Journal of the Egyptian Nat. Cancer Inst., Vol. 19, No. 2, June: 87-95, 2007

Correlation of Karyotype and Immunophenotype in Childhood Acute Lymphoblastic Leukemia; Experience at the National Cancer Institute, Cairo University, Egypt

FAIZA HAMOUDA, M.D.*; AZZA H. EL-SISSY, M.D.*; ASHRAF K. RADWAN, M.D.**; HANY HUSSEIN, M.D.**; FARIDA H. GADALLAH, M.D.*; NAHLA AL-SHARKAWY, M.D.*; EMAN SEDHOM, M.D.**; EMAD EBEID, M.D.**; SHEREEN I. SALEM, M.D.* and AZZA M. KAMEL, M.D.*

The Departments of Clinical Pathology and Pediatric Oncology**, National Cancer Institute, Cairo University.*

ABSTRACT

Purpose: To identify chromosomal pattern among the major immunophenotypic subgroups in Egyptian children with ALL, and its correlation with clinical presentation and disease free survival.

Patients and Methods: Cytogenetic and immunophenotypic analysis were done for all patients. Patients received ALL-PNCI-III/98 chemotherapy protocol used at NCI, Cairo University.

Results: The frequency of pseudodiploidy and normal karyotype in the whole group was 42.9% and 33.3% respectively. The frequency of pseudodiploidy was 36.8% in CALLA positive early pre B, 30.7% in pre B cases, 71.4% in T cell cases and 100% in mature B cell cases. At 12 months, DFS was 50% for pseudodiploid group having pre B phenotype, compared to 16.6% for pseudodiploid group with CALLA positive early pre B ALL. Sixteen percent of the studied cases showed T cell phenotype, 71.4% of them showed pseudodiploid karyotype, all of them had high risk features. Hyperdiploidy was found in 31.5% of CALLA positive early pre B cases and was associated with favorable prognostic features and DFS of 66.6% at 12 months. Hyperdiploidy of >50 chromosome represented 62.5% of hyperdipoid cases, 80% of them were CALLA positive early pre B ALL carrying good risk features. Fifty percent of normal karyotypic patients showed pre B phenotype, while 42.8% showed CALLA positive early pre B ALL. Their age, TLC, DFS, were almost comparable.

Conclusion: CALLA early pre B phenotype has a positive impact on chromosomal pattern having best outcome among patients with hyperdiploidy. The Pseudodiploid karyotype carries a better outcome with pre B phenotype.

Correspondence: Dr Azza H. El-Sissy, Clinical Pathology, National Cancer Institute, Cairo University, aelsissy@yahoo.com

Key Words: *Acute lymphoblastic leukemia – Karyotyping – Immunophenotyping.*

INTRODUCTION

Acute leukemias are a heterogeneous group of neoplasms affecting uncommitted or partially committed haematopoietic stem cells. Leukemia, both lymphoid and myeloid, can be characterized by morphologic assessment, cytochemical, immunological, cytogenetics, ultrastructural 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 outcomes that are predicted by karyotype, early response to therapy, immunophenotype and molecular genetic abnormalities.

Clinically, age, tumor mass index, total white cell count, hepatosplenomegaly and presence of anterior mediastinal mass have been variably reported to confer prognostic significance **[2,3]**. Chromosomal abnormalities have independent prognostic value in childhood acute lymphoblastic leukemia (ALL). Hyperdiploid cases with modal chromosomal unmbers ≥50 fare best, whereas pseudodiploidy and hypodiploidy are associated with generally poor response **[2,3]**. Traslocations within the leukemic cell karyotype were found to be the chromosomal defect with the most profound impact on treatment outcome **[2-4]**. Patients with any translocation have a six-fold greater risk of early

treatment failure than those without such abnormalities [4]. Early pre B cases have the most favorable prognosis and B cell cases have the worst among immunophenotypic subtypes of childhood ALL. By comparison with early pre B; T cell and pre B cases have inferior outcome [5]. Ploidy distribution and recurrent translocations associated with specific morphology and immunophenotypic pattern are well recognized in ALL; their prognostic value was confirmed by several studies [3,5,6]. Some of these studies have also recognized the correlation between cytogenetic findings and some clinical and hematological features as well as the stage of leukemic cell maturation. This contributes significantly in designing the potential therapeutic strategy [3,6].

The aim of this study is to investigate the correlation between karyotype, marker expression and the clinicopathological features of childhood ALL at presentation, duration of first remission and survival.

