. Their mean age was 10.1 years, ranging from 6 months to 16 years. The event free survival (EFS) for the 40 studied patients was 48.65% and 30.1% at 12 and 24 months, respectively (Fig. 1).

Analyzing the patients with respect to their chromosomal pattern, the majority of patients had a pseudodiploid karyotype (17/40 - 42.5%), their mean age was 10.2 years, M/F ratio 2.4:1, massive HSM in 64.7%, the mean TLC = 66.53 ± 5.2 cells per µl, their mean first complete remission (CR1) was 11.05 months, their probability of EFS was 40% at 12 months and 17.78% at 24 months (Table 1 and Fig. 2). Patients with normal karyotype came next, representing 13/40 (32.5%), their mean age was 8.4 years, M/F 0.8:1, massive HSM in 61.5%, their mean TLC was 78.74 ± 3.8 cells per µl, with a mean CR1 of 11.62 months, their EFS was 41.67% at 12 months and 33.33% at 24 months (Table 2 and Fig. 3). The third group comprised patients with hyperdiploidy (8/40 - 20%). Their mean age was 8.8 years, M/F 7:1, massive HSM in 50%, the mean TLC was 45.16 ± 3.1 cells per µl, their mean CR1 was 18.10 months, their EFS was 75% at 12 months and 62.5% at 24 months (Table 3 and Fig. 2). The last group showed the hypodiploid pattern (2/40 - 5%), their mean age was 13 years, all were males, massive HSM in 100%, the mean TLC was 20.00 ± 2.9 cells per mm³, their mean CR1 was 10 months (Table 4).

Anomalies in chromosome 19 (Fig. 3) were encountered in six cases (15%). Their mean TLC was 113.64 ± 6.7 cells per µl, massive HSM in 66.66%. Their mean CR1 was 7.5 months. Five of this group of patients (83.3%) developed either isolated bone marrow or combined bone marrow and testicular relapse. Only one patient of those six, died during induction.

Four patients had t(8;14) (10%) (Fig. 3). Their mean TLC was 112.3 ± 6.7 cells per µl, massive HSM in 100%. Their mean CR1 was 5.5 months. Three patients (75%) developed either isolated bone marrow or bone marrow

and CNS relapse. One patient died during induction.

Six patients showed an abnormal chromosome 9 (15%) (Fig. 3). Their mean TLC was 43.83 cells per μl , massive HSM in 83.33%. Their mean CR1 was 6.2 months. Five patients (83.3%) had bone marrow relapse and one patient died during induction therapy.

Four patients (10%) had abnormalities related to chromosome 21. Their mean TLC was 74.80 ± 4.9 cells per μl , massive HSM in 50%. Their mean CR1 was 15.25 months. Two patients sustained continuous complete remission (CCR), one patient had bone marrow relapse and one patient died during induction therapy.

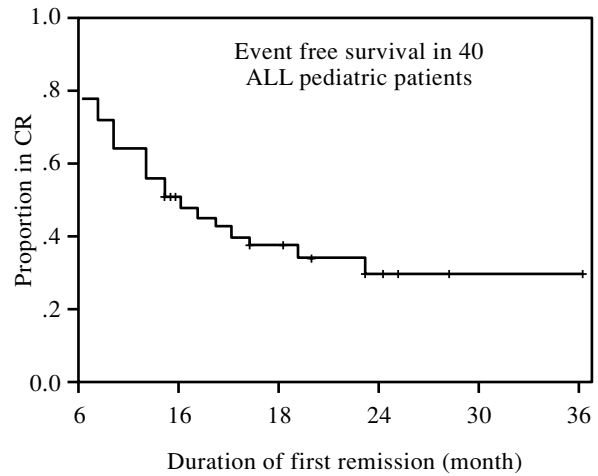


Fig. (1): Event free survival in 40 ALL pediatric patients.

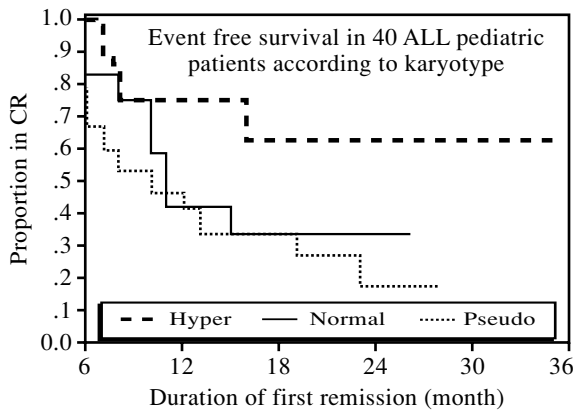


Fig. (2): Event free survival in 40 ALL pediatric patients according to karyotype.

