Academic Journal of Cancer Research 3 (2): 34-43, 2010
ISSN 1995-8943

Bcl-2 Expression and Chromosomal Abnormalities in Childhood Acute Lymphoblastic Leukemia

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Abstract: Acute lymphoblastic leukemia (ALL) is the most common infant malignancy. Several clinical and biologic features have been found to have important prognostic significance in childhood ALL. The aim of this study was to investigate the correlation between numerical chromos aberrations and frequency of translocations among ALL patients and Bcl-2 expression in pediatric ALL. Also, it aimed to determine their impact on patient response to therapy and treatment outcome. The study included 53 cases of de novo ALL. Patients were subjected to karyotyping and Flow Cytometer analysis. Results revealed that 22/53 (41.5%) patients have abnormal karyotype and 31/53(58.5%) are normal. As regarding numerical aberrations, we detected thirteen cases out of fifty three (24.5%) were hyperdiploidy, while, we found only three cases (5.7%) were hypodiploidy. There was a high statistical significant difference in numerical aberrations in metaphases of ALL patients as compared to normal controls. On comparing the metaphases of structural chromosomal abnormalities between healthy controls and patients, there was a statistically significant increase in leukemia patients as compared to the control subjects. There was a highly statistically significant difference in Bcl-2 expression between patients with abnormal karyotype as compared to the control subjects; it was higher in the former. Bcl-2 was significantly lower in B-CALL cases (24.05±23.7) as compared to both pre-B and T cell phenotype. In our study, neither the karyotype nor the levels of bcl-2 in ALL patients appear to correlate with clinical responses to treatment or markers of prognosis.

Key words: Childhood · Acute lymphoblastic leukemia · Cytogenetics · Bcl-2 · Flow Cytometry

INTRODUCTION

Acute lymphoblastic leukemia is the most common malignancy in children, accounting for almost one third of newly diagnosed pediatric cancer cases. The annual incidence is approximately four cases per 100,000 children per year in the National Cancer Institute (NCI), Cairo University, Egypt. ALL constitutes 30% of all pediatric malignancies and 70% of pediatric leukemia. Cases show a male to female ratio of 2.3:1. The 2-10 years age group constitutes 68.5% [1, 2]. Currently, 80% of children with ALL treated in modern centers are alive and disease-free at 5 years. The major contributors to this long-term survival are the improvements in anticancer therapies [3, 4].

Several clinical and biologic features have been found to have important prognostic significance in childhood ALL, including age, presenting leukocyte count, immunophenotype, response to initial therapy and recurrent chromosomal abnormalities [5, 6]. Chromosomal abnormalities have independent prognostic value, especially in childhood ALL.

Patients with any translocation have a six-fold greater risk of early treatment failure than those without such abnormalities. 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 [7]. 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 [5-7]. Some of these studies have also recognized the correlation between cytogenetic findings and some

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clinical and hematological features as well as the stage of leukemic cell maturation. This contributes significantly in designing the potential therapeutic strategy [5, 8].

The bcl-2 gene, an oncogene discovered through its involvement in the t(14:18) translocation in follicular lymphoma acts to prevent apoptosis and, if consistently over-expressed, appears to act as an oncogene. The Bcl-2 gene generates two proteins: p26 bcl-2α and p22 bcl-2β [9]. A role for inherited genetic factors in a minority of cases is supported by the association between certain constitutional chromosomal abnormalities and childhood ALL and by the incidence of familial ALL. Moreover, siblings of children with leukemia have an approximately two-to-four-fold greater risk of developing ALL than do individuals in the general pediatric population [10].

The aim of current study was to investigate the correlation between chromosomal numbers and frequency of translocations among ALL patients with Bcl-2 expression in paediatric ALL, also determination of the correlation with the clinicopathological features of ALL at presentation and their impact on patient response to therapy and treatment outcome was another target of the present study.

MATERIALS AND METHODS

Patients: The present study included 53 cases of de novo acute lymphoblastic leukemia patients. The patients' age ranged between 3 months - 18 years. Ten healthy age- and sex-matched subjects were taken as controls. The patients presented to the outpatient clinic, National Cancer Institute, Cairo University and subjected to the diagnostic work up which included history and clinical examination, laboratory evaluation including complete blood picture, blood chemistry, bone marrow analysis with the proper cytochemical stains were done to confirm the diagnosis, CSF examination, chest X-ray.