PATIENTS AND METHODS

This is a retrospective analysis of 42 pediatric patients with a documented diagnosis of ALL, treated at the Pediatric Oncology Department, National Cancer Institute, Cairo University. The primary goal is to correlate karyotype and surface marker expression in Egyptian pediatric patients with ALL in relation to clinical presentation and treatment outcome.

All patients were subjected to diagnostic work up which included history and clinical examination laboratory evaluation including complete blood picture, blood chemistry, bone marrow analysis, CSF examination, chest X ray, determination of leukemic cell markers (immunophenotyping) using flowcytometer and chromosomal pattern detection (karyotyping); cytogenetic analysis of the cultured bone marrow aspirates or the peripheral blood samples using conventional methods for cytogenetic analysis including banding and karyotyping techniques according to the basic techniques of Moorhead et al. [7]. Culturing: Growth medium: Prepared by mixing the following; 1-RPMI 1640-Earle's base (Gibco laboratories) 100mL. 2- Foetal bovine serum (Gibco laboratories) 25mL. 3- Penicillin 10.000u/mL and streptomycin 10mg/mL (Gibco laboratories) 1.3mL. Procedures: 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 aspirate) in each tube. Three cultures were prepared for each sample, mixed gently and then incubated for 24, 48, 72 hours at 37ºC in slanting position. Harvesting and slide preparation: Solutions used: 1- Colcemid solution (Gibco laboratories) 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 to stop mitosis and were then incubated 45-60 minutes at 37ºC. Tubes were then centrifuged at 1000rpm 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 37ºC. Five mL of the hypotonic solution were added drop by drop to each culture tube with shaking. The cultures were then incubated at 37ºC for 15 minutes, centrifuged for 10 minutes and the supernatant discarded. Five drops of freshly prepared fixative were added to each tube. Tubes were then centrifuged at 1000rpm for 10 minutes and the supernatant discarded. The cells were re-suspended in a small volume of fixative. Three to four drops were dropped on a cold wet slide. The slide was then dried on a hot plate for 15-30 seconds at 40ºC. G-Banding: Slides were aged for one hour in a 90ºC oven, cooled to room temperature in a covered slides box, immersed vertically in 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 photographed and analyzed using a computer image analyzer (Vysis Quips XL=Genetics workstation) according to Pairs conference recommendations and the International System of human Cytogenetic Nomenclature (ISCN) recommendations [8]. For each 20 metaphases, spreads were analyzed to detect any chromosomal aberrations.

Flowcytometric analysis: The coulter Q-PREP EPICS system (Coulter corporation, hialeah, FL, USA) and a reagent system (Coulter

diagnostics, USA) were used to prepare whole blood samples for immunophenotypic analysis by flowcytometry. Peripheral blood or bone marrow samples, drawn on heparin, were used. Immunoprep reagents: Reagent (A) lysing agent for elimination of erythrocytes, reagent (B) a stabilizer for the leukocytes, reagent (C) a fixative to maintain sample integrity. Fluorescent labeled monoclonal antibodies for CD1, CD2, CD3, CD4, CD5, CD7, CD8, CD10, CD13, CD19, CD22, CD33 as well as anti κ, λ and cytoplasmic μ chain were used. The antibodies were obtained from Becton and Dickinson immunocytometry systems (San Jose, CA, USA). The antibodies were either fluorescein-labelled (FITC) or phycoerythrin-labelled (PE). Procedure: For each patient sample, 12x75mm test tubes were labeled for each monoclonal antibody to be tested and appropriate isotypic controls. A 100μl of anticoagulated whole blood or bone marrow were pipetted into the bottom of the orderly labeled test tubes. The inside surface and top of the tube should be free of blood. The 10μl of isotypic control were added to the control tubes and 10μl of the monoclonal antibody to the appropriate test tubes. Anticoagulated whole blood was incubated with antibody for approximately 15 minutes at room temperature. Test tubes were placed into the Q-PREP workstation and the 35s cycle button was pressed. The instrument door was closed to initiate the cycle. The prepared sample can be stored at room temperature if flowcytometric analysis takes place within 2 hours. Otherwise, sample should be covered and refrigerated in the dark at 2-8ºC.