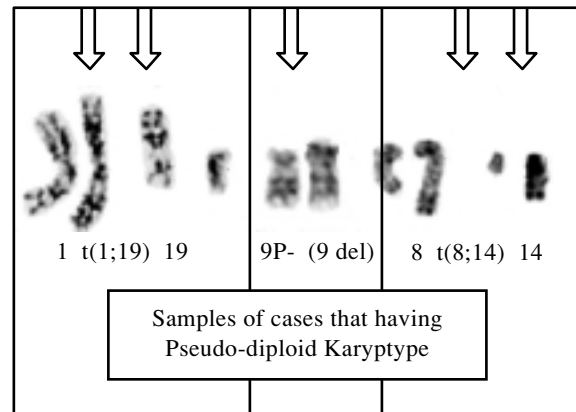


Fig. (3): Samples of cases that having pseudo-diploid karyotype.

Table (1): Characteristics of Pseudo-diploid Patients (17/40 - 42.5%):

UPN	Age (Y)	Sex	TLC (μl)	Karyotype	CR1 (M)	Fate	M	Massive HSM	Immuno-phenotype
1	16	F	210.000	1;19-8;14	6	BM...R	+	+	CALL
2	6	M	18.600	8;14	12	BM...R	+	+	B-cell
3	13	M	14.000	12;21	25	CCR	0	0	CALL
4	5.5	F	101.570	1;19	3	BM...R	0	0	CALL
5	16	F	150.000	1;19	4	BM...R	0	+	CALL
6	4	M	23.000	9p-	0	-----	+	+	T (early)
7	3.5	M	66.000	Abn 9	7	BM...R	0	+	CALL
16	16	M	120.000	7;9	8	BM...R	+	+	T (early)
25	16	F	86.000	12;21	28	CCR	0	0	Pre-B
29	8	M	30.600	8;14	4	BM+CNS	0	+	B-cell
30	10	F	24.600	-10+19	13	BM...R	0	+	T (early)
33	7	M	21.000	9p-	6	BM...R	+	+	T (early)
35	12	M	22.600	14;18	19.8	CCR	0	+	Pre-B
38	16	M	8.000	Dup. 9	10	BM...R	0	0	Pro-B
31	11	M	29.500	1,11+11	23	BM	0	0	Pre-B
34	5	M	190.000	8,14+19+21	0	---	+	+	CALL
32	7	M	5.700	-22+19	19	BM/Test	-	0	Pre-B

UPN: Unique patient number, Age (Y): Years, TLC: Total leukocytic count, CR1 (M): First complete remission in months, M: Mediastinal lymphadenopathy on chest X-ray, Massive HSM: Hepatosplenomegally.

Table (2): Characteristics of Normal diploid Patients (13/40 - 32.5%):

UPN	Age (Y)	Sex	TLC (μ l)	Karyotype	CR1 (M)	Fate	M	Massive HSM	Immuno-phenotype
8	4.5	F	29.500	N	16	CCR	0	0	Pro-B
9	2.5	F	120.000	N	0	-----	+	+	Pro-B
10	5.5	F	115.400	N	15	BM...R	+	+	Pro-B
11	9	M	29.000	N	26	CCR	0	0	Pre-B
13	13	F	89.000	N	23	CCR	+	+	Pre-B
14	3	F	121.000	N	3	BM...R	+	+	Pre-B
17	16	F	41.000	N	11	BM...R	0	0	Pre-B
22	13	M	40.600	N	23	CCR	0	+	CALL
24	5	M	33.000	N	10	BM...R	0	0	Pre-B
26	3	M	137.600	N	11	BM...R	0	+	Pro-B
28	3	M	37.700	N	6	BM...R	0	0	Pre-B
37	16	F	190.000	N	8	BM/CNS	+	+	Biphen
40	15.5	M	40.000	N	10	BM...R	0	+	CALL

UPN: Unique patient number, Age (Y): Years, TLC: Total leukocytic count, CR1 (M): First complete remission in months, M: Mediastinal lymphadenopathy on chest X-ray, Massive HSM: Hepatosplenomegally.