Methods: 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 [11-15].

Immunophenotyping was done for proper lineage affiliation using Flow Cytometer partec III from DAKO cytomation, to confirm the diagnosis of ALL. Heparinized peripheral blood or bone marrow samples were subjected to the following panel of antibodies included CD2, CD3, CD4, CD5, CD7, CD8, CD11c, CD13, CD14, CD16, CD19, CD20, CD22, CD33, CD34, CD45, CD64, CD79a, CD117, Tdt, Mpo, Kappa, Lambda and c IgM. The monoclonal were FITC (fluorescein isothiocyanate)-labeled, clone L27. Moreover, we use anti BCL-2 antibody according to Hartung and Bahler [16] and Infante-Rivard and Guiguct [17].

Statistical Method: SPSS package was used for data management. Means and standard deviation described quantitative data. Frequency and percentages described qualitative data. Non-parametric T-test compared means of two independent groups. Non-parametric ANOVA compared means of more than 2 independent groups Chi-square/Fisher exact test compared independent proportions. P value is significant at 0.05 level.

RESULTS

A total of 53 newly diagnosed ALL patients was selected and analyzed by Conventional Cytogenetics (CC). Bcl-2 expression was analyzed by Flow Cytometry. Table 1 represents the Clinico-pathologic features of our pediatric ALL patients.

CC analysis was carried out, on average; twenty metaphases for each patient sample were analyzed. Fifteen out of 53 (22/53) patients (41.5%) showed an abnormal karyotype and 31/53 (58.5%) were normal.

Table 1: The Clinico-pathologic features of our pediatric ALL patients

	Number of patients (%)
Age range	*3m-18y*
Sex	*31(58%)*
	males
	22(42%)
	females
	M: F= 1.5:1
Clinical findings:	
Splenomegaly	*25/53(47%)*
Hepatomegaly	*22/53(42%)*
Lymphadenopathy	*11/53(20%)*
CSF infiltration	*8/53(15%)*
Total count	*2.3-350*
	$x10^3/mm^3$
Phenotype:	
B-ALL:	*37/53 (70%)*
pro-B	*10/37 (27%)*
common ALL (C-ALL)	*11/37 (30%)*
pre B	*15 (43%)*
T-ALL	*17/53 (30%)*
Bcl-2 expression:	
Positive	*45/53 (85%)*
Negative	*8/53(15%)*

Table 2: Numerical chromosomal aberrations which detected in metaphases of healthy persons and leukemic patients

		Aneuploidy					
Groups	No. of Cases	Hypo	Hyper	Polyploidy	Pseudo.	Total	Mean±SE
Healthy persons	10	2	-	1	-	3	50 ± 0.17
%		0.66	-	0.33	-	1	-
Patients	53	131	157	25	35	348	6.57±1.10*
%		8.23	9.87	1.57	1.16	21.88	-

T-test: - 2.373 p value = 0.021 (significant) Hypo = hypodiploidy, hyper = hyperdiploidy, pseudo = pseudoreduplication. * significant $p < 0.001$

Table 3: Correlation between phenotype of leukemic cells and numerical chromosomal aberration in ALL cases

	B-ALL (37/53=70%)				
	Pre-B	C-ALL	Early Pre-B	B-cell	T-All (16/53=30%)
Hyperdiploid	7/53 (13%)	2/53 (4%)	4/53 (8%)	0(0%)	0(0%)
Hypodiploid	1/53 (2%)	0 (0%)	1/53 (2%)	0(0%)	1/53 (2%)
Normal	6/53 (11%)	10/53 (19%)	5/53 (9%)	0(0%)	16/53 (30%)

Table 4: The relationship between karyotype and response to treatment in ALL cases

	Karyotype		
Response	N	AB	Total
CR	13/53 (25%)	9/53 (17%)	22/53 (42%)
No CR	12/53 (23%)	10/53 (19%)	22/53 (42%)
Died	6/53 (11%)	3/53 (5%)	9/53 (16%)
Total	31/53 (58%)	22/53 (42%)	53 (100%)

P value = 0.57 (N.S) CR:Complete Remission, N=Normal Karyotype, AB=Abnormal Karyotype