Treatment protocol: The 42 ALL patients received the standard pediatric ALL chemotherapy protocol applied at the NCI, Cairo University. The protocol is composed of three phases, omitting the use of radiation therapy for CNS leukemia prophylaxis. The first induction phase is composed of the administration of the basic 4 drugs; Vincristine (VCR): IV, 1.5mg/m^2 and Daunorubicin: IV, 25mg/m^2 given on days 1,8,15. Prednisolone: PO, 40mg/m^2 started on day 1-28 then taper over 10 days. L-asparagenase: IM, 6000u/m^2 alternating days, 3 times a week, for 9 doses, Triple intrathecal: Methotrexate, Cytarabine and Hydrocortisone, given on days 1, 43. Etopside (VP16) and cytarabin (Ara-C): 300mg/m^2 IV, each given on days 22, 25, 29. Bone marrow examination for reevaluation was done on day 43 to determine 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/m^2 over 1 hour followed by 1500mg/m^2 over 23 hours given on days 44 and 51. The third continuation phase is based on using different drug combinations given on weekly bases for a total of 120 weeks. VP16+Cytoxan each 300mg/m^2 IV on weeks 1, 5, 9, 13, 25, 29, 33, 37, 41, 45, 49, 53, 57, 61. Mercaptopurine (6MP) 75mg/m^2 PO, for 7 days +Cytoxan 300mg/m^2 IV. On weeks 65, 69, 73, 77, 81, 85, 89, 93, 97, 101, 105, 109, 113, 117. 6MP 75mg/m^2 PO, for 7 days+MTX 40mg/m^2 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: 40mg/m^2, IM+Ara-C: 300mg/ m^2 IV, on weeks 3, 11, 27, 35, 43, 51, 59, 67, 75, 83, 91, 99, 107, 115. VCR IV, 1.5mg/m^2+L-asparagenase: IM, 10000u/m^2 once+Prednisolone: PO, 40mg/m^2 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/m^2 over 1 hour followed by 1500mg/m^2 over 23 hours +6MP: 75mg/m^2 PO for 7 days on weeks 6, 14, 21, 22, 30, 38, 46, 54. VP16+Ara-C each 300 mg/m^2 IV on weeks 7, 15, 23, 31, 39, 47, 55. 6MP: 75mg/m^2 PO for 7 days+Ara-C: 300 mg/m^2 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 again confirmed by bone marrow analysis, CSF examination and bilateral testicular biopsy, then patients were put under follow-up once monthly by clinical examination + CBC. Complete remission is defined as the disappearance of organomegaly, normalization of hematological indices and bone marrow normocellularity with <5% lymhoblasts.

Statistical methods:

Disease free survival (DFS) was calculated from the time to achieve complete remission till the last follow-up of the patient either in first continuous complete remission (CCR 1) or the occurrence of relapse.

SPSS package was used for data management. Mean and standard deviation were used to describe quantitative data. Chi-square and/or

Fisher-exact test were used to compare proportion. Kaplan-Meier was used to analyze survival data and Breslow test to compare survival curves. *p*-value is always 2-tailed and significant at 0.05 or less.

RESULTS

A total of 42 patients within the pediatric age group were included in this study with a diagnosis of ALL. Their mean age was 9.5±7 years. Patients were divided according to karyotype into 4 groups, hyperdiploidy, hypodiploidy, normal karyotype and pseudodiploidy. The mean age, total leukocytic count (TLC) and the presence of massive hepatomegaly, splenomegaly (HSM), were comparable among the four karyotype groups, with no statistically significant difference (Table 1). Patients phenotype distribution among different karyotype groups is shown in (Table 2).

Pseudodiploid karyotype was the most frequent karyotype in 18/42 (42.85%). Seven of those 18 (38.9%) were CALLA positive pre-B, their mean age was 10±5.79 years, with a M/F of 1.3/1, massive HSM in 57.14%, mean TLC 105.65x10^9/L±81.13, their mean CR1= 7.85 months, bone marrow relapse (BMR) was found in 5/7 (71.4%), 1/7 (14.3%) died during induction. Five of the 18 cases (27.7%) showed T phenotype; their mean age was 10.6±5.36 years with a M/F of 1.5/1, hepatosplenomegally (HSM) was present in 100% of cases, mean TLC 61.7x10^9/L± 53.22, their mean CR1= 7 months, BMR was shown in 4/5 (80%) while 1/5 (20%) died during induction. Four of the 18 (22.2%) were pre B, their mean age was 11.5±3.7 years, with a M/F ratio 3/1, massive HSM was found in 25%, mean TLC was 35.9x 10^9/L, their mean CR1= 22.4 months, BMR in 2/4 (50%) (Table 3). The rest of the 18 cases (2 cases-11.1%) had mature B phenotype, their ages was 6 and 8 years, both were males, massive HSM was found in both, mean TLC 24x 10^9/L, mean CR1 was 8 months, BMR was evident in both (100%) and one case was associated with CNS relapse.