Table (3): Characteristics of Hyper-diploid Patients (8/40 - 20%):

UPN	Age (Y)	Sex	TLC (μ l)	Karyotype	CR1 (M)	Fate	M	Massive HSM	Immuno-phenotype
18	14.5	M	92.000	+22	16	BM...R	0	+	CALL
19	7	M	9.200	+20+21-14	8	BM...R	0	+	T (interm.)
20	3	F	7.300	54xx	20	CCR	0	0	CALL
21	4	M	13.000	57xy	16	CCR	0	0	CALL
23	10.5	M	88.800	51xy	18	CCR	+	+	Pre-B
27	9	M	80.700	51xy	24	CCR	0	0	CALL
36	8	M	10.700	50xy	36	CCR	0	0	Pro-B
39	15	M	60.000	+20	7	BM...R	0	+	CALL

UPN: Unique patient number, Age (Y): Years, TLC: Total leukocytic count, CR1 (M): First complete remission in months, M: Mediastinal lymphadenopathy on chest X-ray, Massive HSM: Hepatosplenomegally.

Table (4): Characteristics of Hypo-diploid Patients (2/40 - 5%):

UPN	Age (Y)	Sex	TLC (μ l)	Karyotype	CR1 (M)	Fate	M	Massive HSM	Immuno-phenotype
15	10	M	15.000	-y	14	BM...R	+	+	T(early)
41	16	M	25.000	-9	6	BM...R	0	+	Pre-B

UPN: Unique patient number, Age (Y): Years, TLC: Total leukocytic count, CR1 (M): First complete remission in months, M: Mediastinal lymphadenopathy on chest X-ray, Massive HSM: Hepatosplenomegally.

DISCUSSION

Multicenter studies have shown that leukemia is a genetic disease. The clinical presentation, as well as the response to therapy, are dictated by the chromosomal pattern of the leukemic cell at diagnosis [6,7]. Current practice is to stratify patients according to a very small number of prognostic variables that are exclusively established as having an important influence on outcome. Achievement and duration of first complete remission (CR1) and survival differed among chromosomal groups. Karyotype is an independent prognostic factor for duration of CR1 and survival even when age, initial leukocytic count (TLC), FAB subtype, and immunologic phenotype are considered [8].

Forty pediatric patients with ALL in whom cytogenetic analysis was performed successfully, were included in this study. All patients received the same ALL chemotherapy protocol (ALL PNCI- III/98). Their mean age was higher (10.1 years) than what was reported in literature, and this could be due to the fact that only patients that have cytogenetic yield were included in this study.

The EFS for the 40 studied patients was 48.65% and 30.1% at 12 and 24 months respectively. Our results are in contrast to what was reported by investigators at St. Jude Children's Research Hospital [9] who reported an EFS of 80% at 24 months. Our poor results could be due to the fact that most of our patients carry high risk features that had a great impact on their survival.

Four main chromosomal patterns were encountered. The majority of our patients (42.5%) had pseudodiploid karyotype in contrast to that reported by Amare et al. [1] who reported that the hypodiploid karyotype was of higher frequency, while Perez et al. reported that hyperdiploid cases were the most frequent [10]. Among the studied patients with pseudodiploid karyotype the mean CR1 was 11.05 months, their EFS was 40% at 12 months and 17.78% at 24 months, 70% of cases had BM marrow relapse with or without CNS relapse. These data are in agreement to those reported by Pui et al. [11] and Hyakura et al. [12] that the worst prognosis was among patients with pseudodiploid karyotype with an EFR of 33%.

Classifying our patients by individual chromosome abnormalities, six of the studied cases (15%) were associated with chromosome 9 anomaly whether total or partial deletion (deletion of the short arm), translocation, duplication or abnormal chromosome. The mean CR1 was 62 months, 83% relapsed and 17% died during induction. This is in agreement with Lai et al. [13], Pui et al. [11], and Harrisson [14] who reported that abnormalities associated with chromosome 9 specially with the short arm appear relatively frequently and are usually associated with some features observed in lymphomatous leukaemia (bulky disease and high total leukocytic count) with poor prognosis and they represent one of the important risk factors.