Numerical Chromosomal Aberrations: In the present study, patients were divided according to their numerical chromosomal aberrations into hypodiploidy 3/53 (5.7%), 13/53 (24.5%) hyperdiploidy and normal 37/53 (69.8%) as shown in Table 2 and Fig. 1. In this study, we found 13 cases of hyperdiploidy (24.5%), seven of them had 47 chromosome, the most common trisomies observed are trisomy chromosome16 in three cases, trisomy 21 (Down Syndrome) in another three cases and trisomy 22 in one case and four cases had 48 chromosome, while the remaining two cases had hyperdiploidy of 51-60 chromosome (one case had 55, XX chromosome and the other case had 57, XXY chromosome). Hyperdiploid of 57 chromosomes was observed in one case of ALL patients (46, XXY/ 57, XXY, +2, +4, +6, +8, +10, +14, +17, +18, +22). On examining the metaphases of such patients and on comparing with metaphases of normal controls, there was a high statistical significant difference in numerical aberrations as compared to the controls, ($p< 0.001$), (Table 2).

On performing immunophenotyping to our ALL cases, 70% showed B-ALL phenotype and 30% were T-ALL. Relationship between phenotype of leukemic cells and numerical chromosomal aberration in ALL cases is illustrated in Table 3.

Regarding the relationship between karyotype of ALL cases and patients' response to treatment, there was no statistical significant difference between karyotype and the group of patients who achieved complete remission and the other 2 groups of patients who did not achieve complete remission or died patients (Table 4).

Structure Chromosomal Aberrations: The percentages of structural chromosomal aberrations of leukemic patients were observed. It was 57% of the examined metaphases. The most common structural chromosomal aberration is the centromeric separation followed by centromeric fusion (Table 5 and Fig. 2), Fig. (1).

a) Polydiploidy

b) Hyperdiploidy (57, XY chromosomes)

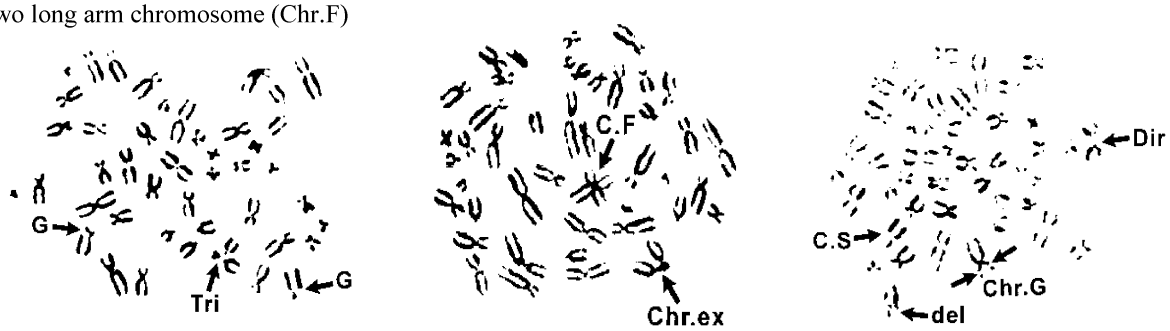
c) Hypodiploidy (45, XX chromosomes)

Fig. 1: Metaphase plates from leukemic patients showing several types of numerical chromosomal aberration

a) Dicentric (Dic), diradial configuration (Dir) and chromatid fusion between two long arm chromosome (Chr.F)

b) Break (B) and triradial configuration (Tri)

c) Chromatid fusion between two long arm chromosome (Chr.F) and acentric fragment (Ac.F)



d) Gap (G), triradial configuration (Tri)

ef) Centric fusion (C.F) and chromatid exchange between short arm of two chromosomes (Chr.ex)

f) Diradial configuration (Dir), deletion (Del) chromatid gap (Chr.G.) and centromeric separation (C.S)

Fig. 2: Metaphase plates from leukemic patients showing several types of structural chromosomal aberration

On comparing the metaphases of structural chromosomal abnormalities between healthy controls and our patients, there was a statistically significant increase in leukemia patients as compared to the control group (Tables 5, a,b).