Normal karyotype was encountered in 14/42 cases (33.3%), 7/14 (50%) were of pre B phenotype their mean age was 7.85±5.05 years, with a M/F ratio of 1.3/1, massive HSM in 28.5% of cases, and mean TLC 50.4x10^9/L± 40.43, their mean CR1= 12.8 months, BMR was found in 5/7 (71.4%) of cases. Six of 14 (42.85%) patients with normal karyotype had CALLA positive early pre B phenotype, their mean age was 7.33±5.52 years with a M/F ratio of 1/1, massive HSM was found in 83.3% and mean TLC of 80.5x10^9/L±48.73 and their mean CR1= 12.5 months, 50% showed BM relapse and one patient (16.6%) died during induction. Only one of the 14 cases had biphenotypic cell surface markers (Table 4).

Hyperdiploid karyotype was encountered in 8/42 (19%) of patients, CALLA positive early pre B phenotype was the predominant phenotype occurring in 6/8 (75%) cases, their mean age was 8.9±5.06 years, with a M/F ratio of 5/1, massive HSM was found in 33.3%, mean TLC was 43.95x10^9/L±38.27, mean CRI= 19.8 months, BMR was found in 2/6 (33.3%) cases (Table 5). One of these 8 hyperdipoloid cases had T-cell surface markers and another patient had pre-B phenotype.

Hypodiploid was documented in 2/42 patients (4.76%) (Table 6 shows their clinicopathological findings).

Fig. (1) shows the disease free survival (DFS) for normal, hyperdiploidy, pseudodiploidy karyotype (*p*=0.15). Fig. (2) shows the DFS in pseudodiploid CALLA, T, pre-B (*p*= 0.21). Fig. (3) shows the DFS in normal karyotype CALLA, pre-B (*p*=0.32). Fig. (4) shows the DFS in CALLA positive, normal, hyperdiploidy, pseudodiploidy karyotype (*p*=0.02).

Table (1): Clinico-pathologic features of pediatric ALL patients among the three major karyotype groups.

Parameter	Pseudo-diploidy (N=18)	Normal (N=14)	Hyper-diploidy (N=8)	*p* value
Age (years) Mean ± S.D	10.44±4.78	8.21±5.34	8.88±4.38	0.43
TLC Mean ± S.D	68.95±65.31	73.29±54.97	45.21±38.79	0.52
M-HSM	12 (66.7%)	8 (57.1%)	4 (50%)	0.72

Age (Y) : Years.
TLC : Total leukocytic count.
M-HSM : Massive hepatosplenomegally.

Table (2): Patient phenotype distribution among different karyotype groups.

	Pre-B (N=13)	CALL early Pre B (N=19)	B-cell (N=2)	T-Cell (N=7)	Bipheno. (N=1)	Total (N=42)
Hyper.	1 (0.08%)	6 (0.32%)	0 (0%)	1 (0.14%)	0 (0%)	8 (0.19%)
Hypo.	1 (0.08%)	0 (0%)	0 (0%)	1 (0.14%)	0 (0%)	2 (0.05%)
Normal	7 (0.54%)	6 (0.32%)	0 (0%)	0 (0%)	1 (100%)	14 (0.33%)
Pseudo.	4 (0.31%)	7 (0.37%)	2 (100%)	5 (0.71%)	0 (0%)	18 (0.43%)

Hyper.: Hyperploidy. Hypo.: Hypoploidy. Pseudo.: Pseudoploidy. Bipheno.: Biphenotypic.

Table (3): Clinico-pathologic findings of patients with pseudodiploid karyotype in relation to CALL early pre B, T cell and pre B immunophenotype (18 case).

	Number of patients (%)	Age (Y) mean ± SD	TLC ($x10^3$) Mean ± SD	M-HSM (%)	Mean CR1 (M)
CALL early pre B	7/18 (38.9%)	10±5.79	105.60±81.13	57.1	7.8
T	5/18 (27.7%)	10.6±5.36	61.70±53.22	100	7.0
Pre-B	4/18 (22.2%)	11.5±3.7	35.9±34.83	25	22.4
p value		0.63	0.27	0.23	

Age (Y): Years. TLC: Total leukocytic count. M-HSM: Massive hepatosplenomegally. CR1 (M): First complete remission in month.

Table (4): Clinico-pathologic findings of patients with normal karyotype in relation to CALL early pre B and pre B immunophenotype (14 case).

	Number of patients (%)	Age (Y) mean ± SD	TLC ($x10^3$) Mean ± SD	M-HSM (%)	Mean CR1 (M)
CALL early pre B	6/14 (42.85%)	7.33±5.52	80.5±48.73	83.3	12.5
Pre-B	7/14 (50%)	7.85±5.05	50.4±40.43	28.5	12.8
p value		0.63	0.30	0.1	

Age (Y): Years. TLC: Total leukocytic count. M-HSM: Massive hepatosplenomegally. CR1 (M): First complete remission in month.