Chromosome 19 anomalies could also be considered as a bad prognostic factor as about 15% of the studied patients compared to 6.5% in the literature [15, 16], having abnormal chromosome 19 either by translocation or an extra chromosome showed short duration of first remission. Among the studied patients with abnormal chromosome 19, the mean CR1 was 13 months, 83% relapsed and 17% died during induction. Our results are in agreement with Secker-Walker et al. [17], Filatov et al. [18], Uekum et al. [19], Chrisl et al. [20], and Erik et al. [21].

However, cytogenetic analysis is not enough for cases having t(1;19) as there is considerable heterogeneity in the location of E2A and PBX1 breakpoints, as a result of the translocation, and in the clinical outcome [15,22]. Patients having t(1;19) (q23; p13) in their leukemic cells but not expressing E2A-PBX1 fusion protein, have good prognosis, whereas the expression of this protein as a chimeric protein appears to have poor prognosis. This may be useful with the phenotypic classification to guide therapeutic decision making in the future. Molecular techniques such as PCR or FISH technique have been used effectively to detect this fusion protein.

Ten percent of our cases had t(8;14) compared to less than 1% in the literature [23], This may be due to the bias in selection of our cases as we are selecting among patients having cytogenetic yield only. All the study patients with t(8;14) had massive HSM and mean TLC of 112.3 cells per μ l. These data are in agreement with the data reported by Mittelman et al. [24]

and Amare et al. [1]. Their mean CR1 was 5.5 months, 70% relapsed and 25% died during induction. Our results were in agreement with Mittelman et al. [24] and Amare et al. [1] who reported that t(8;14) was usually associated with high rates of early treatment failure and poor prognosis and should receive different therapy.

Four of our patients had chromosome 21 abnormality, t(12;21) was encountered in two cases. Their mean CR1 was 26.5 months and both of them sustained CCR. These results are in agreement with those reported by Van der Plas D [25], Shurtleff et al. [26], Martinez-Ramirez et al. [27] and Alexander et al. [28].

Although trisomy 21 in the literature is usually associated with good prognosis [29], we were able to identify two patients with trisomy 21, one of whom was associated with t(8;14) and died during induction, the other had short duration of CR1 and relapsed.

Out of the forty studied patients, the normal karyotype represented 32.5%. This result is in agreement with Perez et al. [10] who reported 30% of their cases with normal karyotype. The mean CR1 was 11.69 months, their EFS was 41.67% at 12 months and 33.33% at 24 months. Jackson et al. [30] reported that the 5 year event free survival was intermediate for patients with normal karyotype representing 58%. We were not able to clarify the significance of normal karyotype on the duration of first remission. The survival rate among the studied patients was variable although it inclined to bad prognosis as almost 61% of cases showed BM relapse, with or without CNS relapse or died with active disease.

Hyperdiploid karyotype was found in 20% of the studied cases compared to 28% in Fletcher et al. [31] and 38% in Pui et al. [11]. Their mean CR1 was 18.1 months, 62.5% of them sustained CCR, and showing the best prognosis of all patients. Their EFS was 75% at 12 months and 62.5% at 24 months. Hyperdiploid cases (≥ 50 chromosomes) represented 62.5% of cases. Their mean CR1 was 22.8 months, and 100% of them were in CCR. This result is in agreement with Hayakura et al. [12], and Harrison [14] who reported that 83% of patients having >50 chromosome had four- year EFS.

The hypodiploid cases represented 5% compared to 4.9% reported by Pui et al. [11]. These cases showed the shortest duration of first remission, their mean CR1 was 10 months and they had 100% relapse rate. This is in agreement with Harbott et al. [32] and Erik et al [21].

Conclusion:

It is clear from the survival curve a trend towards a better outcome among patients with hyperdiploid karyotype, although the EFS at 24 months (62.5%), did not reach statistical significance ($p=0.203$). Among patients with hyperdiploidy, patients with >50 chromosomes showed the best outcome.

Pseudodiploid karyotype is the most frequent among Egyptian ALL patients and this could be the reason for our overall poor results. Normal karyotype carries an intermediate outcome and hence cannot be used as an independent factor to determine the exact treatment outcome. Hypodiploid karyotype carries the worst prognosis.