Giemsa-Trypsin Banding Technique (G/T Banding): By using G/T banding technique of ALL cases we found twenty cases (37.7%) out of fifty three had structural chromosomal abnormalities. Translocation was detected in six cases (11.3%),

Table 5(a): Structural chromosomal abnormalities in acute lymphoblastic leukemia and healthy groups

		Structural chromosomal aberration of ALL and control groups															
Groups	No. of cases	C.S	C.F	A.F	G	Iso.G	Ch.ex	Chr.f	Dic	Del	B	Dir	Tri	Ch.f	Tet	Total	%
Healthy	10	6	3	-	-	-	3	1	-	-	1	2	-	-	-	16	
%		1.33	1	-	-	-	1	0.33	-	-	0.33	0.66	-	-	-	5.3	
Patients	53	394	133	14	5	22	82	88	15	3	13	56	4	69	3	901	56.7
%		24.8	8.4	0.9	0.314	1.38	5.15	5.53	0.943	0.188	0.818	3.52	0.25	4.33	0.188	56.7	
Chi square		63.0	32.16	3.4	1.02	5.13	8.92	10.14	3.09	0.27	0.82	5.52	0.59	19.32	0.38	123.36	
P-value		0.00**	0.00**	0.065	0.311	0.023*	0.002*	0.001**	0.078	0.605	0.364	0.018*	0.44	0.00**	0.532	0.00**	

C.S = centromeric separation, C.F = centric fusion, A.F = Acentric fragment, G = gap, Iso.G = isochromatid gap, Ch.ex = chromatid exchange, Chr.F = chromatid fusion, Dic = dicentric, Del = deletion, B = break, Dir = diradial configuration, Tri = triradial configuration, Ch.F = chromosome fusion, Tet = tetraradial configuration, * = significant, ** = highly significant

Table 5(b): Structural chromosomal abnormalities in acute lymphoblastic leukemia and healthy groups

Groups	Metaphase number (%)	Mean±SD
Healthy group	16 (5%)	1.7±0.15
Patients	901 (57%)	16.81±0.55**

**p<0.001 very highly significant

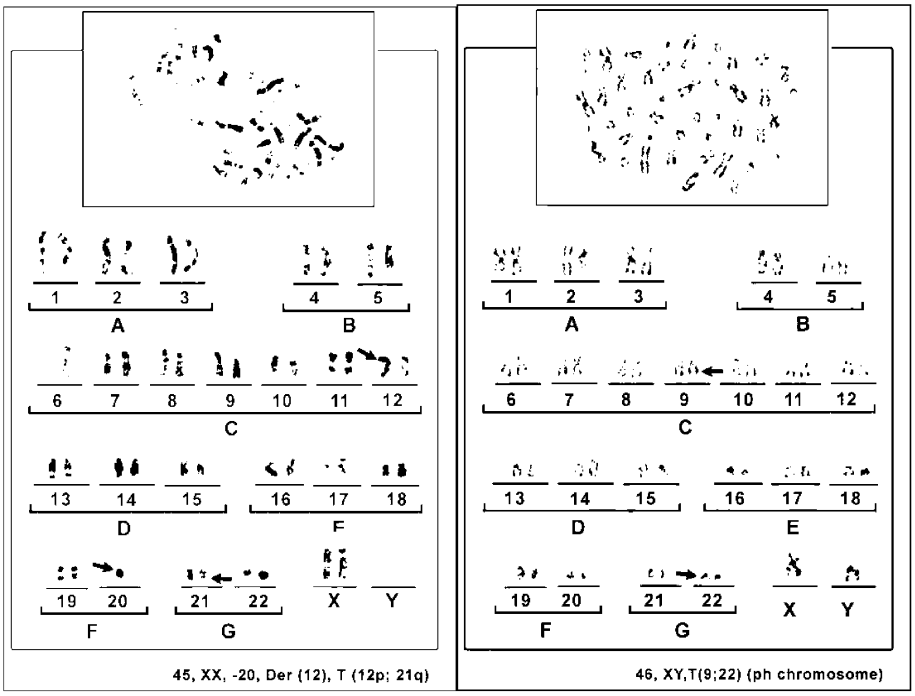


Fig. 3: Structural chromosomal aberration found in ALL

two patients of them (3.8%) had translocation between chromosome 12 and chromosome 21 t(21p12; 21q22) and Philadelphia chromosome (Ph chromosome) (46, XY, t (9q34;22q11) was found in two cases (3.8%), t(3q;16p) was observed in one case (1.9%) and t(5q;15q) in another case (1.9%). Fig. (3) show two examples of structural chromosomal aberration found in ALL.

Family Pedigree of Patients: In the present study, the family pedigree was positive in 4/53 (7.5%) Fig. (4).