Table (5): Clinico-pathologic findings of patients with CALLA early pre B immunophenotype in relation to normal karyotype, hyperdiploidy and pseudodiploidy.

	Normal karyotype	Hyperdiploidy	Pseudodiploidy
Age (Y)	7.3±5.52	8.9±5.06	10.7±5.79
TTLC ($x10^3$) Mean ± SD	80.50±48.73	43.95±38.27	105.7±81.13
M-HSM (%)	83.3	33.3	57.1
Mean CR1 (M)	12.5	19.8	7.8

Age (Y): Years. TLC: Total leukocytic count. M-HSM: Massive hepatosplenomegally. CR1 (M): First complete remission in months.

Table (6): Clinico-pathologic findings of patients with hypodiploidy (2 cases).

Age (Y)	Sex	TLC	M-HSM	Karyotype	Iph	CR1 (M)	Fate
10	M	15.000	+	-y	T	14	BM relapse
16	M	25.000	+	-9	Pre-B	6	BM relapse

Age (Y): Years.
Sex (M): Male.
TLC : Total leukocytic count.
M-HSM: Massive hepatosplenomegally.
Iph: Immunophenotype.
CR1 (M): First complete remission in month.
BM: Bone marrow.

Table (7): Clinico-pathologic findings of patients with t(8; 14) (4 cases).

Age (Y)	Sex	TLC	M-HSM	Karyotype	Iph	CR1 (M)	Fate
16	F	210.000	+	t(1;19) (8;14)	CALL	6	BM relapse
6	M	18.000	+	t(8;14)	B	12	BM relapse
8	M	30.000	+	t(8;14)	B	4	BM+CNS relapse
5	M	190.000	+	t(8;14)+19+21	CALL	0	DDI

Age (Y) : Years.
TLC : Total leukocytic count.
M-HSM : Massive hepatosplenomegally.
Iph : Immunophenotype.
CR1 (M): First complete remission in months.
BM: Bone marrow.
DDI: Died during induction.

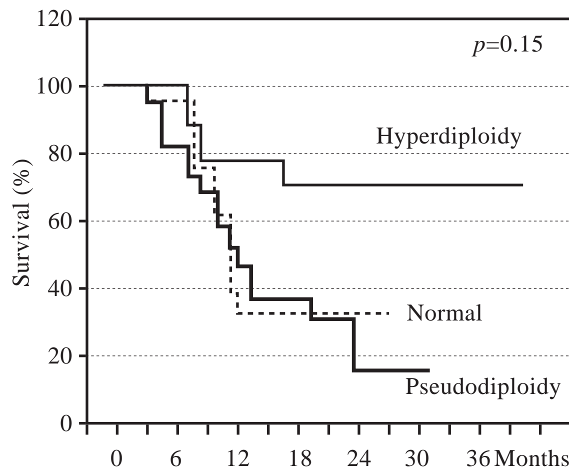

Fig. (1): Disease free survival for normal, hyperdiploid, pseudodiploid karyotype.

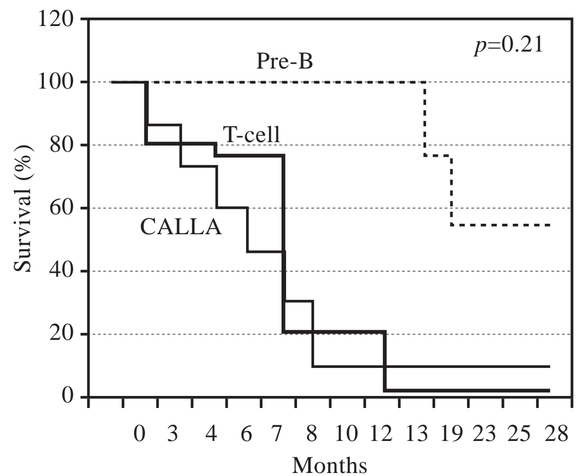

Fig. (2): Disease free survival for pseudodiploid karyotype CALLA, T, Pre-B ALL.