REFERENCES

- 1- Amare P., Gladstone B., Varghese C., Pai S. and Advani S.: Clinical significance of cytogenetic findings at diagnosis and remission in childhood and adult acute lymphoblastic leukaemia: experience from India. *Cancer Genet Cytogenet* 1999, 110 (1): 44-53.
- 2- Crist W., Boyett J., Viesti T., Borowitz M., Chauvenet A., Winick N., et al.: Prognostic significance of the pre B cell immunophenotype and other presenting features in B lineage childhood acute lymphoblastic leukaemia: A Pediatric Oncology Group study. *Blood* 2000, 74: 1252-59.
- 3- Moorhead P.S., Nowell P.C., Mellanon W.J., Battips D.M. and Huneferd D.A.: Chromosome preparation of leucocytes culture from human peripheral blood. *Exp Cell Res.* 1990, 20: 613-6.
- 4- Paris conference. Standardization in human cytogenetics, birth defects. Original article series, New york, The National Foundation 1971, 8 (7) pp. 23-6.
- 5- ISCN. An international system of human cytogenetic nomenclature, birth defects. Original article series. 1985, 21(1): pp. 33-7.
- 6- Sather H.: The use of prognostic factors in clinical trials. *Cancer* 1995, 58: 461-7.
- 7- Chessels J.M., Swansbury G.J., Reeves B., Bailley C.C. and Richard S.M.: Cytogenetics and prognosis in childhood lymphoblastic leukaemia: result of MRC UKALLX. Medical research council working party in childhood leukaemia. *Br. J Haematol* 1997, 99: 93-100,
- 8- Bloomfield C.D., Goldman A.I., Alimena G., Berger