BCL-2 expression in the 53 ALL patients was measured by Flow Cytometry, showed no statistical significant difference in expression with age, Bcl-2 was significantly lower in B-CALL cases (24.05±23.7) compared to both pre-B and T cell phenotype (64.9±25.83, 63.63±29.5), Table (5).

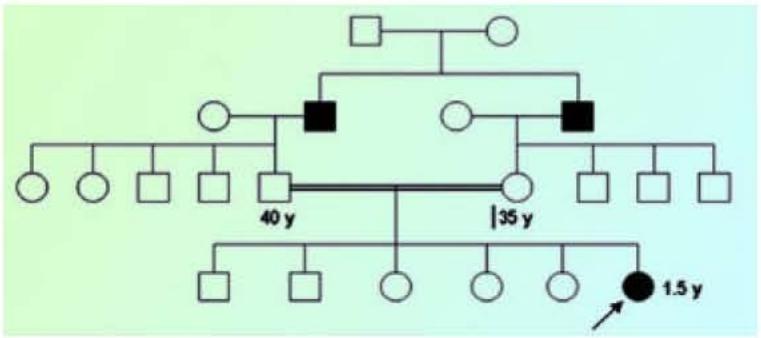


Fig. 4: Family pedigree of leukemia patient

Whereas:

●❑ Represents: female, male suffered from ALL

→ Refers to a proband

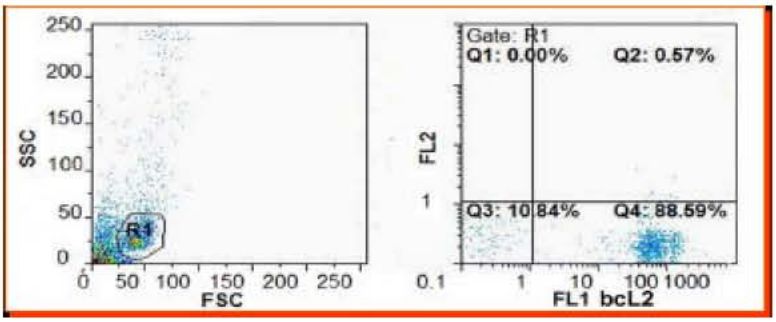


Fig. 5: Show Flow Cytometric analysis case no. (9) Revealed +ve BCL-2 expression (88.59%)

Table 6: The relation between BCL-2 expression and immunophenotypic pattern of ALL cases

		BCL-2	
IPT	N	Mean±SD	P-value
Pro B	12	47.20±32.970	N.S
B C-ALL	11	24.054±23.70	0.002**
Pre B	14	64.92±25.830	N.S
T-ALL	16	63.62±29.470	N.S

**p<0.002 highly significant, IPT=Immunophenotyping

Table 7: The relation between BCL-2 expression, response to treatment

Response	₦	Mean±SD
CR	22/53(42%)	46.56±32.52
No CR	22/53(42%)	58.57±31.27
Died	9/53(16%)	49.44±32.09
Total	53	52.03±31.82

P value = 0.57 (N.S)

Table 8: The relation between BCL-2 expression and the karyotype of ALL patients

		Karyotype	Maen±SD	P value
Bcl-2	N	31/53 (58%)	39.24±30.31	<0.001**
	AB	22/53 (42%)	73.15±21.67	

N: normal karyotype, AB: abnormal karyotype, **: highly significant P value

In this study, 22/53 (42%) of our patients achieved complete remission while the same percent did not achieve complete remission and 9/53 (16%) of our patients were died, Table (7). There was no statistical difference in Bcl-2 expression among different groups sowed different response to therapy.

There was a highly statistically significant difference in Bcl-2 expression, it was higher in the group of abnormal karyotype as compared to the normal one, p=<0.001, Table (8).

DISCUSSION

Cancer is one of the world 's leading cause of death and ocuurs when the homeostatic balance between cell growth and apoptosis is disturbed.

High expression of Bcl-2 protein may be responsible for the occurrence of leukemia and may be used for identification of the disease at earlier time [10].