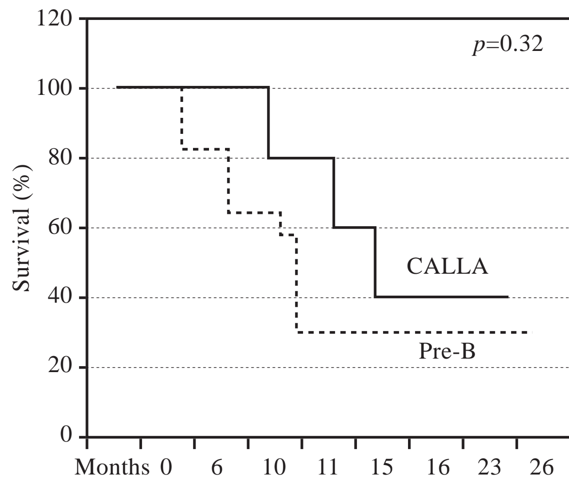

Fig. (3): Disease free survival for normal karyotype CALLA, Pre-B ALL.

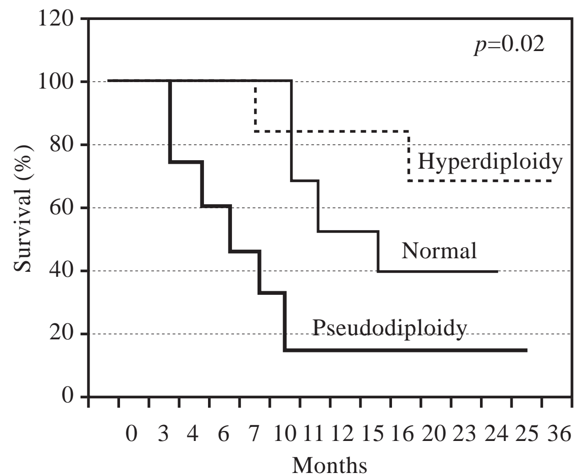

Fig. (4): Disease free survival for CALLA immunophynotype in normal, hyperdiploid, pseudodiploid karyotype.

DISCUSSION

Multi-center 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 at diagnosis **[9-11]**. Aberrant activation of genetic loci near chromosomal breakpoints has a role in malignant transformation or growth characteristic of a particular phenotype **[3]**.

The aim of the current study is to identify the distribution of modal chromosomal numbers and frequency of translocations among the major

immunophenotypic subgroups in Egyptian children with ALL, also to determine the correlation with the clinical presentation and disease free survival.

Forty two pediatric patients with ALL in whom cytogenetic results and immunophenotypic pattern were identified, were included in this study. All patients received a uniform ALL chemotherapy protocol (ALL PNCI 3/98) at the Pediatric Oncology Unit, NCI, Cairo University.

Patients were stratified according to their chromosomal pattern and cell surface marker expression. Among our patient population, great variability was observed concerning the incidence of phenotypic markers with the different karyotypes. These findings are in agreement with other studies [3,12].

Amare et al., [1] reported that pseudodiploidy in B lineage is a common finding among pediatric patients with ALL, in contrast to our finding where the frequency of pseudodiploid and normal karyotype were comparable (38.2%) among B phenotypic groups.

The frequency of pseudodiploidy among the 42 studied patients was 36.8% in CALLA positive early pre B, 30.7% in pre B cases, 71.4% in T cell cases and 100% in mature B cell cases. Pui et al. [3] reported that pseudodiploidy was lowest among CALLA positive pre B cases (35%), intermediate among T cell cases (41%) and pre B cases (59%) and highest among mature B cell ALL cases (100%).

It has been previously reported that pseudodiploidy was predominant in pre B cases (49%) and mature B cell cases 80% [1,13].

On the other hand, Silva et al. [14] reported that 60.9% of CALLA positive early pre B cases had pseudodiploid karyotype, whereas 62.5% of pre B, 100% of mature B and 100% of mature T cases had pseudodiploid karyotype.

The present study showed that among the 18 pseudodiploid cases, the pre B phenotype cases were associated with favorable prognostic features, low total leucocytic count, lower incidence of organomegally and longer duration of CR1, yet this did not reach significance compared to other phenotypic groups.

The DFS for the pseudodiploid group having pre B phenotype was 75% at 13 months, dropping to 50% at 19 months, whereas the DFS for the pseudodiploid group having CALLA positive early pre B markers was 42% at 6 months, dropping to 16.6% at 12 month. From our results, we conclude that the pseudodiploid karyotype with pre B cell phenotype carries the best prognosis among the pseudodiploid group.

Our results were found to be in contrast with Crist et al. [6], reporting that children with pre B phenotype ALL fared worse on intensive multi-agent chemotherapy than did those with CALLA positive early pre B ALL even after adjustment for other strong prognostic factors including karyotype. The difference between our results and other international reports may be due to the presence of high risk features studied among the patients with CALLA early pre B positive cases as age, high TLC and organomegally. This difference also could be due to the low number of patients within each phenotypic group of the pseudodiploid karyotype in our series.