- R., Borgstrom G.H., Brandt L., et al.: Chromosomal abnormalities identify high risk and low risk patients with acute lymphoblastic leukaemia. *Blood* 1986, 67 (2): 415-20.
- 9- George S., Aur R. and Maur A.: A reappraisal of the results of stopping therapy in childhood leukemia. *N Engl J. Med.* 1979, 300: 269-71.
- 10- Perez-Vera P., Mujca-Sanchez M., Carnevale A., Rivera-Luna R., Martinez A. and Frias S.: Cytogenetics in acute lymphoblastic leukemia in Mexican children: an institutional experience. *Arch. Med. Res.* 2001, 32: (30) 202-7.
- 11- Pui C.H. and Crist W.M.: Collaborative study of karyotypes in childhood leukaemias. *Groupe Francais de cytogenetique hematologique. Leukaemia* 1993, 7 (1): 10-9.
- 12- Hyakura N., Naneko Y., Katano N., Iwai T., Nagata T., Sakashita K., et al.: Prognostic significance of chromosome analysis in childhood acute lymphoblastic leukemia. *Children's Cancer and Leukaemia Study Group. Rinsho Ketsueki* 2000, 41 (7): 576-84.
- 13- Lai J.L., Fenaux P., Pollet J.P., Estienne M.H., Savary J.B., Huart J.J., et al.: Adult lymphocytic leukaemia with 9p anomalies. A report of four additional cases and review of literature. *Cancer Genet Cytogenet* 1988, 33 (1): 99-109.
- 14- Harrison C.J.: Acute lymphoblastic leukaemia. *Best Pract Res Clin Haematol* 2001, 14 (3): 593-607.
- 15- Pui C-H., Raimondi S. and Hancock M.: Immunologic, cytogenetic, and clinical characterization of childhood acute lymphoblastic leukemia with t(1;19) (q23;p13) or its derivative. *J. Clin Oncol.* 1994, 12: 2601-7.
- 16- Raimondi S.C., Frestedt J.L., Pui C.H., Downing J.R., Head D., Kersey J.H. et al.: Acute lymphoblastic leukaemia with deletion of 11q23 a novel inversion (p13,q23) lacks MLL gene rearrangement and has favourable clinical features. *Blood* 1995, 86: 1881-6.
- 17- Secker-Walker L.M., Berger R., Fenaux P., Lay J.L., Nelken B., Garson B., et al.: Prognostic significance of the balanced t(1,19) and unbalanced der(19) t(1,19) translocation in acute lymphoblastic leukemia. *Leukemia* 1992, 6: 363-9.
- 18- Filatov L., Behm F., Pui C-H., Head D., Downing J., Raimondi S.: Childhood acute lymphoblastic leukemia with equivocal chromosomal markers of the t(1;19) translocation. *Genes Chromosomes Cancer* 1995, 13: 99-101.
- 19- Uekem F.M., Sensel M.G., Sather H.N., Gayon P.S., Arthur D.C., Lange B.J., et al.: Clinical significance of translocation t(1;19) in childhood acute lymphoblastic leukaemia in the context of contemporary therapies: a report from the Children's Cancer Group. *J. Clin Oncol.* 1998, 16: 527-35.
- 20- Chrisl, W.M., Carrol, A.J., Shuster J.J., Behm F.G., Whitehead M., Vietti T.J., Look, et al.: Poor prognosis of children with pre B acute lymphoblastic leukemia is associated with the t(1;19) (q23;p13) a Pediatric Oncology Group study. *Blood* 1999, 76: 117-122.
- 21- Erik F., Bertil J., Goran G., Georg B., Gitte K. and Johann J.: Prognostic impacts of karyotypic findings in childhood acute lymphoblastic leukaemia, a Nordic series comparing two treatment periods. *Br J. Haematol*, 2000, 110: 147-153.
- 22- Privitera E., Luciano A. and Ronchetti D.: Molecular variants of the 1;19 chromosomal translocation in pediatric acute lymphoblastic leukemia (ALL). *Leukemia* 1994, 8: 554-8.
- 23- Nishida K., Ritterbach J., Repp R. and Harbott J.: Characterization of chromosome 8 abnormalities by fluorescence in situ hybridization in childhood B-acute lymphoblastic leukemia/non Hodgkin lymphoma. *Cancer Genet Cytogenet* 1995, 79: 8-11
- 24- Mittelman F., Mertens F. and Johansson B.: A breakpoint map of recurrent chromosomal rearrangements in human neoplasia. *Nature Genetics* 1997, 15: 417-47.
- 25- Van der Plas D., Dekker I. and Hangermeijer A.: 12p chromosomal aberrations in precursor -B childhood acute lymphoblastic leukemia predict an increased risk of relapse in the central nervous system and are associated with typical blast cell morphology. *Leukemia* 1994, 8: 2041-46.
- 26- Shurtleff S., Bujis A. and Behm F.: TEL/AML1 fusion resulting from a cryptic t(12;21) is the most common genetic lesion in pediatric ALL and defines a subgroup of patients with an excellent prognosis. *Leukemia* 1995, 9: 1985-92.
- 27- Martinez Ramirez A., Urioste M., Contra T., Cantalego A., Tavarez A., Portero J.A. and Lopez-Ibor B.: Fluorescence in situ hybridization study of TEL/AML1 fusion and other abnormalities involving TEL and AML1 genes. Correlation with cytogenetic findings and prognostic value in children with acute lymphatic leukaemia. *Haematologica* 2001, 86 (12): 1245-53.
- 28- Alexandre A.J., Reid G.S., Bader S.A., Massing B.G., Sorensen P.H. and Schultz K.R.: ETV6 (TEL-AML1) pre B acute lymphoblastic leukaemia cells are associated with distinct antigen-presenting phenotype. *Br J. Haematol* 2002, 116 (2): 266-72.
- 29- Papas T., Waston D., Sacchi N.: ETS family of genes in leukemia and Down Syndrome. *Am J. Med. Genet* 1990, 7 (Suppl): 251-7.
- 30- Jackson H.F., Boyett J., Brock B., Patterson R., Land V., Borrow m., et al., Favorable prognosis associated with hyperdiploidy in children with acute lymphocytic leukemia correlates with extra chromosome 6. A Pediatric Oncology Group study. *Cancer* 1990, 66 (6): 1183-9.
- 31- Fletcher J.A., Kimball V.M., Lynch E., Donnelly M., Pavelkac K., Tantravahi R., et al.: Prognostic significance of cytogenetic studies in an intensively treated group of children with acute lymphoblastic leukaemia. *Blood* 1989, 74 (6): 2130-5.
- 32- Harbott F.: Clinical significance of cytogenetic studies in childhood acute lymphoblastic leukaemia of the BFM trials. *Recent Results in Cancer Research* 1993, 131: 123-32.