In this study, B-ALL represent 37/53 (70%) of ALL cases, they were classified as: pro-B-ALL which represent 12/37 (23%); common ALL (C-ALL) represent 11/37 (21%) and pre B found in 14 (26%). On the other hand, T-ALL constituted about 16/53 (30.2%) of ALL cases. Based on

the morphological characteristics, ALL is classified as ALL-1, ALL-2 and ALL-L3. ALL-L2 subtype constituted the majority of ALL cases. This finding was in agreement with Greer Bashir *et al.* [18] In the present study, there was a high statistical significant difference in numerical aberrations as compared to the controls, $p< 0.001$. Where, hyperdiploidy was detected in 24.5% while, hypodiploidy in 5.7%.

Our results was in agreement with Greer Bashir *et al.* [18] and Silva *et al.* [19] who reported numerical abnormalities in 26- 55% of ALL patients, where hyperdiploidy is the most common abnormality in ALL cases.

The hyperdiploidy is associated with the B-cell lineage and none of the T-cell ALL patients. T-cell lineage is commonly associated with a normal karyotype. Our result is in agreement with Paulsoon *et al.* [20].

Compared with other cytogenetic subtypes of childhood ALL, hyperdiploidy is often associated with good risk features, such as a CD10+ common B ALL immunophenotype, low leukocyte count and age between 2 and 10 years [22 - 24].

The chromosomal gain in high hyperdiploid appear to be restricted to certain chromosomes namely chromosomes 6, 9, 10, 14, 17, 18, 21 and X chromosome suggesting that only particular chromosomal configuration are viable. Our findings were in agreement with Vrooman and Silverman [9] Silva *et al.* [19] and Paulsoon *et al.* [20].

We found that the frequency of pseudodiploid and normal karyotype were comparable (38.2%) among B phenotypic groups. This is in agreement with Hamouda *et al.* [7]. On the contrary, Amare *et al.* [21] reported that pseudodiploidy in B lineage is a common finding among pediatric ALL patients.

The variation could be attributed to the laboratory methods applied, factors such as low mitotic index, poor quality of bone marrow specimens or poor morphology of chromosomes and some times the presence of only normal metaphases derived from normal cells after culture.

Children with Down syndrome (DS) who carry trisomy 21 in all their cells have a 20-fold increased risk for childhood ALL and 600-fold risk for acute megakaryocytic leukemia (AMKL)(33-45-48). In the present study, down syndrome (DS) represent three cases of ALL patients (5.7%).

Trisomy 16 was detected in 3 cases (5.7%), trisomy 22, was detected in only one case (1.9%). Our finding was in agreement with Silva *et al.* [19] and Paulsoon *et al.* [20].

Monosmy of X-chromosome was found in 2% of cases. This was supported by the finding of Silva *et al.* [19] and Stanchescu *et al.* [22], who found monosomy X in 2% and 7%, respectively in their ALL cases. Hypodiploid was evident in 3/53 (5.7%) of ALL cases. Our result was in accordance to Vrooman and Silverman [9], Stanchescu *et al.* [22], Raimondi *et al.* [23] and Harrison *et al.* [24] and who found that the frequency of hypodiploidy is from 6-7% of their ALL cases.

We found that hypodiploidy is associated with B and T-phenotype. This is consistent with Harrison *et al.* [24] who found that high hypodiploid include patients with T-lineage ALL in addition with those with pre-B, common phenotype whereas the near haploid and low hypodiploid had common, pre-B phenotype.

As regarding structural abnormalities, we found two patients (3.8%) had translocation between chromosome 12 and chromosome 21, t(12p12; 21q22). This is a lower frequency as compared to Douet-Guilbert *et al.* [25], Berthou *et al.* [26] and Sawinska and Ladon [27] who showed a frequency of 25% in their ALL cases.

Cases with t(12p12; 21q22) displayed the B-cell phenotype, they express CD10, CD19, CD22 antigens on their surface. Most cases with t(12p12; 21q22) have been classified to be L2 subtype according to FAB classification. Our results were in agreement with Stanchescu *et al.* [22] and Sawinska and Ladon [27].

Translocation between short arm is Chromosome 12 and long arm is Chromosome 21 t(12p12; 21q22) (ETV6/RUNX1) was reported to be the most common translocation in childhood ALL and is generally associated with a favorable prognosis. This change is most frequent among the children between 1 and 12 of age with a peak between 2 and 5 years, absent in infancy (age less than 1 year) [9, 22, 23].

In the present study, Philadelphia chromosome (Ph chromosome) that is the result of reciprocat translocation between Ch. 9 and 22 t(9 q34;22 q11) (BCR;ABL), was recorded in 2/53 (3.8%) of our patients. Our result was in agreement with Vrooman and Silverman [9], Greer Bashir *et al.* [18] Harrison *et al.* [24] and Harrison [28] and who reported that Ph chromosome was the most frequent in ALL (6%).