Crist et al. also reported that among factors with a negative influence on prognosis were early pre B phenotype and pseudodiploid karyotype and they concluded that the pseudodiploid karyotype was of independent prognostic importance in CALLA positive early pre B ALL, but not in pre B and this was confirmed in our study [6].

Seven (16.6%) of the 42 cases were of the T cell phenotype, 71.4% of which carried a pseudodiploid karyotype, all of them carried high risk features, one patient died during induction and 6 relapsed. Our results are in contrast with Amare et al. [1], group francais de cytogenetique haematologique [13] and Raimondi [15] who reported that the majority of T cell cases were associated with hypodiploid karyotype and carried better outcome with modern intensive therapy. However we cannot draw solid conclusion from our results due to small number of cases in each category.

Among pseudodiploid cases, 4/18 (22.22%) had t (8; 14). Out of those 4 cases, 2 had additional chromosome 19 anomaly either translocation or addition and were carrying CALLA early pre B surface marker. It is not clear whether the coexistence of the additional chromosomal

anomaly to t (8; 14) is responsible for changing the cell phenotype. Similar cases were reported by Moore et al. [16] and Komrokji et al. [17]. Patients with t (8; 14) associated with mature B and CALLA early pre B phenotype reported in our study showed no significant difference in the clinical presentation except for TLC which showed a higher mean of 200x10^9/L in the CALLA early pre B patients. Also there were no difference in the mean CR1 and survival for those patients. These cases recognize this subset of ALL and emphasize the need for comprehensive diagnostic analysis.

Amare et al. [1], also reported scarcity of the hyperdiploid karyotype and preponderance of the hypodiploid karyotype in contrast to that reported in literature by Jurkowska et al. [18], Zemanova et al. [19] and to our results.

Among our study patients, hyperdiploidy was found in 31.5% of CALLA positive early pre B cases, 7.7% of pre B and 14.2% of T cells. Pui et al., reported that the hyperdiploid karyotype was associated with 52% of CALLA positive early pre B, 34% of pre B cases and 0.09% of T cell cases [3], whereas Silva et al. found hyperdiploidy in 60.9% of CALLA positive early pre B cases [14].

Hyperdiploidy in our CALLA positive early pre B cases was associated with factors known to exert a favourable prognostic influence as lower total leucocytic count, age between 2-10 years and lower incidence of organomegaly. Their DFS was 83% at 6 months dropping to 66.6% at 18 months which is statistically significant compared to other CALLA positive early pre B in normal and pseudodiploid patients.

In our results, the CALLA positive early pre B phenotype behaves differently with different karyotypic patterns, good with hyperdiploid, intermediate with normal karyotype and poor with pseudodiploid. Our results are in agreement with Pui et al. [3], Shuster et al. [21] and Rubin et al. [22].

The present study showed that patients with >50 chromosomes were encountered in 62.5% of hyperdipoid cases, 80% of these cases were CALLA positive early pre B carrying good risk features, in continuous remission with mean CR1 24 months. These findings are in agreement with Pui et al. [3], Raimondi et al. [16], Harrisson et al. [10] and group Francais cytogenetique haematologique [13].

Fifty percent of our normal karyotypic patients showed pre B phenotype, while 42.8% showed CALLA positive early pre B, one patient was biphenotype and none of our normal diploid cases was associated with T cell surface markers, in contrast to that reported by Amare et al. [1] and Pui et al. [3], who reported that the normal diploid karyotype was found in 10% of T lineage cases compared to 5% of B lineage cases.

In our patients, the mean age, TLC, DFS, of pre B and CALLA positive early pre B of normal diploid cases were almost comparable, nullifying the prognostic role of the stage of cell maturation among patients carrying normal karyotype.

Conclusion:

The presence of CALLA has a positive impact on different chromosomal patterns showing the best outcome among patients with hyperdiploid and least among those with pseudodiploid karyotype. The pseudodiploid karyotype carries a better outcome with pre B phenotype only compared to other phenotyps. A large comparative clinical trial is essential to determine whether patients with t (8; 14) with either mature B or CALLA early pre B phenotype may benefit from short intensive chemotherapy regimen. The normal karyotype still carries intermediate an outcome irrespective to phenotype.

REFERENCES

1- Amare P, Gladstone B, Vaghese C, Pai S, Advani S. Clinical significance of cytogenetic findings at diagnosis and remission in childhood and adult acute lymphoblastic leukaemia; experience from India. Cancer Genetic Cytogenetic. 1999, 110 (1): 44-53.

2- Pui CH, Williama DL, Raimmondi SL, Rivera GK, Look AT, Dodge RK, et al. Hypodiploidy is associated with a poor prognosis in childhood acute lymphoblastic leukaemia. Blood. 1987, 7: 247-253.

3- Pui CH, Williams DL, Roberson PK, Raimondi SC, Behm FG, Lewis SH, et al. Correlation of karyotype and immunophenotype in childhood acute lymphoblastic leukaemia. J Clin Oncol. 1988, 6 (1): 56-61.

4- Williams DL, Harber J, Murphy SB, Look AT, Kalwinsky DK, Rivera G, et al. Chromosomal translocations play a unique role in influencing prognosis in childhood acute lymphoblastic leukaemia. Blood. 1986, 68: 205-12.

5- Crist WM, Grossi CE, Pullen DC, Cooper MD. Immunologic markers in childhood acute lymphocytic leukaemia. Semin Oncol. 1985, 12: 105-21.

6- Crist W, Boyett J, Jackson J, Vietti T, Borowitz M, Chauvenet A, 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. Blood. 1989, 74: 1252-9.

7- Moorhead PS, Nowell PC, Mellanon WJ, Battips DM, Huneford DA. Chromosome preparations of leucocytes cultured for human peripheral blood. Exp Cell Res. 1960, 20: 613-6.

8- Paris conference standardization in human cytogenetics, birth defects: Original article series. New York, the national foundation. 1974, Vol. 8 No. 7.

9- Sather H. The use of prognostic factors in clinical trials. Cancer. 1986, 58 (2 Suppl): 461-7.

10- Harrisson CJ, Foroni L. Cytogenetics and molecular genetics of acute lymphoblastic leukaemia. Rev Clin Hematol. 2002, 6 (2): 91-113, discussion 200-2.

11- Nodgren A. Hidden aberrations diagnosed by interphase fluorescence in situ hybridization and spectral karyotyping in childhood acute lymphoblstic leukaemia. Leuk Lymphoma. 2003, 44 (12): 2039-53.

12- Jackson JF, Boyett J, Pullen J, Brock B, Patterson R, Land V, et al. Favorable prognosis associated with hyperdiploidy in children with acute lymphocytic leukaemia correlates with extra chromosome 6. A pediatric oncology group study. Cancer. 1990; 66 (6): 1183-9.

13- Groupe Francais de Cytogenetique Haematologique. Cytogenetic abnormalities in adult lymphoblastic leukaemia, correlations with haematologic findings and outcome. A collaborative study of the groupe Francais de cytogenetique haematologique. Blood. 1996, 87: 3135-42.

14- Silva ML, Ornellas de Souza MH, Ribeiro RC, Land MG, Boulhosa de Azevedo AM, et al. Cytogenetic analysis of 100 consecutive newly diagnosed cases of acute lymphoblastic leukaemia in Rio de Janeiro. Cancer Genetic Cytogenetic. 2002, 137: 85-90.

15- Raimondi SC. Current status of cytogenetic research in childhood acute lymphoblastic leukaemia. Blood. 1993, 81: 2237-51.

16- Moore S, Suttle J, Bain S, Story C, Rice M. Acute lymphoblastic leukaemia characterized by t (8;14) (q11.2; q32). Cancer Genet Cytopgenet. 2003, 141 (1): 1-4.

17- Komrokji R, Lancet J, Felgar R, Wang N, Bennett JM. Bukitt's leukaemia with precursor B-cell immunophenotype and atypical morphology (atypical Burkitt's leukaemia/lymphoma) case report and review of litterature. Leuk Res. 2003, 27 (6): 561-6.

18- Jurkowska M, Malinowska I, Brycz-Witkowska J, Rokicka-Milewska R. Chromosomal aberrations in differential diagnosis and prognosis in childhood acute leukaemia's. Med Wieku Rozwok. 2003, 7 (3): 335-46.

19- Zemanova Z, Michalova K, Sindelavora L, Smisek P, Brezinova J, Ransdorfofova S, et al. Prognostic value of structural chromosomal rearrangements and small cell clones with high hyperdiploidy in children with acute lymphoblastic leukaemia. Leuk Res. 2005, 29 (3): 273-81.

20- Shuster JJ, Fallata JM, Pullen DJ, Crist WM, Humphrey GB, Dowell BL, et al. Prognostic factors in childhood T cell acute lymphoblastic leukaemia: A paediatric oncology group study. Blood. 1990, 75 (1): 166-73.

21- Rubin CM, Le Beau MM, Mick R, Bitter MA, Nachman J, Rudinsky R, et al. Impact of chromosomal translocations on prognosis in childhood acute lymphoblastic leukaemia. J Clin Oncol. 1991, 9 (12): 2183-92.