The Philadelphia chromosome (Ph) is the harbinger of a poor outcome. Polymerase chain reaction (PCR) assays can detect leukaemia-specific genetic lesions down to a sensitivity approaching one leukaemia cell in a background of a million normal cells. In Ph+ ALL, the unique BCR-ABL translocation is thus a specific target for the detection of minimal residual disease (MRD). After chemotherapy or transplantation the detection of residual BCR-ABL transcripts is associated with a

high risk of subsequent relapse. With the advent of novel therapeutics that target the structure and function of BCR - ABL, the detection of MRD may allow for targeted therapy that could abort a potential relapse [9, 20].

We found t(3q; 16p) in one of our cases (1.9%). This translocation resulting from fusion of MRP gene located on chromosomes 3 with CREP gene located on chromosomes 16. Interestingly, 16p13 is the site of the multidrug resistance associated protein gene (MRP) and of the CREB binding protein gene. Translocation of the former gene could result in up-regulation of its expression and hence protect the evolved clone from chemotherapy [29].

In our work, among the 53 ALL cases, 8% of families were consanguineous and 68% were none consanguineous. This is not in agreement with Infant-Rivard and Guiguct [17], Michel *et al.* [30] and Yasmeen and Ashraf, [31], who reported a high percent (80%) of families were consanguineous among 69 cases of ALL while 20% were non- consanguineous.

High expression of bcl-2 protein may be responsible for the occurrence of leukemia and may be used for identification of the disease at earlier time. We found that high bcl-2 expression was not associated with any clinical and laboratory features that have been commonly associated with poor treatment outcome, such as age, gender, high WBC, hepatomegaly, high blast % in Peripheral Blood (PB) or Bone Marrow (BM). These data are supported by Sriniwas *et al.* [32], Sahu and Das, [33] and Hartung and Bahler [34].

Using Flow Cytometry, blasts typically express much significantly higher levels of Bcl-2 compared with normal haematogones. The level of Bcl-2 expression studied by Flow Cytometry was variable among our ALL patients ranging from 0.21 to 95%. Although this variability, it showed significantly higher levels than in any of the lymphocyte population present in the normal specimens examined. These data is in agreement with, Hartung and Bahler [34], McKena *et al.* [35] and Menendez *et al.* [36].

Bcl-2 was significantly higher in B-lineage ALL as compared to T-lineage ALL cases. These data are in agreement with Narayan *et al.* [37] although the difference did not reach the significant level. In our study, Bcl-2 was significantly lower in C-ALL (24.05 ± 23.7) (good prognosis) compared to both pre B, T cell type. While, high bcl-2 expression correlated with T-ALL, pro-B ALL (63.63 ± 29.47; 47.2 ± 32.97) (bad prognosis). Our result is not consistent with Hartung and Bahler [34] who did not find any correlation between Bcl-2 and the phenotype of Bcl-2.

In the present study, high Bcl-2 level was not associated with patient response to treatment and failure to achieve CR. This is in agreement with Hartung and Bahler [34] and Marschitz *et al.* [38] and On the contrary, Del Principe *et al.* [39] found that Bcl-2 positivity was associated with a significantly higher complete remission rate. Moreover, he assumed that Bcl-2 represents sensitive indicators of clinical outcome and potential targets of novel molecules aimed at overcoming chemoresistance and recurrent relapses.

On attempt to find any correlation between Bcl-2 expression and cytogenetic abnormality, we found that chromosomal abnormality was significantly correlated with high Bcl-2 protein expression ($P < 0.001$). Our finding is consistent with Greer Bashir *et al.* [18], Berthou *et al.* [26] and Sawinska and Ladon [27].

Early leukemogenic events should protect lymphoid progenitors with defective rearrangements from apoptosis perhaps through untimely upregulation of Bcl2 [40, 41].

In conclusion, leukemia is inherited disease. It's suggested that Flow Cytometry for measurement of Bcl-2 protein expression may be considered as a biomarker for diagnosis of leukemia and identifying children at risk of leukemia at earlier time. So we recommend Flow Cytometric analysis of BCL-2 expression in large number of ALL patients to detect its diagnostic and prognostic value in this disease.

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